Intégration des bioprocédés de valorisation des résidus organiques pour une gestion durable des agroécosystèmes

Julie Jimenez

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Julie JIMENEZ

Chargée de Recherche-INRAE

Intégration des bioprocédés de valorisation des résidus organiques pour une gestion durable des agroécosystèmes

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INRAE, UR0050 LBE Laboratoire de Biotechnologie de l'Environnement, Narbonne

" Je déclare avoir respecté, dans la conception et la rédaction de ce mémoire d'HDR, les valeurs et principes d'intégrité scientifique destinés à garantir le caractère honnête et scientifiquement rigoureux de tout travail de recherche, visés à l'article L.211-2 du Code de la recherche et énoncés par la Charte nationale de déontologie des métiers de la recherche et la Charte d'intégrité scientifique de l'Université de Montpellier. Je m'engage à les promouvoir dans le cadre de mes activités futures d'encadrement de recherche."

Remerciements

Par où commencer….

Je souhaite dédier ce manuscrit et bilan de mes 15 ans dans le monde de la recherche à deux personnes sans qui je ne serais pas devenue ce que je suis. Un grand, énorme, gigantesque MERCI à **Jean-Philippe Steyer et Dominique Patureau**. Depuis que je suis « toute petite », vous avez cru en moi, avez misé sur moi, m'avez soutenue (dans les bons comme dans les mauvais moments) et m'avez beaucoup apporté. Jean-Phi, tu as toujours su comment me pousser pour avancer, pour m'améliorer et tu y parviens encore et toujours. Domi, sans toi cette thématique de recherche n'existerait pas. Tu es mon soutien number one !

Je souhaite également dédier ce mansucrit **au LBE.** Tout est parti de ce labo … pour finalement y revenir !
Je me souviens encore de ma dernière année à l'IUP de Perpignan délocalisée au LBE en 2004, où déjà, Jean-Phi, tu avais donné le ton et m'avais donné envie de faire ce que je fais aujourd'hui, et toi Domi, faisais office de « maman » pour nous jeunes novices sortis de l'œuf. Tout est parti du LBE. A partir de là et de mon stage à l'INSA de Toulouse (auprès d'**Etienne Paul**, un autre mentor à mes yeux dans le domaine de la recherche), tout s'est enchaîné. Diplomée ingénieure, j'ai eu l'opportunité de rejoindre Veolia et son centre de Recherche « Anjou Recherche » pour sept ans (une pensée pour mon cher collègue de toujours **Pascal Boisson**). Pendant cette période, j'ai eu l'occasion de recroiser Jean-Phi qui arriva à me convaincre de réaliser une thèse (merci **Emmanuel Trouvé et Stephane Déléris**) avec en co-encadrant mon **cher Fabien Vedrenne** (une autre personne ayant marqué ma carrière). Et moi qui ne voulais pas faire de thèse ! Et puis, bizarrement, l'appel du sud et de l'académique se sont fait sentir. Je n'ai pu résister à l'envie de tout laisser, Veolia, CDI, Paris et revenir au LBE avec Domi pour un post-doctorat. Et puis, deux ans après, ce poste de chargée de recherche, en adéquation avec mes compétences et mes envies de recherche, me permet de revenir au LBE pour de bon.

Alors, **MERCI le LBE, à tout le collectif**, ce laboratoire où il fait bon travailler, où le climat familial et bienveillant règne et où la bonne humeur est de mise.

Plus spécifiquement, je souhaiterais remercier **Renaud Escudié et Eric Trably** pour votre bonne humeur, nos échanges (scientifiques ou non) et nos « joutes » verbales ! Je remercie **Nicolas Bernet**, actuel directeur du LBE, pour son écoute bienveillante et ses conseils avisés. Merci à **Rémi Servien** pour ses conseils de « vétéran » et à **Eric Latrille** pour son aide en statistiques et en autres domaines, et ce depuis mes débuts en thèse. Merci à **Diana Garcia Bernet** pour son aide et ses conseils sur la valorisation des résultats et la gestion de projets. Je souhaiterais remercier les techniciens (dont certains sont partis) et gestionnaires pour leur aide précieuse : **Philippe Sousbie, Nadine Delgénès, Pascal Gras, Guillaume Guizard, Denis Loisel, Nadine Le Thinh, Sylvie Farine et Stephanie Harmand**.

Je souhaiterais rendre hommage à **Michel Torrijos** qui est parti bien trop tôt. Nos discussions autour de la bioaccessibilité et l'hydrolyse me manquent et je n'oublie pas que nous voulions travailler ensemble sur la modélisation de la co-digestion…

Je remercie mes collègues devenus amis **Virginie Rossard, Clémence Pagès (Nounoune), Gabriel Capson** pour leur amitié et leur soutien de tous les jours ! Et ceux qui sont partis mais qui m'ont soutenu pendant ces 9 dernières années (**Thibaut Saur, Cyrille Charnier, Florian Paillet, Roman Moscoviz, Lucia Braga Nan, Noémie Figeac, Antoine Haddon, Elsa Rouches, Violette Turon, Alice**

Danel, Quentin Aemig, Morand Severac, Bastien Zennaro, Felipe Guilayn, et mon bon vieux Clément Van Vlierbergue).

Je remercie toutes les personnes que j'ai encadré au LBE et VERI et qui ont participé activement à mes recherches (Bastien, Quentin, Nicolas, Lucie, Chloé, Noémie, Louise, Claire, Clémentine, Tom, Jeanne, Ziren, Han, Camilla, Rémi, Axelle, Albane, Solène, Nicolas, Estelle, Cécile, Ziren, Paolo, Ségolène, Ulysse, Matthjis, Omar).

Je souahite également remercier **Sabine Houot** qui suit ma thématique depuis le début de mon poste de chargée de recherche, avec qui j'ai beaucoup collaboré et qui m'a beaucoup apporté et appris.

Et enfin, je souhaiterais remercier tout particulèrement les trois doctorants que j'ai eus et ai encore le plaisir d'encadrer : **Felipe Guilayn, David Fernandez-Dominguez et Margaud Pereme**. Comme j'ai pu le dire en conclusion, encadrer des jeunes chercheurs, et notamment des doctorants est une expérience à part entière. Pour moi, il s'agit vraiment d'un partage et d'un échange mutuel. Je vous accompagne dans votre démarche scientifique de thèse, vous aiguille tout en vous laissant vous épanouir et mener votre projet (car c'est le vôtre) comme vous voulez le mener. Et vous, vous me nourrissez de réflexions, de discussions, vous me poussez à prendre du recul et à me questionner et cela fait énormément avancer mes réflexions et me fait mûrir. Grâce à vous, je me suis décidée à faire ce bilan de mes recherches afin de rebondir encore plus loin. MERCI !

Plus personnellement, je souhaiterais remercier ma famille, mes proches, qui ont toujours été là quoiqu'il arrive, et une dédicace profonde à mon père qui aurait été, je pense, fier de là où je suis arrivée.

Je souhaiterais remercier mon cher et tendre Romain. Je ne pensais pas prendre autant de temps à rédiger cet HDR ! Et toi non plus je pense ! Que de discussions autour de ça ! Mais que de soutien, de motivation, d'aide et de relecture de ta part !!!! Nos discussions scientifiques (ou non) sont source d'inspiration et je nous souhaite de collaborer ensemble scientifiquement (et moins scientifiquement) sur certains sujets à venir.

Je terminerai par remercier mes animaux (enfin, nos animaux) sans qui la rédaction du manuscrit aurait été impossible : Sirius, Helmy, Pandou et Phantom … un zoo ambulant mais tellement affectueux, drôle et apaisant !

Et… MERCI à l'Echoppe Médiévale et à l'Antre pour le soutien infaillible toutes ces années et ces jeudis soirs. Narbonne ne serait pas Narbonne sans vous !

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Introduction

« La **rétro-ingénierie**, ou **ingénierie inverse**, est l'activité qui consiste à étudier un objet pour en déterminer le fonctionnement interne, la méthode de fabrication, peut-être dans l'intérêt de le modifier. On parle également de **rétro-conception** dans le domaine du vivant. Le terme équivalent en anglais est ***reverse engineering***. »

Cette définition, au cœur de ma thématique de recherche, reflète bien mon parcours en recherche, qu'elle soit dans le domaine privé ou public depuis 15 ans.
En effet, tout au long de mon parcours professionnel, j'ai eu à cœur d'étudier la valorisation des résidus organiques, que ce soit via des approches de caractérisation de la matière, de génie des procédés biologiques afin de « déterminer le fonctionnement interne, la méthode de fabrication » pour mieux comprendre les processus biologiques impliqués, les modéliser « peut-être dans l'intérêt de le modifier », et les optimiser.

Ce manuscrit décrit mes recherches passées, présentes et à venir répondent à cette définition appliquée à la thématique suivante : **« Intégration des bioprocédés de valorisation des résidus organiques pour une gestion durable des agroécosystèmes »** dans un contexte de transition à la fois énergétique et agroécologique. Il décrit également la mise en place de ma stratégie de recherche dédiée à résoudre cette problématique, stratégie appuyée notamment par la mise en place de projets de recherche (que ce soit en tant que coordinatrice ou contributrice), l'encadrement de stagiaires, doctorants, post-doctorants, ingénieurs et la valorisation des résultats afin de prétendre à l'obtention de l'Habilitation à Diriger des Recherches.

Après un Chapitre 1 dédié à la description de mon Curriculum Vitae, la description factuelle de mes activités de recherches et de mes encadrements ainsi que mes productions et valorisations scientifiques, le Chapitre 2 synthétise mes travaux de recherche, basés sur une description préalable du cadre, du contexte et des objectifs de mes recherches. Ce chapitre résume comment j'ai mis en place ma thématique via trois axes de recherches dont les piliers sont associés à mes trois domaines de compétences : la caractérisation matière, le génie des procédés et la modélisation des procédés. Il s'attele à montrer, sous formes de questionnements, comment ces trois compétences sont le socle de ma thématique.
Le Chapitre 3 présente les perspectives de mes recherches sur les bases des résultats et accomplissements décrits dans le Chapitre 2. Dans cette partie, je propose un projet de recherche à court puis long terme autour de l'ingénierie inverse, en utilisant mes compétences pour les approfondir et aller plus loin sur la prise en compte des agroécosystèmes et les impacts environnementaux d'une filière de valorisation des résidus.
Enfin, je terminerai ce manuscrit par une conclusion et analyse plus générales sur mes activités de chercheur.

Chapitre 1. Curriculum Vitae détaillé

1. Curriculum Vitae

Etat Civil

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Parcours professionnel

Depuis 2015 : Chargée de Recherche INRAE- Laboratoire de Biotechnologie de l'Environnement- *Narbonne*

Adaptation et intégration des bioprocédés de traitement des effluents et des résidus pour une gestion durable des agrosystèmes

Mots clés : *Caractérisation, Matière, Modélisation, Méthanisation, Digestat, Bioraffinerie Environnementale, Optimisation, Agroécologie*

2013-2015 : Post-doctorat à INRAE-Laboratoire de Biotechnologie de l'Environnement- *Narbonne*
Caractérisation de résidus organiques et modélisation des processus biologiques d'une filière de traitement de ces résidus – Projet RISQ-PRO (financé par ONEMA) : *Evaluation et réduction des risques de contamination par des polluants organiques dans le contexte de l'usage de Produits Résiduaires Organiques (PRO) sur sols agricoles*

- Devenir de la matière organique avec la **modification de modèles actuels de digestion anaérobie, compostage et d'épandage sur sol** ;
- **Développement d'une méthode de caractérisation** afin d'unifier les variables entrantes dans les modèles
- Devenir des micropolluants organiques (hydrocarbures aromatiques polycycliques) en lien avec la matière organique

2007-2013 : Ingénieure de recherche en modélisation des procédés biologiques de traitement des eaux usées à VEOLIA Environnement Recherche et Innovation-*Maisons Laffitte*

- Etude du procédé **de Digestion anaérobie** des boues d'épuration et d'ordures ménagères résiduelles
- **Modélisation des processus biologiques** de la **digestion anaérobie** des boues d'épuration et des **bioréacteurs à membranes** traitant des eaux usées
- **Caractérisation de la matière organique des résidus organiques liquides et solides** (développement d'outils de caractérisation innovants de la matière organique et sa biodégradabilité)

Diplômes universitaires

2012 **Doctorat en Génie des Procédés** - Université Montpellier II : Caractérisation de la matière organique par spectrofluorimétrie 3D et modélisation de la digestion anaérobie des boues d'épuration***. Collaboration VERI/INRAE LBE Narbonne.***
Directeur de thèse : Jean-Philippe STEYER (Directeur de Recherche, INRAE LBE Narbonne)

Co-encadrant de thèse : Fabien Vedrenne (Chercheur VERI, Maisons Laffitte)
Rapporteurs : Fabrice Béline (Directeur de Recherche, IRSTEA, Rennes) et Peter Vanrolleghem (Professeur, Université de Laval, Québec, Canada)

2007 Ingénieure en Génie des Procédés - Institut National des Sciences Appliquées (INSA) Toulouse

Stage fin étude (6 mois) : « Caractérisation d'effluents agro-alimentaires et modélisation des boues activées » VEOLIA STI (solution et technologie industrie), Plessis Robinson.

Stage technique (3 mois) : « Etude et suivi d'un digesteur anaérobie à lit fixe semi-industriel pour le traitement d'effluents d'abattoirs ». Universidad de Burgos, Espagne

2005 Maîtrise IUP (Institut Université professionnel) Génie des Systèmes Industriels - Université de Perpignan

Stage fin étude (6 mois) : « Traitement thermo-enzymatiques des boues d'épuration » Laboratoire LISBP INSA Toulouse. *Etudes pilotes laboratoires boues activées et fermenteurs*

2001 DEUG 1ere Année Mathématiques et Informatiques appliquées aux Sciences- Université de Perpignan

2. Activités de recherche

Depuis l'obtention de mon diplôme d'ingénieur et dans le cadre de mes activités professionnelles en tant qu'ingénieur de recherche puis chercheur, j'ai développé et consolidé trois expertises majeures, schématisées par la Figure 1 ci-après, en (i) caractérisation de la matière, en génie des procédés (notamment des procédés biologiques de traitement puis valorisation des résidus organiques) pour (ii) modéliser les processus biologiques et à la fois prédire les performances de ces procédés et d'optimiser les filières de valorisation de ces derniers, tant au niveau production de molécules d'intérêt (production d'énergie via la méthanisation par exemple) que leur réutilisation en agronomie en tant que fertilisants organiques (digestats, composts,…). Ces trois expertises interagissent entre elles (i.e. la caractérisation alimente les variables des modèles et aide au suivi des procédés ; la modélisation utilise les données du procédé et en retour peut aider à son pilotage) afin d'être mises au service de l'optimisation des bioprocédés de traitement de tous types de résidus (i.e. urbains, agricoles, agro-alimentaires, liquides ou solides).

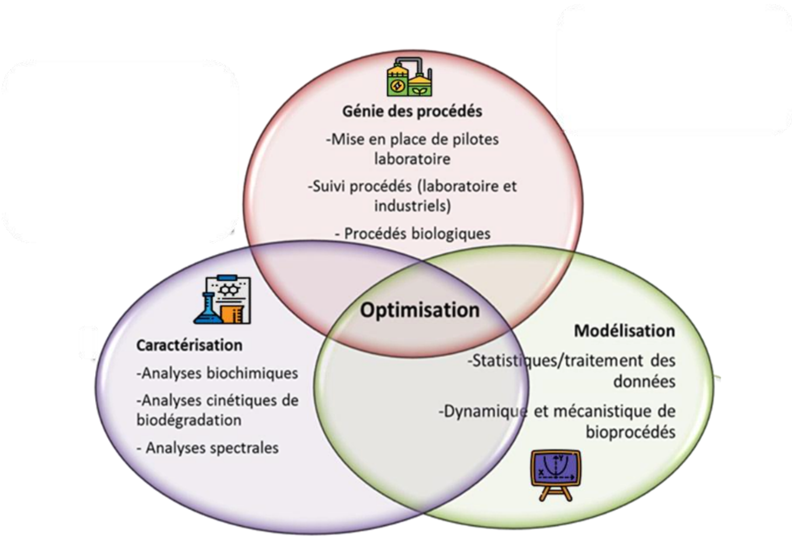

Figure 1 : Expertises développées tout au long de ma carrière professionnelle au sein de la recherche

Ces expertises sont également nécessaires pour répondre à ma thématique de recherche dont j'ai la responsabilité et que je développe depuis 2015 à INRAE autour de « l'adaptation et intégration des bioprocédés de traitement des résidus organiques pour une gestion durable des agrosystèmes », en adéquation avec les objectifs scientifiques INRAE. En effet, lors du traitement et de la valorisation des résidus organiques, les produits résiduaires organiques générés doivent répondre à des critères de qualité agronomique incluant efficacité et innocuité (environnementale et sanitaire). Il est donc obligatoire de concevoir les bioprocédés en fonction des antagonismes entre les services attendus (i.e. dimensions agronomique et écosystémique, traitement de la pollution, et production d'énergie et/ou de molécules plateformes) à la recherche d'un compromis. Trouver un compromis entre valorisation agronomique et énergétique, entre autres services rendus, passe par la modélisation et le contrôle des procédés de traitement des résidus organiques afin de mieux concevoir ces derniers, de les adapter aux besoins en aval et de repenser à des filières innovantes. La connaissance de la qualité des résidus et produits résiduaires organiques est primordiale pour la modélisation des procédés mais aussi pour prédire leur qualité avant retour vers les agrosystèmes (i.e. sols, plantes) et environnement (i.e. eau, air) et ainsi pouvoir utiliser ces critères en tant qu'objectifs dans l'optimisation et le contrôle des procédés de traitements. Ces critères de qualité dépendent des agrosystèmes et environnements ciblés, des plantes cultivées, notamment en termes d'interactions entre les cycles biogéochimiques du carbone (C), de l'azote (N), du phosphore (P) et d'autres éléments primordiaux, enjeu majeur au sein du département AgroEcoSystem d'INRAE.

La suite de ce chapitre décrit succinctement les activités de recherche réalisées que ce soit lors de mon stage de MASTER, mon emploi à VERI, ma thèse de doctorat et les projets de recherche auxquels j'ai participé depuis 2007. Puis, un tableau récapitulatif décrit les encadrements de doctorants, stages Master, et ingénieurs/post-doctorats que j'ai réalisés.

Stage, doctorat et post-doctorat

Stage de fin d'études ingénieure en génie des procédés

Caractérisation d'effluents agro-alimentaires et modélisation des boues activées (février-juin 2007)

INSA Toulouse, Génie des Procédés
VEOLIA STI (Solution et Technologie Industrie), Plessis Robinson
Collaborations : LISBP (INSA Toulouse)
Mots clés : *boues activées, eaux usées industrielles, modélisation, ASM, caractérisation*

L'objectif de mon stage était de prendre en main un outil de modélisation et simulation des processus biologiques d'une boue activée traitant des eaux usées (GPSX), calé et validé pour des eaux usées urbaines et de le caler et le valider sur des eaux usées industrielles. Cela a été réalisé avec les données d'une usine de traitement des eaux usées agroalimentaires située à Sablé sur Sarthe. J'ai pu (i) mettre en place une campagne de prélèvements et d'analyses et de récupération du suivi de la boue activée de l'usine, (ii) réaliser des tests en laboratoires afin de déterminer certains paramètres nécessaires à la modélisation de la boue activée et (iii) me former, caler puis valider le modèle de boues activées ASM1 (Activated Sludge Model n°1)[1] sur la boue activée de l'usine. ***Cette expérience a été la base de ma formation en modélisation des processus biologiques d'un procédé de traitement ainsi que la base de mes connaissances en définition et caractérisation de la matière organique.***

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[1] IWA Task group on mathematical modelling for design and operation of biologic wastewater treatment (1987). *Activated Sludge Models ASM1, ASM2, ASM2d, ASM3*, Scientific and technical Report.

Expérience professionnelle en tant qu'ingénieur de recherche à VERI

Ingénieure de Recherche en modélisation des procédés biologiques de traitement des eaux usées (2007-2013)

VERI, Veolia Recherche et Innovations, Maisons Laffitte
Collaborations : VEOLIA, INRAE-LBE, ENVOLURE, Ecole Polytechnique de Lausanne (Suisse)KWB (Berlin, Allemagne), VITO (Belgique), TTX (Italy), Berlin University of Technology (Allemagne), UNSW (Australie), VERI (France), Aquafin (Belgique), A3 (Allemagne), Polymem (France), Inge (Allemagne), Envi-Pur (République Tchèque)
Mots clés : *eaux usées, station d'épuration, boues activées, bioréacteur à membranes, modélisation, procédés biologiques, méthanisation, ASM1, ADM1, caractérisation, respirométrie, boues*

Dans le cadre de mon expérience d'ingénieur de recherche à VERI, j'ai démarré ma carrière en participant au projet Européen AMEDEUS (projet et participation décrits ci-après) sur la modélisation des processus biologiques des bioréacteurs à membranes (MBR) via un modèle modifié type ASM1 pour traiter des eaux usées urbaines. Cela a donné lieu à des productions scientifiques variées (deux publications et deux conférences internationales). Une des publications a notamment été réalisée via un groupe de travail international sur la modélisation des MBR, formé lors des comités de pilotage du projet. En parallèle, j'ai adapté et mis en place des protocoles de caractérisation de la matière et de détermination expérimentale de paramètres utilisés dans la modélisation des procédés de traitement des boues activées qui ont servi à caractériser puis modéliser le traitement d'un large panel d'eaux usées industrielles auprès de la direction technique de VEOLIA.
Enfin, j'ai été en charge du développement de la modélisation du procédé de digestion anaérobie des boues issues de station d'épuration (STEP) puis de la fraction fermentescible des ordures ménagères (FFOM) impliquant la mise en place de pilotes laboratoires, des suivis analytiques, des caractérisations de la matière organique. Une partie des travaux sur la caractérisation de la matière organique pour la modélisation de la digestion anaérobie des boues a été réalisée via une thèse de doctorat entre 2009 et 2012 décrite ci-après.

Doctorat

Caractérisation de la matière organique par spectrofluorimétrie 3D pour la modélisation de la digestion anaérobie des boues issues de stations d'épuration (2009-2012)

VERI, Maisons Laffitte et INRAE-LBE
Université de Montpellier, Génie des procédés
Collaborations : VERI, Maisons Laffitte et INRAE LBE
Mots clés : *caractérisation, matière organique, bioaccessibilité, méthanisation, modélisation, fluorescence 3D, fractionnement, ADM1*

Afin d'optimiser aussi bien le procédé de digestion anaérobie que la filière complète du traitement des eaux usées, la caractérisation de la matière organique entrante est cruciale afin de contrôler son devenir dans le procédé, notamment grâce à la modélisation. Pour cela une collaboration entre VERI et le laboratoire INRAE LBE a été mise en place et a conduit à une thèse que j'ai réalisée dans le cadre de mes activées d'ingénieur de recherche à VERI en parallèle de mes autres tâches. L'objectif de ma thèse était de développer et d'appliquer une nouvelle méthode de caractérisation de la matière pour faciliter et améliorer la modélisation des processus biologiques se produisant lors de la méthanisation des boues issues STEP. Plus particulièrement, la question de recherche portait sur la notion de bioaccessibilité et de biodégradabilité de la matière organique et comment la décrire puis y associer des indicateurs expérimentaux. C'est ainsi que j'ai développé une nouvelle méthode de caractérisation combinant un fractionnement de la matière organique, basée sur la caractérisation des exopolymères des boues d'épuration, et une analyse spectrale des fractions extraites par fluorimétrie 3D afin de prédire leur biodégradabilité anaérobie. Cette méthodologie a été appliquée et validée via des expérimentations et grâce à la modélisation en utilisant puis modifiant le modèle ADM1 (Anaerobic Digestion Model n°1[2]). Enfin, une analyse de scenarios utilisant le modèle a aussi montré que grâce à la prédiction de la bioaccessibilité et de la biodégradabilité, il était possible d'optimiser le design du procédé. Cela a ouvert des perspectives significatives quant à l'utilisation et l'adaptation de cette méthodologie à d'autres résidus organiques et procédés et à l'élaboration de capteurs et d'indicateurs. Les résultats de ces travaux ont été notamment valorisés par des publications scientifiques, [JJ26], [JJ27], [JJ28], [JJ29] et par des participations à des conférences [PO34], [PO35].

Post-Doctorat

Caractérisation de résidus organiques et Modélisation des processus biologiques d'une filière de traitement de ces résidus (2013-2015)

INRAE-LBE
Collaborations : INRAE ECOSYS, INERIS, INRAE OPAALE (ex-IRSTEA), LDAR, Arvalis, INRAE SAS, Esitpa Rouen, CIRAD Recyclages et Risques, Rittmo Agroenvironnement, Chambre d'Agriculture de Bretagne
Mots clés : *caractérisation, matière organique, méthanisation, compostage, modélisation, fractionnement, ADM1, micropolluants organiques, sol, digestats, fertilisants organiques*

Désireuse de revenir à la recherche académique, j'ai quitté mon poste d'ingénieure de recherche chez VERI pour réaliser un post-doctorat à INRAE LBE. Mon post-doctorat s'est déroulé dans le cadre du projet RISQ-PRO dont l'objectif était de modéliser les procédés d'une filière de traitement des boues (méthanisation et compostage) afin de prédire le devenir à la fois de la matière organique des résidus organiques et de certains micropolluants organiques adsorbées sur la matière. L'idée était de proposer des préconisations et des scenarios sur leur devenir après la valorisation agronomique potentielle des digestats et/ou composts de digestats issus de la filière. La question de recherche sous-jacente a été notamment de coupler les modèles des procédés jusqu'au sol. Pour ce faire, la méthodologie développée pendant mon doctorat a été utilisée et modifiée afin de définir un indicateur de bioaccessibilité de la matière organique pouvant être utilisée à la fois pour étudier la biodégradabilité de cette dernière dans les procédés mais également dans les sols agricoles. Tous ces aspects sont décrits de manière détaillée dans le Chapitre 2. Des publications [JJ24], [JJ25] et des participations à des conférences ont été réalisées [PO29], [PO30], [PO31], [PO32], [PO33], [Poster_5], [Poster_6], [Poster_7].

Projets de Recherche

Les contrats auxquels j'ai participé en tant que porteuse de projet ou contributrice sont décrits ci-dessous (les partenaires porteurs de projet sont soulignés).

AMEDEUS: *Accelerate Membrane Development for Urban Sewage Purification* (2005-2009). Contributrice
Financement : Europe (5 205 k€) ; **Partenaires :** KWB (Berlin, Allemagne), VITO (Belgique), TTX (Italy), Berlin University of Technology (Allemagne), UNSW (Australie), VERI (France), Aquafin (Belgique), A3 (Allemagne), Polymem (France), Inge (Allemagne), Envi-Pur (République Tchèque).

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[2] Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T. M., Siegrist, H., Vavilin, V.A. (2002) Anaerobic Digestion Model No.1. (ADM1). IWA Scientific and Technical Report No. 13. IWA, ISBN:1-900222-78-7.

RISQ-PRO : *Evaluation et réduction des risques de contamination par des polluants organiques dans le contexte de l'usage de Produits Résiduaires Organiques (PRO) sur sols agricoles (2013-2015).* **Contributrice**
Financement : ONEMA (432 k€) ; **Partenaires** : INRAE LBE, INRAE ECOSYS, INERIS

ETYC : *Evaluation intégrée des phases de TraItement et de recYclage agricole des matières organiques pour des systèmes d'élevage moteurs dans l'atténuation du changement Climatique (2012-2015).* **Contributrice**
Financement : ADEME (486 k€) ; **Partenaires** : UMR INRAE/AgroCampus-Ouest, INRAE OPAALE (ex-IRSTEA), INRAE ECOSYS, Chambre d'agriculture de Bretagne, INRAE LBE

PROBIOTIC : Dynamique des pathogènes lors du stockage et de l'épandage des Produits Résiduaires Organiques : influence des déterminants biotiques et de la biodisponibilité de la matière organique (2013-2016). **Contributrice**
Financement : ADEME (249 k€) ; **Partenaires** : INRAE- LBE, INRAE OPAALE (ex-IRSTEA)

PROLAB (financé par l'ADEME-DOSTE) **:** *Protocole de caractérisation des PROs au laboratoire pour prédire leur devenir au champ (2013-2016).* **Contributrice**
Financement : ADEME (637 k€) ; **Partenaires :** INRAE ECOSYS, LDAR, Arvalis, INRAE LBE, INRAE SAS, Esitpa Rouen, CIRAD Recyclages et Risques, Rittmo Agroenvironnement

IBRA : Integral Bioreffinery of the Rapeseed Meal-Bioraffinerie intégrale et durable du tourteau de colza (2017-2018). **Contributrice et responsable d'une tâche**
Financement : 3BCAR Inter-Carnot (275 k€) ; **Partenaires** : LISA, LRGP, INRAE LBE (3BCar)

BiogasMena: Demonstration of dry fermentation and optimization of biogas technology for rural communities in the MENA region (2017-2020)**. Contributrice et responsable du work package sur la valorisation des digestats**
Financement : ERANETMED (1 349k€) ; **Partenaires** : University of Hohenheim (Allemagne), University of Verona (Italie), Agricultural University of Athens (AUA, Grèce), Université des Sciences et Technologies d'Oran (Algérie), INRAE LBE (France), IMDEA (Espagne), Centre de Biotechnologie de Sfax (Tunisie), Nenufar (France), ERM (France), FnBB e. V. (Allemagne), University of Cairo (Egypt)

MAPPED : Outils de pilotage d'unité de méthanisation à l'échelle du territoire (2017-2020). **Contributrice**
Financement : ADEME (3 780 k€) ; **Partenaires** : BioEnTech, INRAE LBE, INRAE OPAALE, INRAE ECOSYS, Akajoule, ENGIE

PROLEG : Intensification écologique des systèmes de culture via le recyclage des produits résiduaires et les légumineuses pour améliorer les services écosystémiques rendus pour et par les systèmes de culture (2016-2020). **Contributrice**
Financement : PSDR (300 k€) ; **Partenaires :** INRAE ECOSYS, INRAE Agronomie, INRAE LBE, Association Patrimoniale de la Plaine de Versailles et du Plateau des Alluets, Terre et Cités, Chambre d'agriculture Interdépartementale d'Ile de France, Terres Inovia, Syndicat mixte d'assainissement de la région ouest de Versailles, Versailles Grand Parc, Bio Yvelines Services

I-MO-POL-DYN : *Interactions micropolluants/matrices organiques au sein des matières fertilisantes d'origine résiduaire : influence sur la dynamique des micropolluants après retour au sol de ces matières* (2016-2019). **Contributrice**
Financement : ADEME (237 k€), **Partenaires** *:* Univ. Limoges, INRAE LBE, IC2M CNRS

MADSLUDGE : *Optimisation de la filière de traitement des boues pour limiter la dissémination de l'antibiorésistance* (2018-2020). **Contributrice**
Financement : ADEME/ANSES (163 k€) ; **Partenaires** *:* INRAE LBE, INTHERES, Laboratoire écologie fonctionnelle et environnement (ENSAT)

DIGESTATE : *Diagnostic des traitements des déchets et comportement des contaminants dans l'environnement* (2015-2020). **Contributrice**
Financement : ANR (691 k€)
Partenaires : CIRAD Unité Recyclage et risque, INRAE ECOSYS, INRAE LBE, CNRS-CEREGE, INRAE EMMAH, LEM

Nut-PRO-Plant: Nutrients accessibility of organic residues from wastes (2017). **Porteuse de projet**
Financement: Agreenskills; Agropolis Fondation, Labex Agro (71 k€); **Partenaires:** INRAE LBE, Department of Agricultural Sciences (DipSA) – Alma Mater Studiorum University of Bologna (Italy)

Concept-Dig : Outil d'aide à la conception de filière pour la valorisation agronomique des digestats, (2016-2019). **Porteuse de projet**
Financement : ADEME (432 k€) **; Partenaires** : INRAE LBE, IRSTEA OPAALE, INRAE ECOSYS, CIRAD BioWooEB, Association des Agriculteurs Méthaniseurs de France (AAMF)

IVA-WASTE: *Integrated VAlorization of liquid and solid WASTE (2021).* **Contributrice**
Financement : Agropolis Fondation (25 k€) ; **Partenaires** : INRAE MISTEA, INRAE-LBE, CERTE (Tunisie), CIRAD Unité Recyclage et risques

BIOGAZ RIO : Recherche et Innovation en Occitanie (2020-2022). **Contributrice et responsable du work package sur l'approche filière et valorisation des co-produits**
Financement : Subventions de la Région Occitanie et de Europe au titre du Programme Opérationnel FEDER-FSE Languedoc Roussillon (2 700k€) ; **Partenaires** : INRAE LBE, BioEnTech, Naskeo, SUEZ, Distillerie Sud Languedoc

COVAFU : *Développement d'une filière de collecte et de valorisation du fumier de cheval à des fins agronomiques* (2021). **Contributrice**
Financement : Région Occitanie ; **Partenaires :** Comité Départemental d'Equitation (CDE), INRAE LBE, plateforme de compostage Aspiran

Compostage_Lallemand : *Optimisation du compostage de fumier équin* (2021-2022). **Porteuse de projet**
Financement : LALLEMAND (23.6 k€) ; **Partenaires :** INRAE LBE, LALLEMAND, Comité Départemental d'Equitation 34

Ferti-Dig : Outil d'aide à la conception de filière pour la valorisation agronomique des digestats, (2021-2024). **Porteuse de projet**
Financement : ADEME (644 k€)
Financement : GRDF (60 k€)
Partenaires : INRAE LBE, Chambre d'Agriculture de Bretagne (CRAB), Chambre d'Agriculture du Grand-Est (CRAGE), INRAE ECOSYS, INRAE SAS, INRAE EMMAH, SOLAGRO, ACTA, AAMF, LDAR, EPLEFPA - Bas Rhin (Obernai), Agro de la Meuse (Bar-le-Duc)

Encadrements de la recherche

L'ensemble des personnes encadrées ou co-encadrées sont listées dans le tableau suivant (trois thèses dont une soutenue, douze stages Master, un stage doctoral, un ingénieur, un post-doctorat et un assistant ingénieur).

Doctorats	
2016-2019	**Felipe Guilayn, SUPAGRO Montpellier (Génie des Procédés), Ecole doctorale GAIA (Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau), Université de Montpellier** *Digestats de méthanisation : Caractérisation et nouvelles voies de valorisation* Financement : Thèse CIFRE INRAE-SUEZ Directeur de thèse : Dominique Patureau (INRAE, LBE Narbonne) Encadrement : Julie Jimenez (INRAE, LBE Narbonne), Marion Crest (CIRSEE, SUEZ), Maxime Rouez (CIRSEE, SUEZ) Collaboration : INRAE LBE, SUEZ, Université de Limoges (Geneviève Feuillade) *Articles acceptés : [JJ09][JJ11][JJ12][JJ13] , conférences : [PO12][PO13][PO15] [PO17][PO20] [PO21][PO22][PO23] [Poster_1]*
2020-2023	**David Fernandez-Dominguez, SUPAGRO Montpellier (Génie des Procédés), Ecole doctorale GAIA, Université de Montpellier** *Characterization, modelling and control of organic matter fate during anaerobic digestion: towards digestates use improvement for agriculture* SUPAGRO Montpellier (Génie des Procédés) Ecole doctorale GAIA, Université de Montpellier Financement : Région et département Agroécosystème (INRAE) Directeur de thès+e : Dominique Patureau (INRAE, LBE) Encadrement : Julie Jimenez (INRAE, LBE) Collaboration: séjour au Department of Thematic Studies-Environmental Change and Biogas Research Center, Linköping University, Sweden (Sepher Yecta) *Articles acceptés : [JJ01][JJ04] , Articles soumis : [36], conférences : [PO5][PO6][PO8]*
2020-2023	**Margaud Pereme, SUPAGRO Montpellier (Génie des Procédés), Ecole doctorale GAIA, Université de Montpellier** *Optimisation multi-objectif pour l'éco-conception d'une filière de valorisation des résidus organiques via la méthanisation* Financement : école doctorale GAIA, Université de Montpellier Directeurs de thèse : Jean-Phillippe Steyer et Julie Jimenez (INRAE, LBE) *Articles soumis : [35] , conférences : [PO4], [PO9]*
Masters	
avril-septembre 2008	**Rémi Lestienne, ENSIACET, Génie Chimique** *Modélisation de la digestion anaérobie* **VERI**
avril – septembre 2010	**Cécile Denis, Master 2 Université Paris 11, Instrumentation et Méthodes d'Analyse Moléculaire** *Caractérisation de la matière organique au cours du traitement des eaux usées : comparaison analyses globales/analyses moléculaires* **VERI** *Co-auteur d'un article publié [JJ29]*

aout 2014-février 2015	**Han Lei, Master 2 ParisTech Science et Technologie de l'Environnement, Gestion et Traitement des Eaux, des Sols et des Déchets** *Lien entre caractérisation des produits résiduaires organiques et stabilité de la matière organique lors de tests d'incubation sur sol agricole (projets RISQ-PRO et ETYC)* **INRAE-LBE** *Co-auteur d'un article publié [JJ18]*
avril-septembre 2015	**Camilla Espinoza Carvajal, Master 2, Pontificia Universidad Catolica de Valparaiso, Escuela de Ingenieria Bioquimica, Chile** *Assessment of the bioaccessibility and biodegradability of organic matter to describe anaerobic digestion process through mathematical modeling* **INRAE-LBE** *Co-auteur d'une conférence internationale [PO31]*
février-juillet 2016	**Ziren Yu, Université Technologique de Compiègne** *Evaluation de l'impact de la préparation d'échantillons sur les analyses de caractérisation des Produits Résiduaires Organiques (projet PROLAB)* **INRAE-LBE**
mars-aout 2017	**Paolo De Toledo Carrenho, Master 2, Ecole Nationale Supérieure de Chimie de Montpellier/ SUPAGRO Montpellier** *Récupération de substances humiques à partir des digestats de méthanisation (Thèse Felipe Guilayn)* **INRAE LBE/SUEZ**
Mars-sept 2017	**Bastien Zennaro, SUPAGRO Montpellier** *Study of the limits of near infrared spectroscopy for the prediction of the biomethane potential of wet organic substrates* **INRAE LBE**
mars-septembre 2018	**Ségolène Fremont, Ecole de Biologie Industrielle Cergy** *Valoriser et concentrer la valeur fertilisante du digestat de méthanisation par séchage thermique en minimisant les pertes de nutriments notamment les pertes d'azote* **INRAE LBE/SUEZ**
mars –aout 2019	**Claire Calmette, Master 2 DTEC-Bio, Université de Montpellier 2** *Caractérisation des digestats de méthanisation agricole : vers une meilleure gestion de leur valorisation agronomique (projet Concept-Dig)* **INRAE-LBE**
2019	**Matthijs Somers, Department of Chemical Engineering KU Leuven (Belgique), stage doctoral** *Implementation of digestate treatment and recirculation: influence on the anaerobic digestion* Financement: Research Foundation - Flanders (FWO) Directeur de thèse : Lise Appels Encadrement durant un séjour au LBE en 2019 *Article en commun [JJ06]*
avril-septembre 2020	**Omar Cherad,** *Modélisation et contrôle de la digestion anaérobie des boues : application sur un cas industriel* **INRAE LBE/SIAAP**
avril-septembre 2021	**Tom Iche-Puig, Ecole d'ingénieur PURPAN Toulouse** *Etude de la transformation du fumier équin héraultais par le compostage en vue d'une valorisation en viticulture (projet COVAFU)* **INRAE-LBE/CDE 34**

mars-septembre 2021	**Jeanne Bouvard, Master 2 Gestion des Territoires et développement local, Institut Agronomique Méditerranéen de Montpellier, Université Paul Valéry de Montpellier III** *Diagnostic agricole et agronomique du potentiel de la fertilisation circulaire en oléiculture tunisienne (projet IVA-WASTE)* **INRAE LBE, CIRAD Recyclages et risques, CERTE (Tunisie)**
septembre 2021- février 2022	**Nicolas Bourdin, Mastère spécialisé Génie des procédés biotechnologiques CPE Lyon et ENSIC Nancy** *Valorisation des digestats de méthanisation : montée en échelle de concept innovant (projet Biogaz-RIO)* **INRAE LBE, SUEZ**
Post-doctorats, Ingénieurs, Assistant-ingénieurs	
2017-2019	**Bastien Zennaro, Ingénieur SUPAGRO Montpellier** *Caractérisation des résidus organiques et des digestats pour la modélisation de leur méthanisation afin de prédire production de méthane et la qualité agronomique des digestats (projet MAPPED)* **INRAE LBE, INRAE ECOSYS, INRAE OPAALE, BIOENTECH, ENGIE, Akajoule**
2016-2018	**Quentin Aemig, post-doctorat** *Interactions micropolluants/matrices organiques au sein des matières fertilisantes d'origine résiduaire : influence sur la dynamique des micropolluants après retour au sol de ces matières (projet I-MO-POL-DYN)* **INRAE LBE, ENSIL**
2019-2022	**Lucie Sauvadet, assistant ingénieur** *Valorisation des effluents de distillerie pour une meilleure gestion agronomique des co-produits (projet Biogaz-RIO)* **INRAE LBE, Distillerie Sud Languedoc**
Mars-décembre 2022	**Nicolas Bourdin, Ingénieur CPE Lyon** *Valorisation des digestats de méthanisation : montée en échelle de concept innovant (projet Biogaz-RIO)* **INRAE LBE, SUEZ**
Autres encadrements de stage	
2008-2009	Estelle Gonidec, Licence Pro (alternance) Procédés de Traitements et de Valorisation des Rejets, IUT Pontivy, VERI
2014	Nicolas Doussiet, Master 1 DTEC-Bio, Université de Montpellier 2, projet RISQ-PRO, INRAE LBE
2017	Albane Loménède, Master 1, UTC Compiègne, projet IBRA, INRAE LBE
2017	Axelle Herbette, Master 1, UTC Compiègne, projet Concept-Dig, INRAE LBE Anne Wallrich, Master 1, Ecole UniLaSalle, Rouen, projet Concept-Dig, INRAE LBE, INRAE ECOSYS, TRAME
2018	David Fernandez-Dominguez, Master 1 Environmental Enginnering University of Barcelona Spain, projet Concept-Dig, INRAE LBE
2019	Noémie Lakehal, Master 1, UTC Compiègne, projet Concept-Dig, INRAE LBE
2020	Solène Vaudron, Master 1, UTC Compiègne, projet BIOGAS-MENA, INRAE LBE
2021	Chloé Hanania, Master 1, UTC Compiègne, projet FertiDig, INRAE LBE Clémentine Londero, Master 1, UTC Compiègne, INRAE LBE, LALLEMAND
2022	Louise Thomas (IUT Génie Chimique Génie des Procédés Narbonne), projet FertiDig, INRAE LBE Thomas Lacombe (IUT Génie Chimique Génie des Procédés Narbonne), projet Biogaz-RIO, INRAE LBE

3. Principales collaborations

Tout au long de ma carrière et grâce à ma participation dans 18 projets principaux dont 3 que j'ai coordonnés complètement, j'ai collaboré avec divers acteurs au sein de mon unité, au sein de l'INRAE et à l'extérieur de l'INRAE, que ce soient partenaires publics ou privés et acteurs de la filière de valorisation des résidus organiques de manière générale. Ainsi, le tableau suivant présente l'ensemble des collaborations que j'ai eues depuis mon doctorat.

Collaborations INRAE	Collaborations avec les instituts publics/associations et collectivités	Collaborations avec des partenaires privés
ECOSYS Grignon OPAALE Rennes SAS Rennes EMMAH Avignon FARE Reims MISTEA Montpellier ITAP Montpellier	**Nationales :** AAMF (Association des Agriculteurs Méthaniseurs de France) ENSIL Limoges CIRAD BIOWOOEB CIRAD Recyclages et risques ECOLAB INSA Toulouse Université de Lorraine EPLEFPA (Obernai, Bar le duc) SIAAP **Internationales :** Université de Laval (Canada) AUA (Grèce) IMDEA (Espagne) University of Honenheim (Allemagne) University of Bologna (Italie) University of Verona (Italy) KU Leuven (Belgique) EGE University (Turquie) CBS (Centre de Biotechnologie de Sfax, Tunisie) Nireas-IWRC (University of Cyprus) CERTE (Tunisie) University of Cairo (Egypt)	VEOLIA (VERI) SUEZ GRDF ENGIE BIOENTECH Distillerie Sud Languedoc LALLEMAND RITTMO LDAR

4. Expertises et participation à des comités et jurys de thèse

Expertises

J'ai réalisé des expertises de projets et de rapports scientifiques dans les cas suivants :

- Expertises des rapports rédigés dans le cadre du Comité Stratégique de Filière (CSF), Axe Méthanisation Action « Externalité » (2020) en tant que membre du conseil scientifique du CSF (groupes de travail : Gaz à effet de serre, Déchets, Qualité de l'eau, Résilience des exploitations)
- Expertises de projets ADEME (appels à projets GRAINE et PIA)

Je suis également reviewer d'articles pour les revues suivantes : *Bioresource Technology, Waste Management, Water Research, Waste and Biomass Valorization, Environmental Science and Pollution Research, Soil and Tillage Research, Frontiers in Environmental Science.*

Participation aux comités de thèse

Dans le cadre d'autres activités de recherche, j'ai participé aux comités de thèse des doctorants suivants :

Daniela-Alejandra Mora-Salguero (2021-2024), *Impact des digestats de méthanisation sur la qualité microbiologique des sols agricoles*, INRAE Dijon

Joshua Cooke (2020-2023), *Etude et prédiction de l'impact des paramètres de digestion anaérobie sur la composition carbonée des digestats et leur effet sur la stabilité structurale des sols*, INRAE OPAALE et SAS

Victor Moinard (2017-2021), *Conséquences de l'introduction de la méthanisation dans une exploitation de polyculture-élevage sur les cycles du carbone et de l'azote. Combinaison de l'expérimentation et de la modélisation à l'échelle de la ferme*, INRAE ECOSYS, CIRAD-ISTOM

Robin Beghin (2017-2020), *Etude des conséquences de la digestion anaérobie du maïs sur ses caractéristiques amendantes: devenir des matières organiques, stockage du carbone, vie microbienne, capacité d'adsorption et stabilité structurale de l'horizon de surface de Luvisols cultivés,* Ecole Purpan Toulouse

Alexandre Mallet (2018-2021), *Caractérisation spectroscopique robuste des déchets organiques: comment s'affranchir des effets de l'eau*, INRAE, BIOENTECH

Younes Bareha (2015-2018), *Modélisation des processus de transformation de l'azote en digestion anaérobie: application à l'optimisation de la valorisation des digestats*, INRAE OPAALE

Afifi Bin Akhiar (2014-2017), *Caractérisation de la fraction liquid des digestats issus de la codigestion de résidus solides*, INRAE LBE

Cyrille Charnier (2013-2016), *Fast characterization of the organic matter, instrumentation and modelling for the anaerobic digestion process performances prediction*, INRAE, BIOENTECH

Quentin Aemig (2012-2015), *Dynamique des micropolluants organiques au cours du traitement biologique de résidus solides : lien entre caractérisation du résidu, localisation des micropolluants organiques au sein des compartiments du résidu (sorption, biodégradation)*, INRAE LBE

Sophie Descremps (2012-2015), *Caractérisation du résidu particulaire et étude des mécanismes limitant la biodégradation des boues d'épuration*, LISBP INSA Toulouse

Participation à des jurys de thèse

J'ai également participé à quatre jurys de thèses, deux nationaux en tant qu'examinateur et deux internationaux en tant que rapporteur.

- Examinatrice dans le jury de thèse de Maël Mercier-Huat (2021) « Développement et validation d'un bioprocédé à l'échelle pilote de valorisation de biomasses conchylicoles par digestion anaérobie bi-étagée ou fermentation acidogène », Université de technologie de Compiègne
- Examinatrice dans le jury de thèse de Fida Mrad (2018) « Décomposition de résidus de culture et de matériaux biosourcés : impact sur les communautés microbiennes des sols agricoles et les fonctions associées », Université de Rouen-UniLaSalle, spécialité sciences agronomiques, biotechnologies agro-alimentaires
- Examinateur/Rapporteur dans le jury de thèse de Juan Cubero Cardoso (2021) « Valorization of residual strawberry extrudate throught obtaining high added value compounds and methane production by anaerobic digestion », Universidad de Huelva (Espagne)

- Examinateur/Rapporteur dans le jury de thèse de Mercedes Llamas Redondo (2021) « Microalgae biomass valorization for volatile fatty acids, microbial oils and biogas production in a biorefinery context », Universidad Computense de Madrid (Espagne)

5. Activités pédagogiques et administratives

Contribution aux enseignements

Dans le cadre du Master SPA2E (Sciences et Procédés des Agroressources pour l'Alimentation et l'Environnement) de l'Université de Montpellier, participation dans l'UE « Gestion intégrée des sous-produits et co-produits de l'agro-industrie », je donne un cours magistral de 4h30 sur la « Valorisation des digestats de méthanisation » que j'ai mis en place en 2017 et reconduit chaque année depuis.

Je participe également à la formation « Modélisation et croissance microbienne » pour l'école doctorale GAIA de l'Université de Montpellier, cours/TD de 4h sur la modélisation des bioprocédés de traitement des résidus organiques, reconduite chaque année depuis 2015 avec mon collègue Eric Latrille (INRAE LBE).

Activités collectives au sein de l'unité

Depuis début 2019, je suis en charge de deux groupes de travail au sein de la démarche qualité du LBE. Le premier est la co-animation du groupe de travail « gestion de projets ». Dans ce cadre, des solutions et propositions d'amélioration et d'outils sont recherchées afin d'améliorer la traçabilité des projets ainsi que leur gestion. Via ce groupe de travail, j'ai suivi une formation pour devenir référente LBE de la méthode EUREQUA, méthode d'aide à la gestion et conduite de projet. Le second groupe de travail que je co-anime est celui de la « gestion des équipements » où l'objectif est d'améliorer la traçabilité des divers équipements utilisés au laboratoire ainsi que leur bonne utilisation.

Activités d'animations

Depuis 2020, je co-anime un groupe de travail sur la Matière Organique (GT MO) au sein du Bureau de Normalisation Fertilisation (BNFERTI) avec Fiona Ehrhardt (RITTMO). L'objectif de ce groupe de travail est de réunir divers acteurs (laboratoires, instituts publics, privés, industriels) autour de la thématique de caractérisation de la matière organique afin de mettre en place et/ou améliorer des méthodes de caractérisation selon les besoins identifiés (ex : indice de stabilité de la matière organique dans les sols, carbone organique labile, biodégradabilité, etc…) et de générer des modifications ou de nouvelles méthodes normalisées.

Participation à des réseaux thématiques

Depuis 2015, je participe à des réseaux thématiques comme listés ci-après :

- Réseaux RMT COMIFER Bouclage des cycles ;
- Groupe de travail Matière organique, Bureau de Normalisation Fertilisation (puis co-animatrice du groupe de travail GTMO depuis 2020);
- Membre de l'observatoire SOERE-PRO Système d'Observation et d'Expérimentation, sur le long terme, pour la recherche en environnement « Impacts environnementaux du recyclage de produits résiduaires organiques sur les écosystèmes cultivés », participation au directoire scientifique et à l'assemblée générale (depuis 2018) ;
- Membre du réseau sur les matières organiques RESMO : participation aux workshops (2015 et 2018) ;
- Membre du conseil scientifique du Comité Stratégique de Filière (CSF), Axe Méthanisation, Action « Externalité » (2020) ;
- Membre du Conseil Scientifique de RITTMO (depuis 2019).

6. Production scientifique

Les noms soulignés sont les étudiants encadrés lors de la publication.

Production de connaissances originales, reconnues dans la/les communauté(s) scientifique(s) de référence

[JJ01] David Fernández-Domínguez, Felipe Guilayn, Dominique Patureau, **Julie Jimenez** (2022). Characterising the stability of the organic matter during anaerobic digestion: a selective review on the major spectroscopic techniques. *Reviews in Environmental Science and Bio/technology,* 21, 691-726, IF 14,284, https://dx.doi.org/10.1007/s11157-022-09623-2,https://hal.inrae.fr/hal-03691866

[JJ02] Bastien Zennaro, Paul Marchand, Eric Latrille, Jeanne-Chantal Thoisy-Dur, Sabine Houot, Cyril Girardin, Jean-Philippe Steyer, Fabrice Béline, Cyrille Charnier, Charlotte Richard, Guillaume Accarion, **Julie Jimenez** (2022). Agronomic characterization of anaerobic digestates with near-infrared spectroscopy. *Journal of Environmental Management,* 317, 115393, IF 8,91, https://dx.doi.org/10.1016/j.jenvman.2022.115393,https://hal.inrae.fr/hal-03709209

[JJ03] Ulysse Bremond, Aude Bertrandias, Raphaëlle de Buyer, Eric Latrille, **Julie Jimenez**, Renaud Escudié, Jean-Philippe Steyer, Nicolas Bernet, Hélène Carrère (2021). Recirculation of solid digestate to enhance energy efficiency of biogas plants: Strategies, conditions and impacts. *Energy Conversion and Management,* 231, 113759, IF 11,533, https://dx.doi.org/10.1016/j.enconman.2020.113759, https://hal.inrae.fr/hal-03192581

[JJ04] David Fernández-Domínguez, Dominique Patureau, Sabine Houot, Nicolas Sertillanges, Bastien Zennaro, **Julie Jimenez** (2021). Prediction of organic matter accessibility and complexity in anaerobic digestates. *Waste Management,* 136, 132-142, 8,816, https://dx.doi.org/10.1016/j.wasman.2021.10.004,https://hal.inrae.fr/hal-03473235

[JJ05] Roman Moscoviz, **Julie Jimenez** (2021). Improving anaerobic digestion mass balance calculations through stoichiometry and usual substrate characterization. *Bioresource Technology,* 337, 125402, IF, 10,89, https://dx.doi.org/10.1016/j.biortech.2021.125402, https://hal.inrae.fr/hal-03319269

[JJ06] Matthijs H Somers, **Julie Jimenez**, Samet Azman, Jean-Philippe Steyer, Jan Baeyens, Lise Appels (2021). Ultrasonication affects the bio-accessibility of primary dairy cow manure digestate for secondary post-digestion. *Fuel,* 291, 9, IF 6,609, https://dx.doi.org/10.1016/j.fuel.2021.120140, https://hal.inrae.fr/hal-03130716

[JJ07] **Julie Jimenez**, Marco Grigatti, Elisa Boanini, Dominique Patureau, Nicolas Bernet (2020). *The impact of biogas digestate typology on nutrient recovery for plant growth: Accessibility indicators for first fertilization prediction. Waste Management,* 117 18-31, IF 8,816, https://dx.doi.org/10.1016/j.wasman.2020.07.052, https://hal.inrae.fr/hal-02921497

[JJ08] Ulysse Bremond, Aude Bertrandias, Denis Loisel, **Julie Jimenez**, Jean-Philippe Steyer, Nicolas Bernet, Hélène Carrère (2020). Assessment of fungal and thermo-alkaline post-treatments of solid digestate in a recirculation scheme to increase flexibility in feedstocks supply management of biogas plants. *Renewable Energy,* 149 641-651, IF 8,634, https://dx.doi.org/10.1016/j.renene.2019.12.062, https://hal.inrae.fr/hal-02555213

[JJ09] Felipe Guilayn, M. Rouez, M. Crest, Dominique Patureau, **Julie Jimenez** (2020*). Valorization of digestates from urban or centralized biogas plants: a critical review. Reviews in Environmental Science* *and Bio/technology,* IF 14,284, https://dx.doi.org/10.1007/s11157-020-09531-3, https://hal.inrae.fr/hal-02571756

[JJ10] **Julie Jimenez**, Cyrille Charnier, Mokhles Kouas, Eric Latrille, Michel Torrijos, Jérôme Harmand, Dominique Patureau, Mathieu Sperandio, Eberhard Morgenroth, Fabrice Béline, George Ekama, Peter Vanrolleghem, Ángel Robles, Aurora Seco, Damien Batstone, Jean-Philippe Steyer (2020-01). Modelling hydrolysis: Simultaneous versus sequential biodegradation of the hydrolysable fractions. *Waste Management,* 101 150-160, IF 8,816 https://dx.doi.org/10.1016/j.wasman.2019.10.004, https://hal.insa-toulouse.fr/hal-02904361

[JJ11] Felipe Guilayn, Mohamed Benbrahim, Maxime Rouez, Marion Crest, Dominique Patureau, **Julie Jimenez** (2020). Humic-like substances extracted from different digestates: First trials of lettuce biostimulation in hydroponic culture. *Waste Management,* 104 239-245, IF 8,816, https://dx.doi.org/10.1016/j.wasman.2020.01.025, https://hal.inrae.fr/hal-02627274

[JJ12] Felipe Guilayn, **Julie Jimenez**, Jean-Luc Martel, Maxime Rouez, Marion Crest, Dominique Patureau (2019). First fertilizing-value typology of digestates: A decision-making tool for regulation. *Waste Management,* 86 67-79, IF 8,816 , https://dx.doi.org/10.1016/j.wasman.2019.01.032, https://hal.inrae.fr/hal-02627223

[JJ13] Felipe Guilayn, **Julie Jimenez**, Maxime Rouez, Marion Crest, Dominique Patureau (2019b). *Digestate mechanical separation: Efficiency profiles based on anaerobic digestion feedstock and equipment choice. Bioresource Technology,* 274 180-189, https://dx.doi.org/10.1016/j.biortech.2018.11.090, https://hal.inrae.fr/hal-02627289

[JJ14] Quentin Aemig, Nicolas Doussiet, Alice Danel, Nadine Delgenes, **Julie Jimenez**, Sabine Houot, Dominique Patureau (2019). Organic micropollutants' distribution within sludge organic matter fractions explains their dynamic during sewage sludge anaerobic digestion followed by composting. *Environmental Science and Pollution Research,* 26 5820-5830, IF 3,056, https://dx.doi.org/10.1007/s11356-018-4014-7, https://hal.inrae.fr/hal-02629338

[JJ15] Younes Bareha, Romain Girault, **Julie Jimenez**, Anne Trémier (2018). Characterization and prediction of organic nitrogen biodegradability during anaerobic digestion: A bioaccessibility approach. Bioresource Technology, 263, 425-436, IF 1089, https://dx.doi.org/10.1016/j.biortech.2018.04.085, https://hal.inrae/hal-02940628

[JJ16] Charlotte Rennuit, Jin Mi Triolo, Søren Eriksen, **Julie Jimenez**, Hélène Carrère, Sasha D. Hafner (2018). Comparison of pre- and inter-stage aerobic treatment of wastewater sludge: effects on biogas production and COD remova*l. Bioresource Technology,* 247 332-339, IF 10,89, https://dx.doi.org/10.1016/j.biortech.2017.08.128, https://hal.inrae.fr/hal-02620984

[JJ17] Anais Goulas, Marjolaine Deschamps, Sylvie Nelieu, **Julie Jimenez**, Dominique Patureau, Claire-Sophie Haudin, Pierre Benoit (2017). Development of a soft extraction method for sulfamethoxazole and transformation products from agricultural soils: Effects of organic matter co-extraction on the environmental availability assessment. *Science of the Total Environment,* 607-608 1037-1048, IF 10,753, https://dx.doi.org/10.1016/j.scitotenv.2017.06.192, https://hal.inrae.fr/hal-01570306

[JJ18] **Julie Jimenez**, Lei Han, Jean-Philippe Steyer, Sabine Houot, Dominique Patureau (2017). Methane production and fertilizing value of organic waste: organic matter characterization for a better prediction of valorization pathways. *Bioresource Technology,* 241 1012-1021, IF 10,89, https://dx.doi.org/10.1016/j.biortech.2017.05.176, https://hal.inrae.fr/hal-01543454

[JJ19] Cyrille Charnier, Eric Latrille, **Julie Jimenez**, Margaux Lemoine, Jean Claude Boulet, Jérémie Miroux, Jean-Philippe Steyer (2017). Fast characterization of solid organic waste content with near infrared spectroscopy in anaerobic digestion. *Waste Management,* 59 140-148, IF 8,816, https://dx.doi.org/10.1016/j.wasman.2016.10.029, https://hal.inrae.fr/hal-01605641

[JJ20] Géraldine Maynaud, Dominique Steyer, Céline Druilhe, Christine Ziebal, **Julie Jimenez**, Michel Torrijos, Anne Marie Pourcher, Nathalie Wéry (2017). Caractéristiques physico-chimiques et microbiologiques de digestats bruts et post-traités destinés à l'épandage agricole. *Techniques Sciences Méthodes,* (5), 33-50, https://dx.doi.org/10.1051/tsm/201705033, https://hal.inrae.fr/hal-01605927

[JJ21] Géraldine Maynaud, Céline Druilhe, Mylène Daumoin, **Julie Jimenez**, Dominique Patureau, Michel Torrijos, Anne-Marie Pourcher, Nathalie Wéry (2017). Characterisation of the biodegradability of post-treated digestates via the chemical accessibility and complexity of organic matter*. Bioresource Technology,* 213 65–74, IF 10,89, https://dx.doi.org/10.1016/j.biortech.2017.01.057, https://hal.inrae.fr/hal-01606007

[JJ22] Cyrille Charnier, Eric Latrille, **Julie Jimenez**, Michel Torrijos, Philippe Sousbie, Jérémie Miroux, Jean-Philippe Steyer (2017). *Fast ADM1 implementation for the optimization of feeding strategy using near infrared spectroscopy*. *Water Research,* 122 27-35, IF 13,400, https://dx.doi.org/10.1016/j.watres.2017.05.051, https://hal.inrae.fr/hal-01603275

[JJ23] Quentin Aemig, Claire Cheron, Nadine Cathala-Delgenès, **Julie Jimenez**, Sabine Houot, Jean-Philippe Steyer, Dominique Patureau (2016). Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in sludge organic matter pools as a driving force of their fate during anaerobic digestion. *Waste Management,* 48 389-396, IF 8,816, https://dx.doi.org/10.1016/j.wasman.2015.11.045, https://hal.inrae.fr/hal-01533862

[JJ24] **Julie Jimenez**, Eric Latrille, Jérome Harmand, Daniel Gaida, Christian Wolf, Francis Mairet, Olivier Bernard, Victor Alcaraz-Gonzalez, Hugo Mendez-Acosta, Daniel Zitomer, Dennis Totzke, Henri Spanjers, Fabian Jacobi, Alan Guwy, Richard Dinsdale, Sofiane Mazhegrane, Gonzalo Ruiz-Filippi, Aurora Seco, Thierry Ribeiro, André Pauss, Jean-Philippe Steyer (2015). Instrumentation and control of anaerobic digestion processes: a review and some research challenges*. Reviews in Environmental Science and Bio/technology,* 14 615-648, IF 14,284, https://dx.doi.org/10.1007/s11157-015-9382-6, https://hal.inria.fr/hal-01256735

[JJ25] **Julie Jimenez**, Quentin Aemig, Nicolas Doussiet, Jean-Philippe Steyer, Sabine Houot, Dominique Patureau (2015). A new organic matter fractionation methodology for organic wastes: Bioaccessibility and complexity characterization for treatment optimization. *Bioresource Technology,* 194 344-353, IF 10,89, https://dx.doi.org/10.1016/j.biortech.2015.07.037, https://hal.inrae.fr/hal-01535235

[JJ26] Mathieu Muller, **Julie Jimenez**, Maxime Antonini, Yves Dudal, Eric E. Latrille, Fabien Vedrenne, Jean-Philippe Steyer, Dominique Patureau (2014). Combining chemical sequential extractions with 3D fluorescence spectroscopy to characterize sludge organic matter. *Waste Management,* 34 (12), 2572-2580, IF 8,816, https://dx.doi.org/10.1016/j.wasman.2014.07.028, https://hal.inrae.fr/hal-02636065

[JJ27] **Julie Jimenez**, Estelle Gonidec, Jesus Cacho Rivero, Eric Latrille, Fabien Vedrenne, Jean-Philippe Steyer, (2014). Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: towards ADM1 variables. *Water Research* 50, 359-372, IF 13,400, https://doi.org/10.1016/j.watres.2013.10.048

[JJ28] Alexis Mottet, Ivan Ramirez, Hélène Carrère, Stéphane Déléris, Fabien Vedrenne, **Julie Jimenez**, Jean-Philippe Steyer (2013*).* New fractionation for a better bioaccessibility description of particulate organic matter in a modified ADM1 model. Chemical Engineering Journal, 2013, vol. 228, p. 871-881, IF 13,273, https://doi.org/10.1016/j.cej.2013.05.082

[JJ29] **Julie Jimenez**, Fabien Vedrenne, Cecile Denis, Alexis Mottet, Stéphane Déléris, Jean-Philippe Steyer, Jesus Andres Cacho Rivero (2013). A statistical comparison of protein and carbohydrate characterisation methodology applied on sewage sludge samples. *Water Research,* 47 (5), 1751 - 1762, IF 13,400, https://dx.doi.org/10.1016/j.watres.2012.11.052, https://hal.inrae.fr/hal-02647946

[JJ30] Alessio Fenu, Giuseppe Guglielmi, **Julie Jimenez**, Mathieu Sperandio, Devendra Saroj, Boris Lesjean, Christoph Brepols, Chris Thoeye, Ingmar Nopens (2010). Activated sludge model (ASM) based modelling of membrane bioreactor (MBR) processes: A critical review with special regard to MBR specificities. *Water Research,* 44 (15), 4272-4294, IF 13,400, https://dx.doi.org/10.1016/j.watres.2010.06.007, https://hal.inrae.fr/hal-02661330

[JJ31] **Julie Jimenez**, Patricia Grelier, Jens Meinhold, Annie Tazi-Pain (2010). Biological modelling of MBR and impact of primary sedimentation. *Desalination,* 250 (2), 562-567, IF 11,211, https://dx.doi.org/10.1016/j.desal.2009.09.024, https://hal.inrae.fr/hal-02660708

Publications en cours de soumission

[JJ32] **Julie Jimenez**, Dominique Patureau, Françoise Watteau, Thierry Morvan (en cours de soumission). Deciphering the organic matter mineralsation kinetics of cattle farmyard manure thanks to organic matter fractionation and litterbags soil assay. *Biol. Fertil. Soils.*

[JJ33] **Julie Jimenez**, Virginie Rossard, Romain Girault, Anne Wallrich, Florian Christ, Denis Ollivier, Dominique Patureau, Eric Latrille, Sabine Houot (en cours de soumission). Concept-Dig: design support tool for the agronomic valorization of digestates from agriculture residues. *Waste Management.*

[JJ34] **Julie Jimenez**, Quentin Aemig, Jean-Philippe Steyer, Dominique Patureau (en cours de soumission). Modelling anaerobic digestion and composting of organic residus: towards organic matter fate prediction in soils. *Bioresource Technology.*

[JJ35] Margaud Pereme, Antoine Haddon, Jean-Philippe Steyer, **Julie Jimenez** (soumis). SoilFrac: A mechanistic model accounting for the fate of exogenous organic matter in soil carbon and nitrogen cycles. *Waste Management.*

[JJ36] David Fernández-Domínguez, Dominique Patureau, **Julie Jimenez** (soumis). Impact of substrate biodegradability on the identification of endogenous compounds during anaerobic digestion. *Biomass and Bioenergy.*

Chapitres d'Ouvrages

[CO01] Julie Jimenez (2022). *Anaerobic digestate as fertilizer for land application* in: Anaerobic Digestate Management. IWA Publishing. https://doi.org/10.2166/9781789062755_0025

[CO02] Felipe Guilayn, **Julie Jimenez**, Florian Monlau, Céline Vaneeckhaute (2022). *Valorisation of Anaerobic Digestate: Towards Value-Added Product*s. In: Renewable Energy Technologies for Energy Efficient Sustainable Development. Springer International Publishing, https://dx.doi.org/10.1007/978-3-030-87633-3_9,https://hal.inrae.fr/hal-03546575

[CO03] Hélène Carrère, Georgia Antonopoulou, Céline Druilhe, Eric Latrille, Gérasimos Lyberatos, **Julie Jimenez**, Ioanna Ntaikou, Konstantina Papadopoulou, Eric Trably, Anne Trémier (2020*). Methods to*

Assess Biological Transformation of Biomass. In: Handbook on Characterization of Biomass, Biowaste and Related By-products. Springer International Publishing, 641-730, https://dx.doi.org/10.1007/978-3-030-35020-8_5, https://hal.inrae.fr/hal-02918268

Communications à des congrès et colloques : conférence invitée

[PO1] **Julie Jimenez**, Pascal Peu (2018). *Etat des lieux des connaissances sur les facteurs de contrôle du potentiel méthanogène d'un substrat et de la composition de son biogaz*, Presented at : Journées Recherche Innovation Biogaz Méthanisation - JRI 2018, fr, (2018-10-02), https://hal.inrae.fr/hal-02940648

[PO2] Mathieu Sperandio, **Julie Jimenez**, Diana Garcia-Bernet (2016). *Programmes de recherche sur la valorisation des nutriments.* np, Presented at : Journées Techniques Eau et Déchets JTED 2016, fr, (2016-11-16), https://hal.inrae.fr/hal-01608024

[PO3] Jean-Philippe Steyer, **Julie Jimenez**, Eric Latrille, Jérôme Harmand, Angel Robles Martinez (2015) Keynote: *Instrumentation and control of anaerobic digestion processes: A review and some research challenges*. 14. World Congress on Anaerobic Digestion (AD14), International Water Association (IWA). INT., (2015-11-17), Viña del Mar, Chile, https://hal.inrae.fr/hal-02742240

Communication à des congrès et colloques : abstract et full paper

[PO4] Margaud Pérémé, Antoine Haddon, Jean-Philippe Steyer, **Julie Jimenez** (2022). *Anaerobic Digestion Modelling: From Energetic Value Of Wastes To Agronomic Fate Of Digestates*. Presented at: WasteEng22 Copenhagen, Denmark (2022-06- 27 – 2022-06-29).

[PO5] David Fernández-Domínguez, Dominique Patureau, **Julie Jimenez** (2022). *Insights into the organic matter quality during anaerobic digestion by coupling biochemical fractionation with 3D fluorescence and FTIR-PAS*. Presented at: WasteEng22 Copenhagen, Denmark (2022-06- 27 – 2022-06-29).

[PO6] David Fernández-Domínguez, Dominique Patureau, Sepher Shakeri Yekta, **Julie Jimenez** (2022). *Deciphering the organic matter stabilization during anaerobic digestion according to substrate type*. Presented at: 17th World Congress on Anaerobic Digestion – AD17 2022, Michigan, USA (2022-06-19-2022-06-21)

[PO7] **Julie Jimenez**, Virginie Rossard, Romain Girault, Anne Wallrich, Florian Christ, Denis Ollivier, Dominique Patureau, Eric Latrille, Sabine Houot (2022). *Concept-Dig: design support tool for the agronomic valorization of digestates from agriculture residues*. Presented at : 17th World Congress on Anaerobic Digestion – AD17 2022, Michigan, USA (2022-06-19- 2022-06-21)

[PO8] David Fernández-Domínguez, Dominique Patureau, Sabine Houot, Nicolas Sertillanges, Bastien Zennaro, **Julie Jimenez** (2022). *Prédire l'accessibilité et la complexité de la matière organique des digestats pour l'évaluation de leur stabilité en carbone organique au sol*. Presented at: Journées Recherche Innovation Biogaz Méthanisation - JRI 2022, Lyon, France (2022-03-14-2022-03-16).

[PO9] Margaud Pérémé, Antoine Haddon, Jean-Philippe Steyer, **Julie Jimenez** (2022). *Modélisation de la filière de méthanisation : de la valorisation énergétique à la valorisation agronomique des résidus organiques. Journées Recherche Innovation Biogaz et méthanisation* (JRI2022). Presented at : Journées Recherche Innovation Biogaz Méthanisation - JRI 2022, Lyon, France (2022-03-14-2022-03-16).

[PO10] **Julie Jimenez**, Romain Girault, Anne Tremier, Anne Wallrich, Claire Calmette, Florian Christ, Denis Ollivier, Dominique Patureau, Eric Latrille, Jean-Michel Commandre, Sabine Houot (2020). *Concept-Dig, outil d'aide à la conception de filière pour la valorisation agronomique des digestats.* Presented at : Journées Recherche Innovation Biogaz Méthanisation - JRI 2020, Toulouse, France (2020-09-08 - 2020-09-10), https://hal.inrae.fr/hal-02940672

[PO11] **Julie Jimenez**, Dominique Patureau, Marco Grigatti, Nicolas Bernet (2019). *Impact de la typologie des digestats sur les nutriments disponibles pour la plante : utilisation d'indicateurs de caractérisation pour prédire la fertilisation minérale.* Presented at : 14èmes rencontres de la fertilisation raisonnée et de l'analyse, Dijon, France (2019-11-20 - 2019-11-21), https://hal.inrae.fr/hal-03749961

[PO12] Felipe Guilayn, Dominique Patureau, **Julie Jimenez**, Maxime Rouez (2019). *Substances humiques à partir de digestats : biostimulation racinaire et croissance de production légumière en culture hydroponique.* Presented at : Les Intrants durables au service des sols agricoles urbains et périurbains, Romainville, France (2019-05-14 - 2019-05-16), https://dx.doi.org/10.15454/244e-f426, https://hal.inrae.fr/hal-03359974

[PO13] Felipe Guilayn, **Julie Jimenez**, Dominique Patureau, Maxime Rouez, Marion Crest (2019). *Les digestats : un produit au regard de la nouvelle réglementation européenne sur les fertilisants ?* Presented at : Les Intrants durables au service des sols agricoles urbains et périurbains, Romainville, France (2019-05-14 - 2019-05-16), https://dx.doi.org/10.15454/bwwn-da81, https://hal.inrae.fr/hal-03359753

[PO14] **Julie Jimenez**, Dominique Patureau (2019). *Digestats de méthanisation ou comment la variabilité des sous-produits de la valorisation énergétique des résidus organiques peut combler les divers besoins des agrosystèmes.* Presented at : Les Intrants durables au service des sols agricoles urbains et périurbains, Romainville, France (2019-05-14 - 2019-05-16)

[PO15] Felipe Guilayn, Mohammed Benbrahim, Maxime Rouez, Marion Crest, Dominique Patureau, **Julie Jimenez** (2019-06-23). *Digestate soluble organic matter extracts versus commercial humic substances for biostimulation of hydroponic systems.* Presented at : 16th IWA World Conference on Anaerobic Digestion, Delft, Pays-Bas (2019-06-23 - 2019-06-27), https://dx.doi.org/10.15454/3n40-0g03, https://hal.inrae.fr/hal-03360094

[PO16] Bastien Zennaro, **Julie Jimenez**, Jean-Philippe Steyer, Sabine Houot, Cyril Girardin, Paul Marchand, Jeanne-Chantal Thoisy-Dur, Henry Fisgativa, Fabrice Béline, Jeremy Miroux, Cyrille Carnier, Julien Budin, Charlotte Richard, Elodie Le Cadre Loret, Geoffrey Karakachian, Julien Werly, Fabien Rupin, Fréderic Fallot, Guillaume Accarion, Emmanuelle Baudu, Eric Latrille (2019). *Caractérisation rapide des digestats de méthanisation par proche infrarouge pour une meilleure valorisation.* Presented at : Colloque APIVALE 2019, Produits Résiduaires Organiques : Ingrédients Clés de la Bioéconomie circulaire, (2019 - 2019), https://hal.inrae.fr/hal-02791340

[PO17] Felipe Guilayn, **Julie Jimenez**, Maxime Rouez, Marion Crest, Dominique Patureau (2018). *Séparation de phases et digestats : profils d'efficacité basés sur les intrants de digestion anaérobie et le choix du séparateur.* Presented at : Journées Recherche Innovation Biogaz Méthanisation - JRI 2018, Rennes, France (2018-10-02 - 2018-10-04), https://dx.doi.org/10.15454/0egj-ss72, https://hal.inrae.fr/hal-01976248

[PO18] **Julie Jimenez**, Nicolas Sertillanges, Sabine Houot, Dominique Patureau (2018). *Caractérisation de la matière organique : outil d'aide à la compréhension des mécanismes de la digestion anaérobie et* *de la qualité du digestat.* Presented at : Journées Recherche Innovation Biogaz Méthanisation - JRI 2018, Rennes, France (2018-10-02 - 2018-10-04), https://hal.inrae.fr/hal-02940648

[PO19] Anne Wallrich, Denis Ollivier, **Julie Jimenez**, Sabine Houot (2018). *Définition de classes de digestats agricoles en vue dla préconisation de leur insertion dans les pratiques de fertilisat*ion. Presented at : JRI biogaz méthanisation 2018, Rennes, France (2018-10-02 - 2018-10-04), https://hal.inrae.fr/hal-02737352

[PO20] Felipe Guilayn, **Julie Jimenez**, Maxime Rouez, Marion Crest, Dominique Patureau (2018). *Mass balance and performance of common digestate mechanical separation equipment and fertilizing-value typology of liquid and solid fractions.* Presented at : 7. International Conference on Engineering for Waste and Biomass Valorisation (WasteEng2018), Prague, Tchéquie (2018-07-02 - 2018-07-05), https://dx.doi.org/10.15454/ha2n-1v41, https://hal.inrae.fr/hal-02734540

[PO21] Felipe Guilayn, **Julie Jimenez**, Maxime Rouez, Marion Crest, Dominique Patureau (2017). *Digestate typology from common fertilizing-value parameters: feedstock influence and recovery potentials.* Presented at : 15. IWA World Conference on Anaerobic Digestion (AD-15), Beijing, Chine (2017-10-17 - 2017-10-20), https://dx.doi.org/10.15454/sd7q-3e22, https://hal.inrae.fr/hal-02737702

[PO22] Felipe Guilayn, **Julie Jimenez**, M. Rouez, Dominique Patureau (2017). *Typologie des digestats de méthanisation à partir de paramètres usuels de valeur amendement/fertilisante.* Presented at : Journées Recherche et Industrie biogaz méthanisation - JRI 2017, Beauvais, France (2017-04-11 - 2017-04-13), https://dx.doi.org/10.15454/s0dn-wk41, https://hal.inrae.fr/hal-01605862

[PO23] Felipe Guilayn, **Julie Jimenez**, M. Rouez, Dominique Patureau (2017). *Typologie des digestats de méthanisation à partir de paramètres usuels de valeur amendement/fertilisante.* Presented at : Journées Recherche et Industrie biogaz méthanisation - JRI 2017, Beauvais, France (2017-04-11 - 2017-04-13), https://dx.doi.org/10.15454/s0dn-wk41, https://hal.inrae.fr/hal-01605862

[PO24] Sabine Houot, **Julie Jimenez**, Dominique Patureau (2016). *Quelle place pour la méthanisation dans la gestion de la matière organique à l'échelle de l'agrosystème ?* Presented at : Journées Recherche et Industrie Biogaz méthanisation, Limoges, France (2016-02-10 - 2016-02-12), https://hal.inrae.fr/hal-01594815

[PO25] **Julie Jimenez**, Quentin Aemig, Jean-Philippe Steyer, Sabine Houot, Dominique Patureau (2016). *Modelling anaerobic digestion and compost of organic residues: towards organic matter fate prediction in soils during land spreading.* Presented at: WasteEng2016. 6. International Conference on Engineering for Waste and Biomass Valorisation, Albi, France (2016-05-23 - 2016-05-26), https://hal.inrae.fr/hal-02744020

[PO26] **Julie Jimenez**, Quentin Aemig, Jean-Philippe Steyer, Dominique Patureau, Sabine Houot (2016). *Modelling anaerobic digestion and compost of organic residues: towards organic matter fate prediction in soils.* Presented at: ORBIT 2016 Organic Resources and Biological Treatment - 10. International Conference on Circular Economy and Organic Waste, Heraklion, Grèce (2016-05-25 - 2016-05-28), https://hal.inrae.fr/hal-02739210

[PO27] Cyrille Charnier, Eric Latrille, **Julie Jimenez**, Michel Torrijos, Margaux Lemoine, Jérémie Miroux, Jean-Philippe Steyer (2016). *Analyse rapide des substrats pour la planification des recettes de mélanges.* Presented at : Journées Recherche et Industrie Biogaz méthanisation, Limoges, France (2016-02-10 - 2016-02-12), https://hal.inrae.fr/hal-01595183

[PO28] **Julie Jimenez**, Cyrille Charnier, Eric Latrille, Michel Torrijos, Jérôme Harmand, Dominique Patureau, Mathieu Sperandio, Eberhard Morgenroth, Fabrice Béline, George Ekama, Peter Vanrolleghem, Angel Robles Martinez, Aurora Seco, Damien J. Batstone, Jean-Philippe Steyer (2016). *Modelling hydrolysis: simultaneous versus sequential biodegradation of the hydrolysable fractions.* Presented at: 5. Wastewater Treatment Modelling Seminar. WWTmod2016, Annecy, France (2016-04-02 - 2016-04-06), https://hal.inrae.fr/hal-01886388

[PO29] Anne-Marie Pourcher, Christine Ziebal, Géraldine Maynaud, Celine Druilhe, Eric Latrille, Dominique Patureau, **Julie Jimenez**, Nathalie Wéry (2015). *Persistence of Salmonella Derby and Listeria monocytogenes in digestates derived from pig and dairy farms.* Presented at : XVII International Congress on Animal Hygiene 2015, ISAH 2015, Košice, Slovaquie (2015-06-07 - 2015-06-11), https://hal.inrae.fr/hal-02739657

[PO30] Quentin Aemig, **Julie Jimenez**, Dominique Patureau, Julien Michel, Pauline Molina, Marjolaine Marjolaine Deschamps, Sabrina Ferhi, Sabine Houot (2015). *Evaluation et réduction des risques de contamination par des polluants organiques dans le contexte de l'usage de produits résiduaires organiques sur sols agricoles.* Presented at : Assemblée Générale du SOERE PRO, Colmar, France (2015-11-24 - 2015-11-24), https://hal.inrae.fr/hal-02797417

[PO31] **Julie Jimenez**, Camila Espinoza Cavajal, Quentin Aemig, Sabine Houot, Jean-Philippe Steyer, Fabien Vedrenne, Dominique Patureau (2015). *Organic matter characterization: towards a unified methodology for biological treatments modelling.* Presented at : 14. World Congress on Anaerobic Digestion (AD14), Viña del Mar, Chili (2015-11-15 - 2015-11-18), https://hal.inrae.fr/hal-02743262

[PO32] **Julie Jimenez**, Han Lei, Jean-Philippe Steyer, Sabine Houot, Dominique Patureau (2015). *Organic matter stability and accessibility characterization - Towards a tool for organic residue diagnostic before land spreading.* Presented at : RAMIRAN 2015 16. International Conference Rural-Urban Symbiosis, Hambourg, Allemagne (2015-09-08 - 2015-09-10), https://hal.inrae.fr/hal-02740786

[PO33] **Julie Jimenez**, Quentin Aemig, Jean-Philippe Steyer, Dominique Patureau (2014). *Anaerobic digestion modelling: innovative characterization tool and extension to micropollutant fate.* Presented at: 2. IWA Specialized International Conference. EcoSTP2014, EcoTechnologies for Wastewater Treatment. Technical, Environmental & Economic Challenges, Verona, Italie (2014-06-23 - 2014-06-27), https://hal.inrae.fr/hal-02739270

[PO34] **Julie Jimenez**, Estelle Gonidec, Jean-Philippe Steyer, Eric E. Latrille, Fabien Vedrenne, Jesús Andrés Cacho Rivero (2013-06-25). *Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: towards ADM1 implementation.* Presented at: 13. World Congress on Anaerobic Digestion: Recovering (bio) Resources for the World. AD13, Santiago de Compostela, Espagne (2013-06-25 - 2013-06-28), https://hal.inrae.fr/hal-02745513

[PO35] Alexis Mottet, Iván Ramírez, Hélène Carrère, **Julie Jimenez**, Stephano Déléris, Fabien Vedrenne, Jean-Philippe Steyer (2010). *New particulate organic matter mapping in ADM1 for sludge digestion.* Presented at : 12th World Congress on anaerobic digestion, Guadalajara, Mexique (2010-10-31 - 2010-11-04), https://hal.inrae.fr/hal-02753303

Communication à des congrès et colloques : poster

[Poster_1] Felipe Guilayn, Mohammed Benbrahim, **Julie Jimenez**, Maxime Rouez, Dominique Patureau, Marion Crest (2019). *Extraits de matière organique soluble de digestats de méthanisation pour la biostimulation de la croissance de plantes cultivées en système hydroponique.* Presented at :

14èmes rencontres de la fertilisation raisonnée et de l'analyse, Dijon, France (2019-11-20 - 2019-11-21), https://dx.doi.org/10.15454/mamn-6923, https://hal.inrae.fr/hal-03360110

[Poster_2] **Julie Jimenez**, Marco Grigatti, Dominique Patureau, Nicolas Bernet (2018). *Nutrients accessibility analysis of organic residues from wastes: fertilizing indicators?* Presented at : 5. AgreenSkills Annual Meeting, Édimbourg, Royaume-Uni (2018-06-11 - 2018-06-14), https://hal.inrae.fr/hal-02790493

[Poster_3] Sabine Houot, **Julie Jimenez** (2017). *Qualité agronomique des produits résiduaires PRO : comment l'évaluer et comment l'inclure dans les outils de gestion de la fertilisation ?* Presented at : Rencontre Recyclage des produits résiduaires organiques en agriculture, INRA Transfert Environnement, Institut Carnot 3BCAR, Paris, France (2017-12-01 - 2017-12-01), https://hal.inrae.fr/hal-01708611

[Poster_4] **Julie Jimenez**, Dominique Patureau, Thierry Morvan (2017). *Deciphering the organic matter kinetics of fresh and dried cattle farmyard manure thanks to organic matter fractionation and litter bags soil incubation assay.* Presented at : 17. International Ramiran Conference, Wexford, Irlande (2017-09-04 - 2017-09-06), https://hal.inrae.fr/hal-02790182

[Poster_5] **Julie Jimenez**, Quentin Aemig, Jean-Philippe Steyer, Dominique Patureau (2014). *ADM1 implementation with an innovative organic matter characterization methodology based on chemical sequential extractions and 3D fluorescence spectroscopy: extension to organic micropollutants fate.* Presented at: 4. IWA/WEF Wastewater Treatment Modellling Seminar. WWTmod2014, Spa, Belgique (2014-03-30 - 2014-04-02), https://hal.inrae.fr/hal-02793861

[Poster_6] **Julie Jimenez** (2015). *Caractérisation de la matière organique des produits résiduaires organiques : outil d'optimisation des filières de traitements jusqu'à leur devenir au sol.* Presented at : Journées Recherche Industrie Biogaz-Méthanisation (JRI2015), Rennes, France (2015-02-03 - 2015-02-06), conférence invitée. https://hal.inrae.fr/hal-01163011

[Poster_7] **Julie Jimenez**, Quentin Aemig, Jean-Philippe Steyer, Dominique Patureau (2014). *ADM1 implementation with an innovative organic matter characterization methodology based on chemical sequential extractions and 3D fluorescence spectroscopy: extension to organic micropollutants fate.* Presented at: 4. IWA/WEF Wastewater Treatment Modellling Seminar. WWTmod2014, Belgium (2014-03-30 -), https://hal.inrae.fr/hal-02793861

Thèse pour l'obtention du grade de docteur

[Thèse_JJ] Julie Jimenez (2012). *Organic matter characterization with 3D fluorescence spectroscopy for anaerobic digestion modeling of wastewater treatment sludge.* (Université Montpellier 2 (Sciences et Techniques). 267 p., https://hal.inrae.fr/tel-02810690

Déclarations d'inventions (brevets, logiciels, marques)

[Brevet_1] Cyrille Charnier, Eric Latrille, Julie Jimenez, Jérémie Miroux, Jean-Philippe Steyer, Michel Torrijos, Philippe Sousbie, Margaux Lemoine (2016). *Procédé de configuration d'un modèle de prédiction des caractéristiques d'un substrat de méthaniseur et utilisation de modèles pour l'estimation de la biodégradation d'un substrat.* FR3047562A1, 52 p., https://hal.inrae.fr/hal-02786650

Déclarations d'invention et de résultats valorisables :

[DIRV_1] Julie Jimenez, Dominique Patureau, Quentin Aemig, Jean-Philippe Steyer, Mathieu Muller (2019). **Indice de Stabilité et de Bio-accessibilité de la Matière Organique (ISBaMO).** *Marque ISBaMO déposée (20/05/21), N°018474884*

[DIRV_2] Julie Jimenez, Virginie Rossard, Eric Latrille, Florian Christ, Romain Girault, Jean-Michel Commandre, Sabine Houot (2019). **Outil d'aide à la conception de filière pour la valorisation agronomique des digestats : Concept-Dig.** *Marque CONCEPT DIG déposée (23/11/21), N°214819581*

[DIRV_3] Miniaturisation du procédé de compostage (en cours)

Dépôt Agence pour la Protection des Programmes : CONCEPT DIG, N°IDDN.FR.001.280008.000. S.P.2022.000.10000 (11/07/2022)

Chapitre 2. Présentation des travaux de recherche

1. Contexte

1.1. Changements globaux et agroécologie

Pour faire face aux changements globaux, changement climatique, croissance démographique mondiale, sécurité alimentaire, préservation des ressources, de nouveaux enjeux de recherche sont apparus. Dans le domaine de l'agriculture, la spécialisation et l'intensification (via l'utilisation excessive d'intrants chimiques) ont généré des impacts environnementaux majeurs (sols, eaux, climat, biodiversité). Ainsi, un des enjeux majeurs est de concilier une production agricole suffisante (en quantité et en qualité) pour satisfaire à la fois une demande mondiale en croissance, permettre le développement économique et social, et assurer la préservation de l'environnement et la limitation des risques pour la santé humaine et les écosystèmes. Une des solutions repose sur un changement de paradigme afin de s'orienter vers l'**Agroécologie** (Altieri, 2002).

D'après (Malézieux and Lafontaine, 2013), l'hypothèse principale sur laquelle repose l'agroécologie est qu'il est possible d'augmenter les productions agricoles en quantité et en qualité, d'assurer une meilleure maîtrise des populations de ravageurs et de diminuer la dépendance vis-à-vis des intrants, en accroissant la diversité biologique dans les agroécosystèmes et en optimisant les interactions biologiques au sein de ces derniers. Les systèmes agroécologiques peuvent notamment participer à une gestion plus durable du cycle de l'eau et des cycles biogéochimiques de l'azote, du phosphore et du carbone. De plus, lors de la COP 21 (2015), et sur la base d'une étude menée par INRAE via une modélisation long terme, le cap de 4 ‰ a été fixé pour neutraliser l'augmentation annuelle du carbone atmosphérique et compléter l'objectif de réduction des émissions de gaz à effet de serre (GES) (Minasny et al., 2017) . En effet, l'initiative « 4 ‰ » consiste à promouvoir le stockage du Carbone, à hauteur de 4 ‰ /an, sous forme de matière organique (MO) dans le sol (via l'apport en intrants organiques, la couverture des sols, la production de légumineuses, l'agroforesterie augmentant la photosynthèse) et ainsi compenser l'augmentation des émissions de CO_2 dans l'atmosphère. D'après cette initiative, en augmentant la teneur en MO dans les sols, leurs fertilités augmentent et la préservation de la sécurité alimentaire et du climat est supportée. En France, d'après INRAE (Pellerin et al., 2019), 41% des émissions de carbone agricole pourrait être compensés avec un stockage additionnel de 1,9‰ sur l'ensemble des surfaces agricoles et forestières (3,3 ‰ pour les seules surfaces agricoles et 5,2 ‰ pour les grandes cultures). Les pratiques conseillées sont la mise en place de couverts intercalaires et intermédiaires, l'introduction et allongement des prairies temporaires dans les rotations culturales, le développement de l'agroforesterie, l'apport de composts ou produits résiduaires organiques issus de la valorisation de résidus organiques.

La transition agroécologique nécessite donc des pratiques de production agricole qui préservent mieux l'environnement et des alternatives aux intrants chimiques, par exemple grâce à un recyclage accru des déchets pour une gestion plus durable de la nutrition des plantes et de la qualité des sols.

1.2. Déchets organiques et Bioraffinerie Environnementale : vers un changement de paradigme

L'utilisation de matières organiques dans l'agriculture en tant que fertilisants était pratiquée au XIXe siècle et auparavant. Au XXe siècle, les produits résiduaires organiques urbains ont été considérés comme « déchets » et ont été écartés de leur utilisation agronomique au profit d'engrais chimiques (Barles, 2005). Dans les années 1980, les pratiques de réutilisation agricole des résidus urbains sont à nouveau à l'ordre du jour. Par ailleurs, sous l'effet de l'urbanisation rapide et de la croissance démographique, il est prévu que la production mondiale de déchets augmente de 70 % d'ici 2050, soit une moyenne de plus de 2 milliards de tonnes de déchets par an, selon un rapport de la Banque mondiale (Kaza et al., 2018).

En France, l'équivalent de 343 millions de tonnes de déchets sont produits chaque année (Bilan 2018 de la production de déchets en France, Ministère de la transition écologique). Il existe trois catégories de déchets : déchets dangereux (3% du tonnage en France, 2018), déchets non dangereux (28% du tonnage en France, 2018) et déchets non dangereux inertes (69% du tonnage en France, 2018). Ces derniers sont essentiellement issus des activités du bâtiment. Le gisement intéressant ici est celui contenu dans les déchets non dangereux et plus particulièrement les déchets organiques. Ces derniers peuvent provenir d'activités urbaines et agricoles ou assimilées générant une variabilité de ressources potentielles significative. Tout au long de ce mémoire, ces déchets qui seront appelés par la suite **résidus organiques** peuvent être classés en trois grandes catégories : les résidus urbains, agricoles et ceux issus des activités industrielles (agro-industries). Parmi les déchets urbains, on retrouve la fraction fermentescible des ordures ménagères (FFOM), les résidus provenant de l'assainissement (boues de station d'épuration STEP) et les biodéchets (déchets verts, déchets alimentaires provenant des restaurants ou cantines, déchets alimentaires triés à la source, déchets alimentaires des grandes et moyennes surfaces). Parmi les déchets agricoles, on peut citer les déjections animales produites par les installations d'élevage (fumiers, lisiers) ou encore les résidus de récolte et enfin les résidus issus des activités industrielles notamment d'industries agro-alimentaires.

Une gestion durable de ces déchets est gouvernée par deux enjeux majeurs : la limitation de leurs productions et leur valorisation et réutilisation. En effet, les déchets organiques provenant des diverses sources citées précédemment sont riches en MO et nutriments et valorisables à différents niveaux selon leur qualité. Certains résidus organiques ont des potentiels énergétiques non négligeables (tel qu'un potentiel de production de méthane via la méthanisation par exemple), d'autres ont des potentiels agronomiques évidents (qualité de MO en adéquation avec les besoins des sols en termes d'amendements et/ou sources de nutriments NPKS en adéquation avec les besoins en fertilisation chimique des plantes). Certains résidus possèdent également des molécules d'intérêt (précurseurs de bioplastiques, substances humiques, molécules organiques réutilisables en industrie chimique, etc…) extractibles via des procédés dédiés. Ainsi, ces résidus organiques, bien qu'à limiter et diminuer, constituent aussi une opportunité pour répondre aux services rendus à la fois agronomiques, énergétiques, chimiques. En les recyclant et les valorisant au maximum, les enjeux environnementaux et climatiques associés pourraient être atténués dans une logique de **bioéconomie circulaire**.

Classiquement, les deux grandes voies principales de valorisation des déchets organiques non dangereux sont le compostage et la valorisation énergétique via la méthanisation (Ministère de la Transition écologique et de la Cohésion des territoires and Ministère de la Transition Energétique, 2021). Longtemps utilisée, la mise en décharge dans des centres de stockage est désormais réservée aux déchets dit « ultimes », à savoir pour lesquels aucune autre valorisation n'est possible. La loi de transition énergétique pour la croissance verte a notamment fixé un objectif de réduction de l'élimination par stockage de moitié en 2025 avec une étape intermédiaire de -30 % en 2020, par rapport aux quantités admises en décharge en 2010.

Le compostage consiste en une biodégradation en deux temps, en présence d'oxygène, dans des conditions opératoires données (ratio C/N, taux d'humidité, tassement), de la MO afin d'à la fois hygiéniser (via une réaction exothermique réalisée par des bactéries dans un premier temps) puis stabiliser la matière (i.e. humifier la matière via l'action de plusieurs micro-organismes dont les bactéries, les champignons et actinomycètes) avec un objectif de retour au sol. Ce retour au sol doit présenter les garanties nécessaires en termes d'innocuité sanitaire et de qualité agronomique. Les normes NF U 44-095 sur les composts de boues et NF U 44-051 sur les amendements organiques, ont été rendues d'application obligatoire et fixent des critères à respecter pour ces composts afin d'encadrer l'innocuité et l'efficacité de ces derniers en agriculture.

La méthanisation ou digestion anaérobie consiste en une succession de réactions biologiques de la MO en l'absence d'oxygène réalisées par des bactéries et Archae afin de produire un mélange gazeux composé en majorité de méthane et de gaz carbonique, de transformer la MO initiale en un produit humide, partiellement stabilisé, appelé le digestat. En plus de la production de biogaz qui fait l'objet d'une valorisation énergétique intéressante, le digestat, qui est un déchet aux caractéristiques fertilisantes, peut être épandu sur les sols agricoles quand il respecte la réglementation du code rural. Sa valorisation agronomique est possible de différentes manières : après une phase de maturation par compostage, ou directement par son épandage sur les sols agricoles, s'il respecte le cahier des charges CDIGAgri (Journal Officiel de la République Française, 2020) concernant les digestats d'origine agricole, dans le cadre d'un plan d'épandage, ou bien encore s'il est homologué pour devenir un produit commercialisable. Ce procédé connaît un fort essor depuis 2013 et le lancement du Plan Energie Méthanisation Autonomie Azote (EMAA) adopté par le gouvernement visant la mise en place d'installations de méthanisation à la ferme, les autres types de méthanisation étant également encouragés et faisant l'objet de subventions de la part de l'ADEME. De plus, le tarif intéressant de rachat de l'électricité produite par co-génération combinée à l'augmentation des sites de méthanisations favorisant l'injection de gaz directement dans le réseau de gaz naturel font de ce procédé un atout économique non négligeable. **Au 1er janvier 2022, on dénombrait en France 1 308 unités de méthanisation dont 371 en injection et 759 en cogénération**. En 2022, 71 nouveaux sites d'injection de biométhane ont été mis en service et 3,1 TWh de biométhane ont été injectés depuis le début de l'année (GRDF, 2022). A la fin juin 2022, **442 sites ont été** raccordés à tout réseau gazier dont **376 sites** raccordés au réseau GRDF. Cela représente une production de près de **7,6 TWh/an**, soit l'équivalent de la consommation annuelle de plus de 1 900 000 logements neufs (GRDF, 2022).

La valorisation de ce gisement de résidus organiques en énergie et/ou en engrais organique est un enjeu majeur dans le recyclage des produits résiduaires pour boucler les cycles géochimiques (C, N, P, K et S) et préserver l'environnement via une bioéconomie circulaire. Pour réussir cette transition agroécologique, le développement de procédés permettant à la fois la production d'énergies renouvelables et de fertilisants organiques comme la méthanisation est un des leviers d'action. A titre d'exemple, plus de 200 unités de méthanisation implantées dans les exploitations agricoles françaises et raccordées au réseau de gaz ont permis d'économiser 450 000 tonnes d'équivalent CO_2 en 2020 (données GRDF, 2021). Un exemple de bioéconomie circulaire réussie dans un territoire à forte empreinte animale est représenté par la Figure 2. Cet exemple associe des activités de production de chaleur, de biogaz, d'abattage d'animaux, de traitement et recyclage de l'eau, de traitements d'effluents et de production d'engrais organiques via notamment l'utilisation de la méthanisation.

La gestion des déchets organiques longtemps considérée plutôt comme un traitement de ces derniers est devenue maintenant une véritable valorisation où les résidus organiques sont devenus des ressources à exploiter. D'après l'ADEME, « la valorisation des déchets organiques doit passer par une logique de déchet à une logique de produit. Pour pouvoir être recyclés ou valorisés, les « déchets » organiques ne doivent plus être considérés comme des résidus dont il faut se débarrasser mais comme des matières premières qui peuvent présenter un intérêt soit agronomique (fertilisation, apport de MO…), soit matière (alimentaire ou industriel), soit énergétique» (Chauvin, 2004).

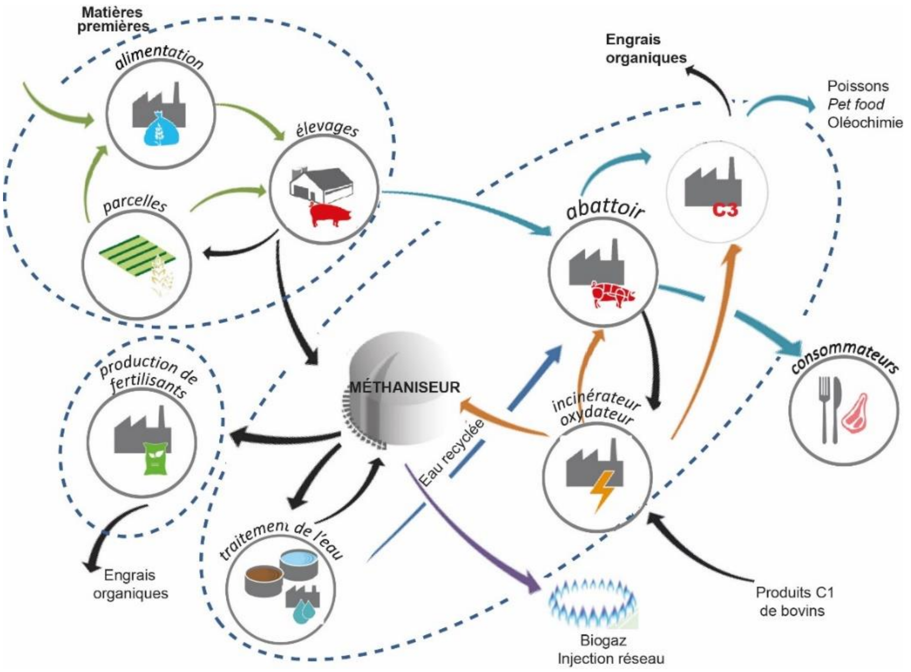

Figure 2 *:* Exemple de bioéconomie circulaire dans un territoire à forte empreinte animale (Dourmad et al., 2019)

C'est dans ce contexte de changement de paradigme qu'un concept innovant est apparu : la **Bioraffinerie Environnementale** (Garcia-Bernet and Daboussi, 2016). **Les bioraffineries ont pour vocation de transformer l'ensemble des fractions constitutives d'une ressource organique dans l'optique d'une valorisation complète et dans un contexte de bioéconomie circulaire**. Cet ensemble peut combiner plusieurs outils tels que des extractions de molécules à haute valeur ajoutée, la production de bio-carburant, d'énergie, de biomatériaux, de fertilisants organiques, etc… Chaque coproduit devient alors une « matière première » à part entière à valoriser. L'optimisation de cette valorisation repose néanmoins sur la connaissance des résidus organiques afin de cibler des services rendus pertinents, l'utilisation et/ou la création d'un panel de procédés ciblant les dits-services rendus et la mise en place d'une filière de traitement respectueuse de l'environnement (minimisation des impacts environnementaux, innocuité, …). Tout autant de connaissances à apporter que de questions de recherche associées sous le couvert de la recherche du compromis.

1.3. Problématiques de recherche : INRAE et LBE

La transition agroécologique est devenue une des orientations scientifiques majeures au sein de **INRAE**. En effet, INRAE étudie les conditions d'une transition de l'agriculture vers des **systèmes multi-performants**, **bas carbone et bas intrants**, en mobilisant les principes de l'agroécologie, notamment via les enjeux scientifiques portés par le département AgroEcoSystem. En parallèle, INRAE se positionne comme un acteur de la **bioéconomie circulaire** en proposant quatre questions de recherche portées par le département Transform : « Comment produire et mobiliser plus de biomasse sous contrainte climatique tout en préservant écosystèmes et ressources ? Comment optimiser la transformation des biomasses dans toute leur diversité ? Comment assurer le recyclage des matériaux afin de boucler les cycles biologiques du carbone, de l'azote et du phosphore ? Comment anticiper, organiser et gérer les flux, les échanges et les marchés dans un contexte d'incertitude ? »

Au carrefour de ces départements et de leurs enjeux se situent les recherches menées par l'unité **LBE** (Laboratoire de Biotechnologie de l'Environnement) de Narbonne, à l'origine du concept de **Bioraffinerie Environnementale (Figure 3),** valorisant un large panel de résidus organiques en divers services rendus (bioénergies, biomolécules, amendement et fertilisant organique) tout en minimisant leur impact environnemental et sanitaire, avec un focus sur les procédés biologiques classiques (fermentation, méthanisation, compostage) et innovants (bioélectrochimie, culture de microalgues).

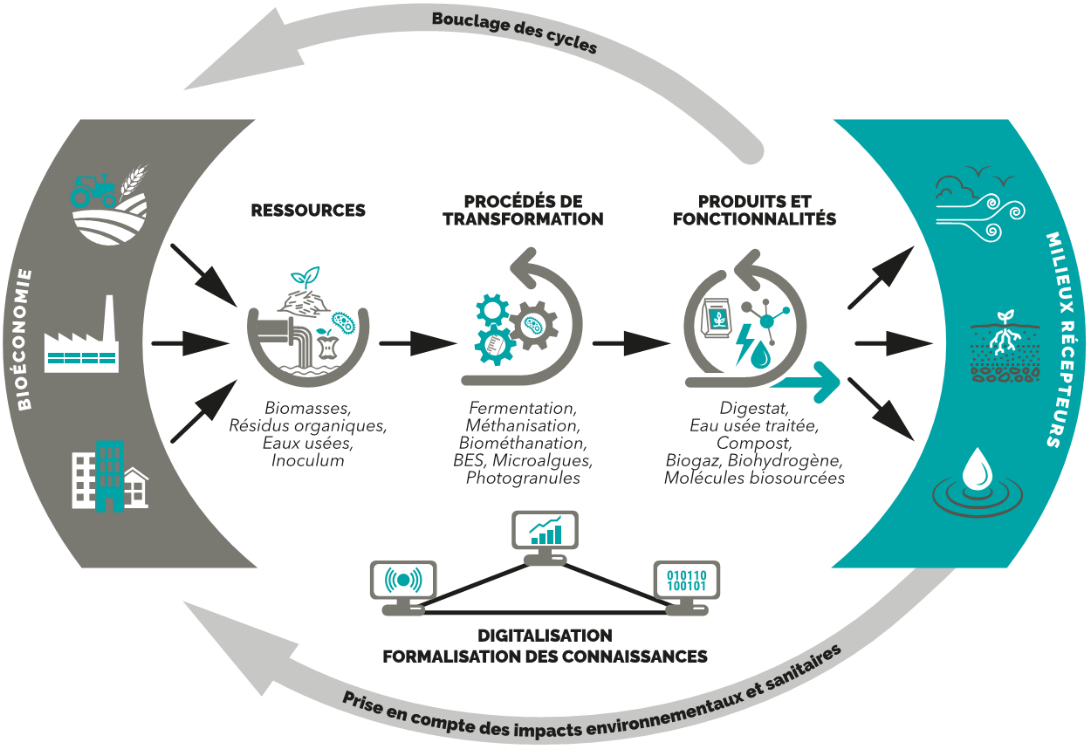

Figure 3 : Schéma de la Bioraffinerie Environnementale, thématique centrale au sein des recherches du LBE

1.4. Stratégie de recherche

Ainsi, une gestion durable des déchets organiques permettrait de répondre aux besoins de la transition agroécologique via la production de fertilisants organiques nécessaires à la substitution d'engrais minéraux et participant au bouclage des cycles géochimiques (CNPKS) et notamment au stockage de C dans les sols. Le schéma de la Figure 4 présente cette adéquation, comprenant d'une part les enjeux majeurs de la gestion durable des déchets organiques et d'autre part ceux de la transition agroécologique et leurs moyens associés.

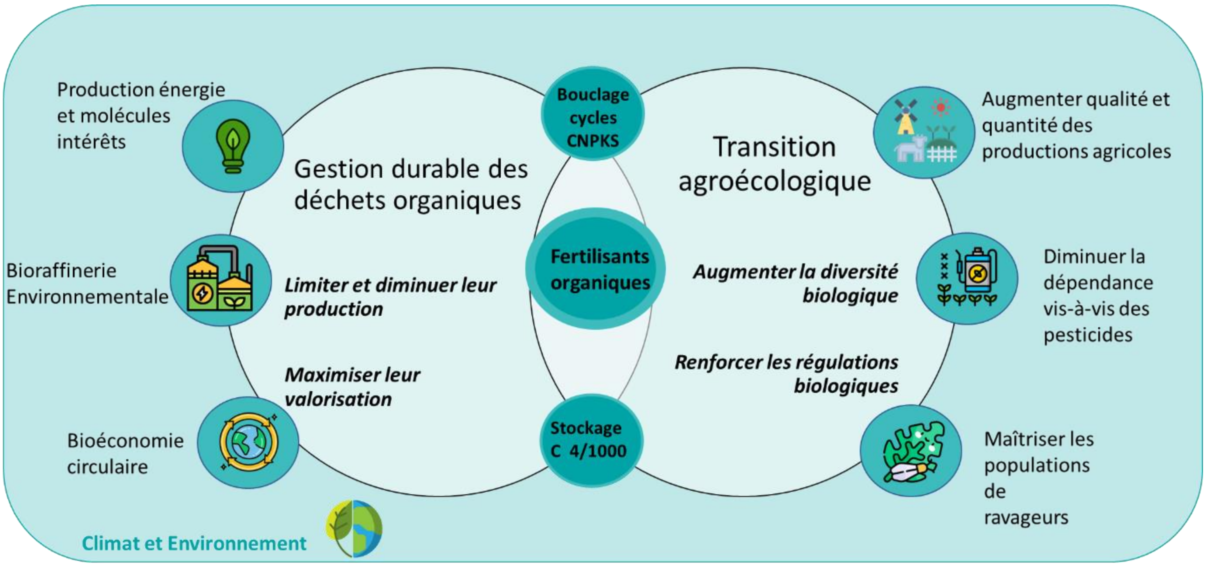

Figure 4 : Schématisation de l'adéquation entre Gestion durable des déchets organiques et la Transition Agroécologique (Source : schéma personnel)

C’est dans ce contexte que j’ai été recrutée au LBE en tant que chargée de recherche sur la thématique “**Adaptation et intégration des procédés biologiques de traitement des résidus organiques pour une gestion durable des agroécosystèmes**”.

Le concept de Bioraffinerie Environnementale repose sur l’idée d'utiliser et de combiner la meilleure configuration de procédés constituant une filière pour maximiser cette valorisation. Cependant, pendant des décennies, l'optimisation du traitement s'est concentrée uniquement sur l'amélioration des produits à haute valeur ajoutée au détriment de la qualité de la MO non transformée restante, appelée par la suite **Produits Résiduaires Organiques** (PRO).

Dans un contexte de bioéconomie circulaire, l'optimisation de la qualité des produits issus de résidus organiques (tant l'efficacité agronomique et environnementale que l’innocuité) est une préoccupation majeure. Il est donc crucial de concevoir des bioprocédés favorisant un compromis adéquat entre les services attendus (i.e. dimensions agronomiques et écosystémiques, traitement des pollutions, et production d'énergie et/ou de molécules à haute valeur ajoutée). La prise en compte d'un tel compromis n'était pas inclus dans la conception et le contrôle des bioprocédés jusqu'à mon recrutement et l’établissement de ce nouveau sujet de recherche. Pour répondre à cette problématique, l'idée innovante de ma thématique de recherche est de proposer une approche d'**ingénierie inverse** et de concevoir une bioraffinerie environnementale en fonction des services rendus attendus et des besoins des agroécosystèmes ciblés (Figure 5).

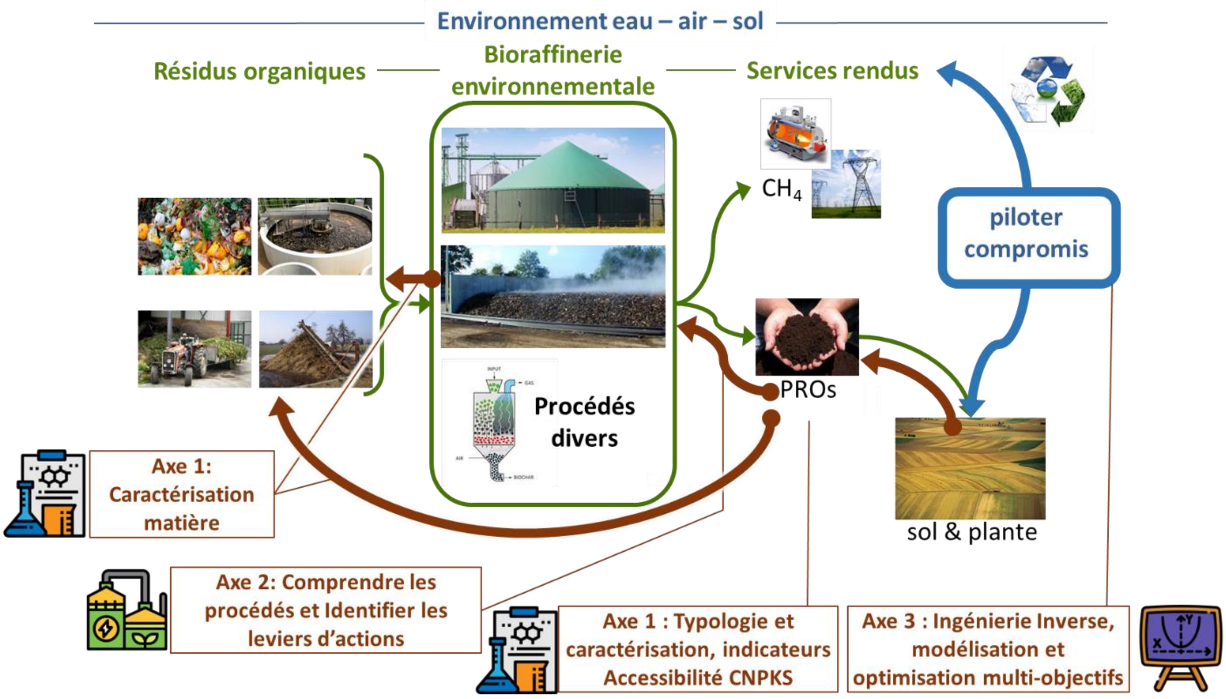

Figure 5 : Concept de l’« Ingénierie inverse » au service de la Bioraffinerie Environnementale et définition de ma stratégie de recherche

En effet, l’idée est de concevoir des filières de valorisation non plus à partir uniquement de la qualité des intrants mais également à partir des services rendus ciblés et du « cahier des charges » associé, dans une logique « produit ». Il s’agit donc d’un exercice d’optimisation multi-objectif, sous contraintes environnementales à la recherche de compromis entre services rendus.
Tout au long de ma carrière (cf. Chapitre 1), que ce soit pendant mon poste d’ingénieure de recherche à VEOLIA, ma thèse et mon post-doctorat au LBE, j’ai acquis et développé trois domaines d’expertises : la caractérisation de la matière, le génie des procédés et la modélisation des bioprocédés.

Afin de répondre aux enjeux associés à ma problématique de recherche, j’ai proposé une stratégie de recherche innovante et ambitieuse basée sur trois axes de recherche complémentaires (Figure 5) :

(i) Vers une meilleure connaissance des résidus organiques et des produits résiduaires organiques via la caractérisation matière : comment les outils de caractérisation de la matière peuvent nous aider à mieux définir et comprendre leur potentiel de valorisation et l’identification de paramètes clés ?

(ii) L’identification des leviers d’actions des procédés de valorisation : comprendre les mécanismes de transformation de la matière pour l’optimiser ;

(iii) Optimisation de la conception de la Bioraffinerie Environnementale afin d’appliquer une stratégie d’ingénierie inverse, en utilisant la modélisation et l'optimisation multi-objectifs en fonction des besoins des agroécosystèmes.

L’axe 1 basé sur la caractérisation de la matière alimente à la fois l’axe 2 sur les procédés et l’axe 3 sur l’optimisation filière de valorisation. En effet, la connaissance de la matière permet de mieux comprendre les processus impliqués dans sa transformation dans les procédés via l’étude du devenir de cette matière au cours du temps mais également permet de définir des objectifs et/ou contraintes dans l’exercice d’optimisation. De même, l’axe 2 alimente directement l’axe 3. Une meilleure connaissance des procédés permet une meilleure modélisation de ces derniers afin d’identifier les leviers d’action possibles et les antagonismes vis-à-vis des services rendus et de réussir une conception de filière de valorisation optimale sous contrainte environnementale.

Ma thématique de recherche concernant la Bioraffinerie Environnementale est assez large du point de vue conception de filières, services rendus et procédés à considérer. J’ai choisi, dans un premier temps, de développer ma stratégie et ma démarche scientifique sur la filière de méthanisation, étant donné qu’elle répond aux enjeux scientifiques à la fois de la gestion des déchets et de la transition agroécologique. Cette filière est également source d’antagonismes et de complémentarités à définir et à comprendre afin d’être optimisée. L'identification des antagonismes et la gestion des compromis sont des enjeux clés pour optimiser à la fois la production d'énergie et celle d'engrais organiques. En effet, lorsque le devenir de la matière au sein d’un méthaniseur est étudié de plus près (Figure 6), il apparaît que la MO biodégradable en anaérobie est transformée en biogaz valorisable (selon les conditions procédés) et que la MO résiduelle peut être composée de plusieurs types de MO plus ou moins complexes.

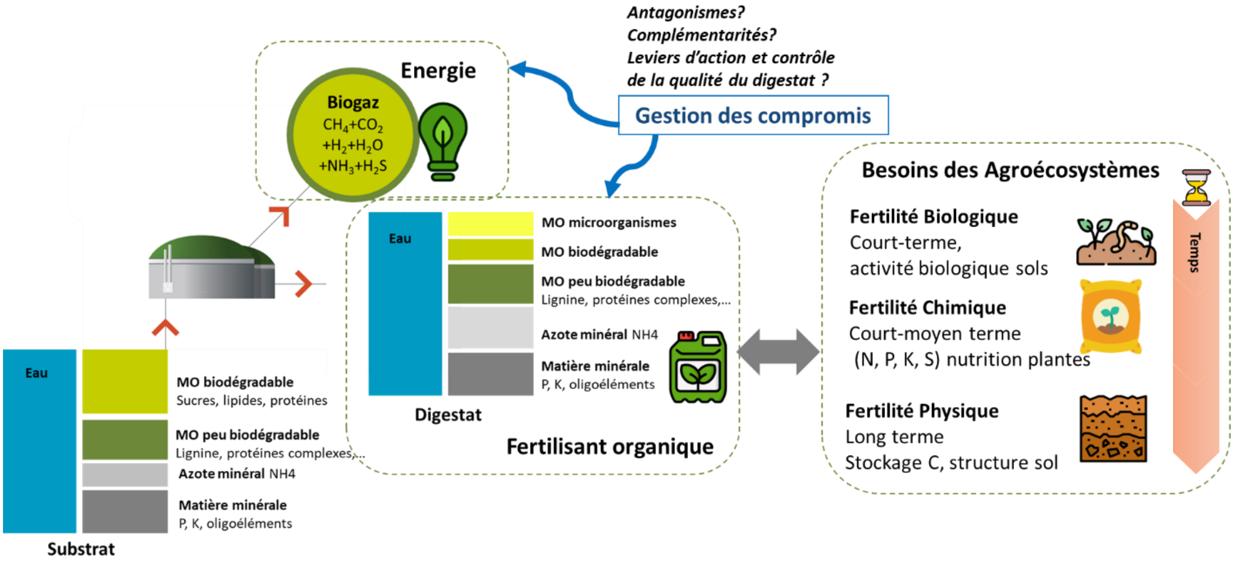

Figure 6 : Devenir de la matière pendant la méthanisation : application de ma thématique de recherche à la filière méthanisation et mes questions de recherches associées

Cette MO résiduelle est également intéressante dans les digestats puisqu’elle peut participer à favoriser la vie du sol et/ou améliorer la structure du sol selon sa qualité (plus ou moins labile/stable). Ainsi, un des antagonismes ou complémentarités à évaluer repose sur la qualité de la MO entrante en méthanisation et comment cette dernière est transformée par la biologie et va se comporter ensuite après épandage au sol. De même, les nutriments contenus dans les résidus organiques valorisés sont conservés lors de la méthanisation. Toutefois, certains éléments associés à des formes organiques (exemple : azote organique) subissent une minéralisation ayant pour conséquence une transformation d’éléments moins disponibles à disponibles et assimilables par les plantes (azote organique transformé en azote ammoniacal). Ce processus est une opportunité à saisir concernant le service rendu « fertilisant ». Or, certaines formes minérales comme l’azote ammoniacal peuvent être également inhibitrices de la méthanogénèse. Ainsi, un compromis entre bon fonctionnement du digesteur et production de fertilisants est à prendre en compte. Ce raisonnement est également valable pour d’autres éléments, plus ou moins inhibiteurs, tels que le soufre.
De plus, comme souligné dans la Figure 4, ma thématique de recherche s’inscrit à l’interface entre « Gestion des déchets organiques » et « Transition agroécologique » que l’on peut associer aux départements Transform et AgroEcoSystem de INRAE respectivement. J’ai donc relevé le défi de travailler à l'interface entre deux disciplines qui interagissaient rarement : le génie des procédés pour la valorisation des résidus organiques et l'agronomie. Ainsi, j’ai développé une stratégie de collaboration entre l'expertise du LBE en génie des procédés biologiques et des experts agronomes (issus d'INRAE et d'autres instituts de recherche nationaux et structures de recherche internationales). Cette stratégie de collaboration m’a permis de partager, renforcer et combiner mon expertise d’ingénierie inverse pour la conception de la bioraffinerie environnementale (Figure 7).

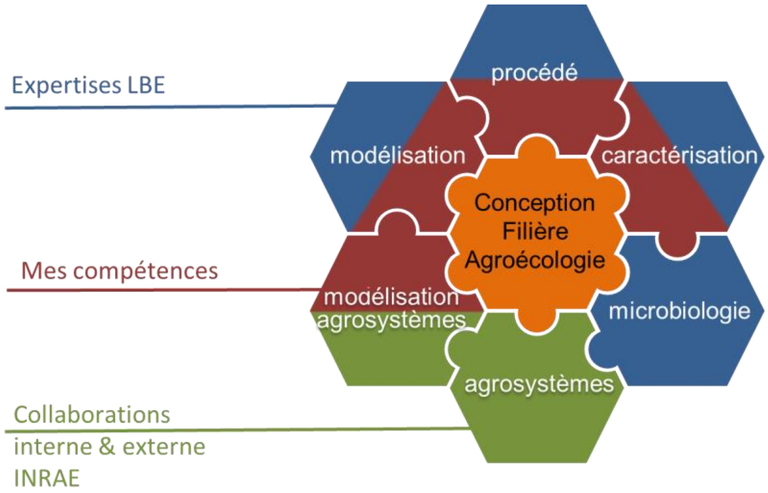

Figure 7 : Représentation schématique des collaborations mises en place durant mes activités de recherche complémentaires à mes domaines d’expertises

Ainsi, la structure de ce Chapitre suivra la logique de mes axes de recherche dans lesquels je décrirai les principales actions et les développements menés depuis le début de ma carrière à INRAE, rythmés par les diverses collaborations mises en place pour répondre à mes questions de recherche.

2. Axe 1 : Comment développer de nouveaux indicateurs pour optimiser la conception de la Bioraffinerie Environnementale ?

Au départ, mon expertise en matière de caractérisation de la matière était principalement axée sur la MO pour la modélisation des stations d'épuration, y compris la méthanisation dans un objectif de production d’énergie. Par conséquent, j’ai dû faire face au challenge d’étendre mes connaissances et concepts à d’autres éléments (N, P, K, S), pour décrire d’autres procédés et d’autres services rendus ce qui a considérablement élargi mes domaines d'application. Dès lors, dans mes premières années d'investigation, j’ai concentré mes efforts sur le développement de nouveaux indicateurs adaptés à ce nouveau domaine d'application.

2.1. Démarche scientifique

Objectifs

Une définition de l’ingénierie inverse ou rétro-ingénierie est la suivante : *« Pratique qui consiste à analyser un produit fini pour connaître la manière dont celui-ci a été conçu ou fabriqué ».* Cette définition brute met en évidence que pour concevoir un procédé visant à produire un produit fini, il faut non seulement partir dudit produit pour « remonter » les étapes de production mais également analyser sa composition.

La thématique principale de mon premier axe de recherche est la caractérisation de la matière. En effet, optimiser la conception de filières de valorisation de résidus organiques passe d’abord par la connaissance de la matière qu’ils contiennent afin de connaître leur potentiel valorisable. Le schéma de la Figure 8 présente la place de la caractérisation de la matière au sein de mes thématiques de recherche et les questions de recherche associées.

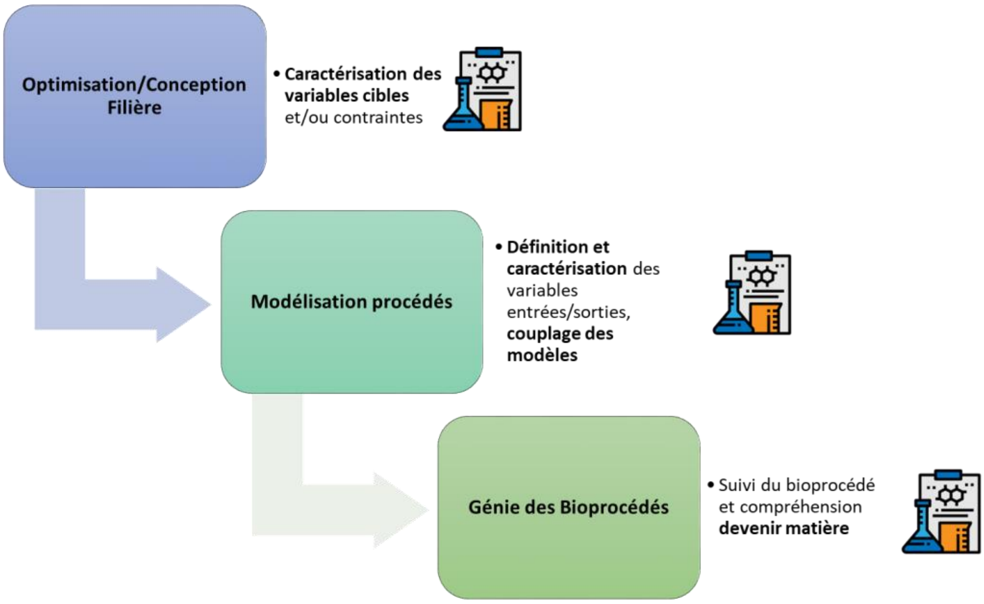

Figure 8 : Place de la caractérisation au sein de la démarche scientifique de ma thématique de recherche

Cet exercice d’optimisation tel que je le conçois passe par la mise en place de modèles de prédiction des performances et des services rendus de chaque procédé de la Bioraffinerie Environnementale afin de pouvoir générer des scénarios et optimiser les paramètres opératoires et succession de procédés selon un cahier des charges donné par les services rendus, comme par exemple les besoins des agroécoystèmes et la production de méthane.

Or, afin de modéliser les bioprocédés, il faut tout d’abord les comprendre afin de proposer des équations mathématiques susceptibles de reproduire les processus biologiques impliqués et pouvoir prédire l’impact de ces derniers sur la qualité de la matière entrante et leur potentiel valorisable. Cette étape ne peut être réalisée qu’à partir d’une définition pertinente des composantes de la matière à biodégrader et donc d'une caractérisation associée mesurable. De même, afin d'atteindre les spécifications de qualité d'un produit, la définition de ces variables cibles mesurables expérimentalement est cruciale.

Dans le cas de ma thématique de recherche, j'ai choisi d'investiguer et de définir des variables communes et utilisables dans chaque bioprocédé jusqu'au devenir de la matière dans les sols afin de suivre le devenir de la matière. Ce choix aura également le mérite de répondre à la problématique autour de la modélisation de la filière via le couplage de modèles, que ce soit des modèles de procédés ou de sol/culture (cf. axe 3).
Ces variables devront être associées à des méthodes de caractérisation pertinentes permettant de décrire également les services rendus.

<u>Définitions des concepts</u>

La première étape a été d'identifier et de mettre en place des indicateurs pertinents capables de décrire les services rendus ciblés et les procédés impliqués dans la Bioraffinerie Environnementale : (i) qualité de la MO pour la conversion de la MO en méthane, (ii) stabilité du C, N, P, K, S, l'accessibilité et la disponibilité pour la production d'engrais organiques, (iii) la mobilité et la persistance des micropolluants (organiques, métalliques) et la survie des contaminants microbiens pour l'innocuité et (iv) les indicateurs d'impacts environnementaux.
Partant de cette stratégie, j'ai proposé d'associer chaque définition d'indicateur à des outils de caractérisation.
En effet, j'ai tout d'abord choisi d'identifier et de définir deux concepts que sont la **Bioaccessibilité** de la matière et sa **Biodisponibilité**, notions associées au concept de **Biodégradabilité** des MO dans les processus biologiques (digestion anaérobie, compostage, sol).

Tout d'abord, il est à noter que le type de résidus organiques à traiter, et notamment via la méthanisation a évolué depuis des décennies. En effet, au départ appliquée sur des eaux usées industrielles chargées en MO mais plutôt liquides, la méthanisation est désormais utilisée pour valoriser un très large panel de résidus organiques complexes, plus ou moins solides [JJ24].
Ensuite, basée sur cette précédente observation, l'étape limitante de la biodégradation des résidus organiques dépend de la nature de ces derniers. En effet, l'hydrolyse est l'étape limitante dans la biodégradation des molécules particulaires ou solides (Vavilin et al., 1996; [JJ10]) étant donné que la structure physique de ces dernières est plus lentement accessible et donc hydrolysable. Cette notion de cinétique de biodégradation est cruciale dans le design de bioprocédés et le bon fonctionnement d'un bioréacteur. C'est pourquoi la compréhension et l'optimisation d'un procédé comme la méthanisation passe par la compréhension et l'optimisation de l'hydrolyse de la matière et de sa vitesse de biodégradation.
Si l'on étudie de plus près la biodégradabilité d'une eau usée par boues activées aérobie en réalisant de la respirométrie par exemple (Spérandio, 1998) ou encore celle d'un résidu organique par méthanisation via un test de potentiel méthane (BMP), les courbes expérimentales associées de vitesse de consommation d'oxygène et de vitesse de production de méthane (Figure 9) respectivement montrent qu'il existe plusieurs « fractions » de MO correspondant aux fractions hydrolysables et biodégradables de cette matière ((Yasui et al., 2006); [JJ27] et [JJ10]).
Les modélisateurs des processus biologiques impliqués dans les bioprocédés ont d'ailleurs inclus ce fractionnement de la matière à plusieurs reprises, que ce soit pour la modélisation des boues activées d'une station d'épuration via le modèle mécanistique Activated Sludge Model (ASM) (Henze et al., 2000; Spérandio, 1998), la modélisation de la digestion anaérobie via le modèle ADM1 (Batstone et al., 2002) et ses modifications (Vavilin et al., 2008; Yasui et al., 2006 ; [JJ28] et [JJ10]). Toutefois, il n'existe pas de méthodologie de caractérisation de cette matière qui fait consensus.
Le verrou existant alors réside dans la compréhension de ce fractionnement : quel est/sont le ou les concepts qui gouvernent le fractionnement de la matière en fractions plus ou moins hydrolysables et biodégradables ? Est-ce que ces fractions sont dégradées simultanément ou séquentiellement ?

Comment mesurer ces fractions afin de modéliser au plus juste les bioprocédés ?

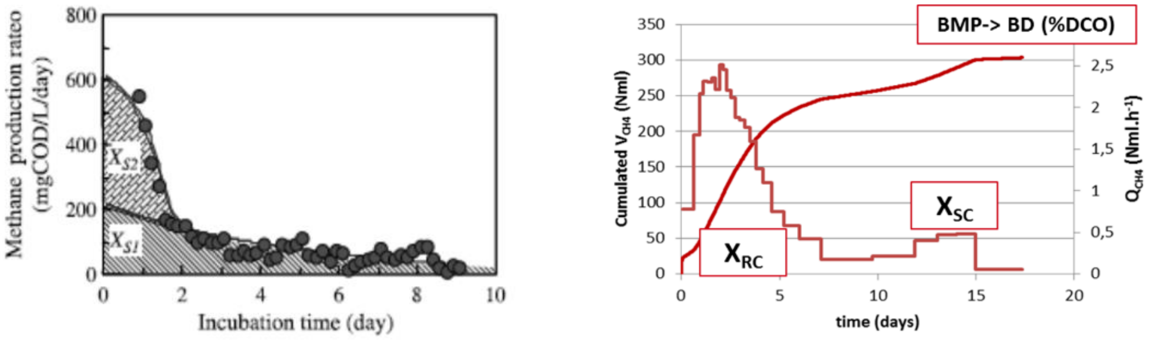

Figure 9 : Figures extraites de Yasui et al. (2006) et [JJ27]

Basé sur la définition proposée par (Aquino et al., 2008), j'ai entrepris dans le cadre de ma thèse d'abord [These_JJ] puis dans mes divers travaux de recherche par la suite ([JJ27], [JJ25], [JJ18] et [JJ10]) de répondre à ces questions, pour le procédé de méthanisation dans un premier temps, et de proposer les définitions suivantes schématisées par la Figure 10.

De manière vulgarisée, prenons deux fruits que sont la pêche et la noix de coco. Faisons l'hypothèse que ces deux fruits ont une valeur nutritionnelle similaire. Dans le cas de la noix de coco, cette valeur nutritionnelle n'est pas directement accessible car elle possède une coquille qui en limite l'accès et qu'il faut d'abord « enlever » alors que la même valeur nutritionnelle est directement accessible dans le cas d'un autre fruit tel que la pêche ou la pomme. Cette métaphore simpliste a le mérite d'introduire le concept de Bioaccessibilité. Ainsi les microorganismes contenus dans un système biologique ont un possible accès à certaines molécules organiques selon divers facteurs tels que leurs structures physiques, le temps de séjour dans le procédé, l'activité hydrolytique présente dans le système ou encore les pré-traitements appliqués. Ces molécules accessibles peuvent être biodisponibles si elles ont des caractéristiques suffisantes pour être consommées par les microorganismes (par exemple, si leur poids moléculaire (< 1kDa) leur permet de traverser la paroi cellulaire d'un microorganisme). Enfin, la biodégradabilité concerne la consommation à proprement parlé de la molécule si cette dernière n'est pas inhibitrice, si sa qualité le permet et si le système microbien possède les enzymes nécessaires.

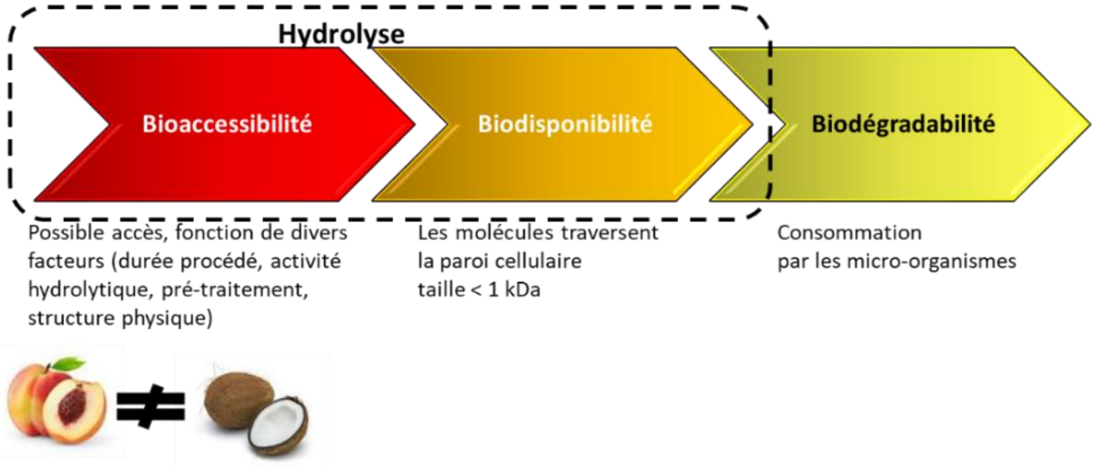

Figure 10 : Notions de Bioaccessibilité, Biodisponibilité et Biodégradabilité nécessaires à la description des processus de biodégradation dans un procédé biologique

Toutefois, la notion de bioaccessibilité seule ne permet pas de prédire la biodégradabilité des boues. En effet, comme cela sera décrit ultérieurement, le fractionnement développé dans ma thèse puis par la suite (décrit et détaillé dans le paragraphe 2.2) permet de donner des tailles de compartiments plus ou moins accessibles associés à des cinétiques de dégradation différentes mais pas leur qualité en termes de molécules organiques. Par exemple, le fait de rendre bioaccessible la matière comme dans le cas d'un traitement thermique où une partie de la matière est solubilisée ne favorise pas systématiquement sa biodégradabilité car les molécules biodisponibles (glycoprotéines complexes issues des réactions de Maillard) ne sont pas consommables par les microorganismes [JJ26]. Ainsi, la notion de complexité de la matière est à associer à chaque fraction afin d'en prédire sa biodégradation.

Dans le reste de la section dédiée à l'axe 1, la biodégradabilité de la MO est considérée comme le résultat de la combinaison des deux concepts que sont la Bioaccessibilité de la MO (taille de compartiments) et la complexité de la MO (nature biochimique du compartiment), comme le montre le schéma proposé de la Figure 11.

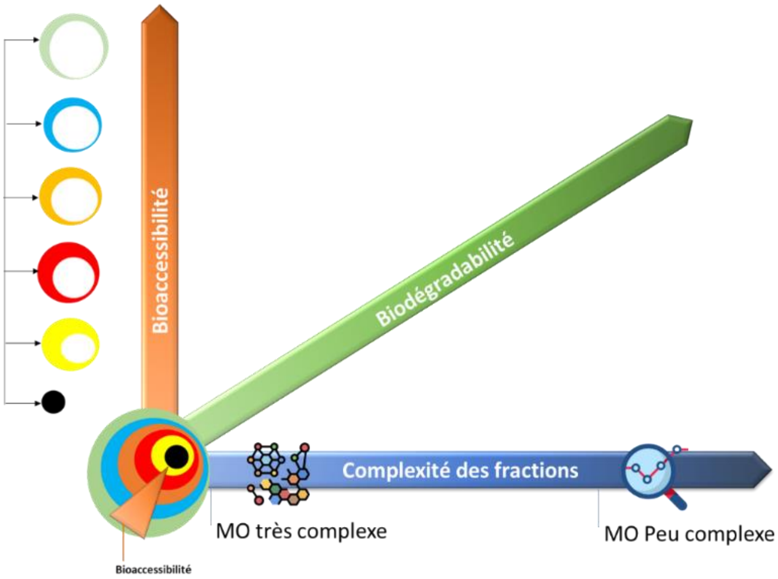

Figure 11 : Définition de la Biodégradabilité d'un intrant organique suivant deux axes : Bioaccessibilité (taille de compartiments) et Complexité de la MO des fractions (qualité)

<u>Stratégie proposée</u>

En suivant la méthodologie suggérée précédemment et en listant les services rendus à caractériser et les concepts associés, des méthodes de caractérisation pertinentes peuvent être proposées afin de créer des indicateurs. Par exemple la notion de biodégradabilité, que ce soit anaérobie dans un méthaniseur, aérobie dans un compost ou au sol, requière des tests biologiques expérimentaux trop coûteux en temps (tests d'incubation entre 30 et 300 jours) pour en faire des indicateurs de routine [JJ18]. Ces derniers doivent être pertinents, suffisamment descriptifs et rapides pour prédire la réponse biologique désirée. Enfin, une étape de validation des indicateurs est nécessaire vis-à-vis des réponses biologiques. Cette dernière peut être réalisée via des tests de biodégradation suivis dans l'application ciblée (par exemple au sein d'un méthaniseur, lors de tests de potentiel méthane, pendant le compostage ou encore dans le sol après épandage d'un produit).

La Figure 12 schématise cette stratégie appliquée à la conception optimale d'une filière de méthanisation pour la production concomitante d'énergie via le méthane, de MO utilisable à la fois pour la fertilité biologique et physique des sols et de nutriments. Pour chacun des services rendus, j'ai défini des notions comme la biodégradabilité de la MO, sa bioaccessibilité et complexité ou encore la disponibilité des nutriments pour le service rendu “fertilisant”.

Ensuite, j'ai proposé des méthodes de caractérisation en tant que proxys de ces notions. La contrainte principale est que dans un but de modélisation de filière, les indicateurs doivent pouvoir décrire le devenir de la matière à la fois dans les bioprocédés mais également dans le sol. Enfin, l'étape de validation expérimentale permet ou non de tester la qualité des indicateurs en tant que prédicteurs des références ou réponses biologiques. Si la validation est un échec, les indicateurs sont revus.

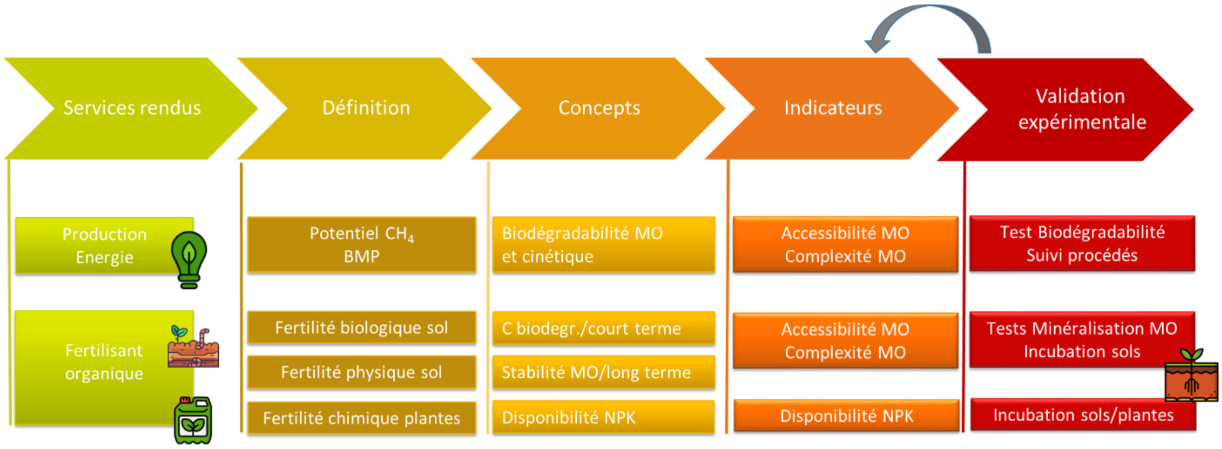

Figure 12 : Démarche suivi pour la définition des concepts associés aux services rendus et procédés à caractériser

Les paragraphes suivants reprennent la démarche proposée avec la description de mes travaux afin de définir et de développer des indicateurs de caractérisation pertinents, que ce soit pour la MO (2.2) ou pour décrire la disponibilité des nutriments (2.3). Enfin, une section est dédiée aux travaux pour la validation expérimentale de ces indicateurs (2.4).

2.2. Caractérisation de la matière organique et Indicateurs associés

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Afin d'associer des indicateurs analytiques à ces concepts, j'ai travaillé à l'élaboration d'un fractionnement de la matière afin de décrire sa Bioaccessibilité lors de ma thèse (collaboration VERI/INRAE LBE) concernant le service rendu « production d'énergie ».

L'hypothèse de la méthodologie développée repose sur la simulation de la bioaccessibilité par l'accessibilité chimique de la matière. Pour ce faire, un protocole de fractionnement a été mis en place basé sur des extractions chimiques séquentielles. Le résidu organique ciblé étant les boues issues de station d'épuration (STEP), le protocole se base sur des extractions réalisées sur les boues afin d'en extraire des substances exo-polymériques (protéines, sucres, lipides et des substances humiques-like, provenant de la MO non biodégradable et/ou de la MO sécrétée par les microorganismes afin de conférer aux boues biologiques une structure de floc microbien, [JJ27]). La boue est d'abord centrifugée et filtré afin d'obtenir une première fraction appelée Dissolved Organic Matter (DOM). Sur la fraction particulaire, quatre extractions successives à l'eau mélangée à une solution de NaCl+$NaHCO_3$ à 4mM (afin de maintenir une force ionique adaptée et prévenir la rupture des parois cellulaires) sont réalisées (15 minutes, 200 rpm, 30°C), inspirées des travaux de (Esparza-Soto and Westerhoff, 2001) sur les extractions de substances exopolymériques (EPS). Sur le culot restant, quatre extractions successives d'un mélange de NaOH et NaCl à 10mM sont réalisées afin d'extraire les EPS faiblement liés aux flocs (Esparza-Soto and Westerhoff 2001) et enfin quatre extractions à la soude à 100mM visent à solubiliser et récupérer les substances humique-like après un prétraitement acide (HCl, 0.1M, 1h, 30°C) visant à précipiter les acides humiques comme préconisé dans les travaux de (Swift, 2018) et (Giovanela et al., 2004) sur les sols et sédiments. Après chaque extraction, les surnageants sont récupérés et représentent chacun une fraction de la matière. La fraction non extractible restante de la matière est supposée contenir la MO non accessible telle que la lignine insoluble à la soude ou les humines.

Une fois ce protocole testé et optimisé, j'ai appliqué ce dernier sur 52 boues d'origines variées et des expérimentations en réacteur pilote ont permis de mettre en évidence que l'accessibilité chimique des boues mimait leur bioaccessibilité [These_JJ], [JJ27].

Concernant la qualité de la MO contenue dans chaque compartiment, la seule connaissance des molécules biochimiques présentes (protéines, sucres, lipides totaux) ne permet pas de décrire leur complexité [These_JJ] et le fractionnement pour simuler la bioaccessibilité n'est pas suffisant pour prédire la biodégradabilité d'un échantillon de boues ou d'autres résidus comme montré dans [JJ27] et [JJ18]. Certaines de ces molécules sont en effet plus ou moins complexes et donc plus ou moins biodégradables.

Afin de caractériser la nature des fractions, j'ai choisi de travailler avec la spectrofluorimétrie en 3 dimensions (3D). En effet, mon objet d'étude étant les boues issues de station d'épuration, en particulier les boues biologiques, ces dernières sont composées en majorité de protéines. La fluorescence permet d'analyser qualitativement ces dernières ainsi que les produits microbiens et de mettre en lumière la complexité de la MO telle que les substances humiques ou encore les produits de la réaction de Maillard (i.e. glycoprotéines récalitrantes produites à haute température). En collaboration avec le LBE, une méthodologie d'analyse de la fluorescence a pu être développée et des indicateurs de complexité associés également [JJ26]. Un spectre de fluorescence en 3D permet de balayer plusieurs longueurs d'onde d'excitation et donc d'avoir une cartographie de la MO. Cette méthodologie a été associée au fractionnement précédemment décrit en réalisant un spectre de fluorescence sur chaque fraction extraite. La Figure 13 montre sur un exemple de caractérisation de boues que la signature de fluorescence est différente par fraction chimique et que la complexité évolue avec l'accessibilité chimique de ce résidu organique. Il est à noter que toutes les molécules organiques ne sont pas fluorescentes naturellement (telles que les acides gras ou encore les sucres).

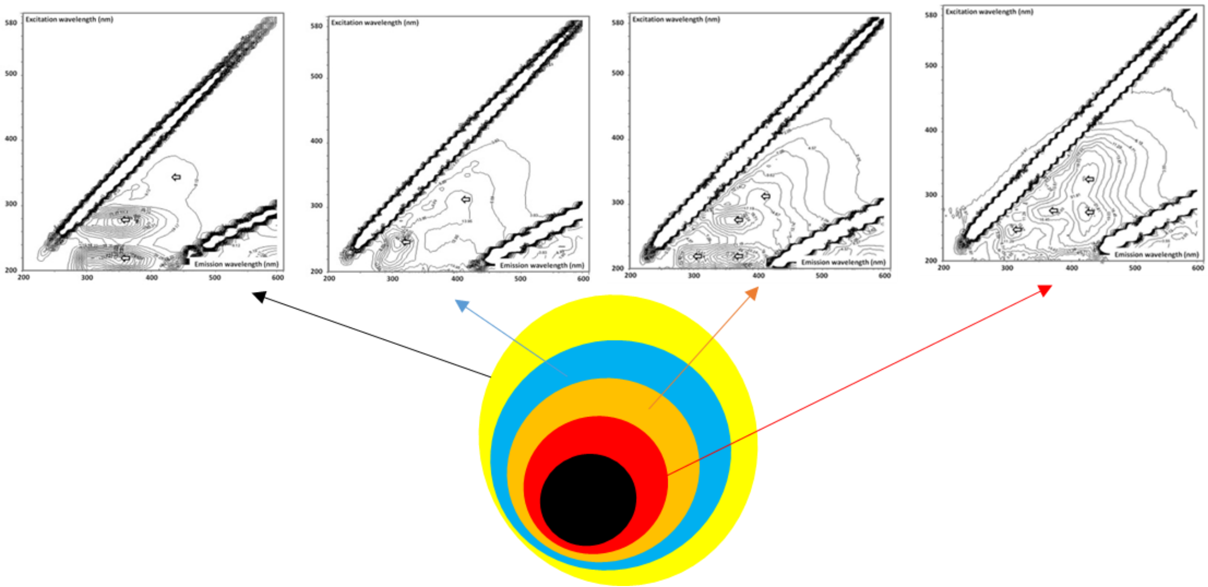

Figure 13 : Spectres de fluorescence 3D obtenus lors du fractionnement d'une boue [These_JJ]

Comme explicité ci-après, la combinaison des deux techniques d'analyses, à savoir les extractions chimiques séquentielles combinées à la fluorescence 3D a permis de prédire via des modèles statistiques la biodégradabilité anaérobie des boues et leurs fractions facilement hydrolysables [JJ27].

Concernant le traitement des spectres de fluorescence 3D, nous nous sommes basés sur les travaux de (Chen et al., 2003), et avons opté pour la régionalisation des zones de fluorescence. A partir de données issues de la littérature et de tests internes avec des molécules modèles, nous avons proposé une nouvelle régionalisation du spectre basée sur 7 zones [JJ26] comme décrite par la (Figure 14) du Fait Marquant 1. A partir du calcul de volumes de chaque zone (polygone) et de la zone des volumes de fluorescence, un pourcentage de fluorescence d'une zone est obtenu. Ce sont ces pourcentages, pour chaque zone et chaque fraction extraite que nous utilisons ensuite dans les modèles de prédiction.

N.B : d'autres techniques analytiques spectrales ont été testées sur l'analyse des extraits mais finalement mises de côté telles que la spectroscopie proche infra-rouge possible à réaliser sur des échantillons non humides et broyés. Cette technique s'est révélée très prometteuse afin de prédire la biodégradabilité des résidus organiques non fractionnés, que ce soit pour le potentiel méthane (Lesteur et al., 2011*; [JJ19]) ou la minéralisation du carbone organique au sol* (Peltre et al., 2011) *sur des échantillons totaux. Toutefois, l'associer au fractionnement signifie réaliser le spectre sur les résidus solides de chaque extraction et non sur chaque extrait (liquide) et les premiers tests réalisés n'ont été que peu concluants.*

Lors de mes premières activités à INRAE, un des premiers challenges à relever a été d'élargir le panel de résidus organiques (urbains et agricoles, au-delà des boues) ainsi que d'élargir les procédés et services rendus associés, à savoir considérer la filière méthanisation dans son ensemble (valorisation énergétique et agronomique) via les bioprocédés de méthanisation et compostage et la considération du retour au sol des Produits Résiduaires Organiques (PRO) associés. Ceci implique d'étudier et de définir les besoins des agroécosystèmes en termes de MO puis de nutriments. Toutefois, fait intéressant, les concepts définis précédemment et qui ont permis le développement d'un nouvel indicateur, rejoignent ceux de la description des engrais organiques. En effet, d'un point de vue agronomique, l'accessibilité et la stabilité de la MO sont cruciales pour mieux gérer la fertilité des sols, sommant la fertilité biologique (via une MO labile qui active la biologie du sol) et la fertilité physique (via une valeur d'amendement élevée et un stockage du C organique permettant l'amélioration de la structure du sol). Ainsi, les indicateurs associés aux nouveaux services rendus élargis, reprennent les méthodologies développées pendant ma thèse, notamment le fractionnement pour mimer la bioaccessibilité et la fluorescence pour évaluer la complexité de la MO.

Afin d'élargir la méthodologie au panel de résidus organiques variables, j'ai dû adapter la méthodologie et la tester puis la valider afin de pouvoir prédire à la fois le potentiel méthane de ces résidus (en plus des boues) et prédire le devenir de la MO de ces résidus dans d'autres bioprocédés que la méthanisation ainsi que leur devenir après épandage au sol.
Pour cela, je me suis inspirée d'une autre technique analytique de fractionnement de la matière appliquée à la caractérisation des fibres pour prédire la digestibilité des fourrages (Van Soest, 1963). Cet indicateur a déjà été utilisé pour prédire la biodégradabilité anaérobie de certains résidus lignocellulosiques (Bayard et al., 2018; Gunaseelan, 2007; Liu et al., 2015; Triolo et al., 2011) et la minéralisation du carbone au sol de résidus organiques riches en fibres (résidus agricoles, composts) via un indice de stabilité de la MO (ISMO) décrit par (Lashermes et al., 2009). Toutefois, comme montré par (Mottet et al., 2010 ; [JJ25] ; [JJ15]) ce protocole n'est pas suffisamment fin pour décrire les fractions facilement disponibles puis accessibles, que ce soit pour le carbone ou l'azote car les extractants chimiques sont trop agressifs.

Ainsi, en combinant le fractionnement appliqué aux boues, ciblant l'extraction de matière facilement bioaccessibles riches en sucres, protéines puis en substances humiques-like, avec le fractionnement Van Soest ciblant les fibres, nous avons ajouté l'extraction à l'acide sulfurique 72% afin de considérer les hemicelluloses et celluloses (holocelluloses) biodégradables afin de les différencier de la lignine restant dans la fraction non extractible, peu biodégradable en anaérobie et agissant comme une barrière protectrice limitant l'accessibilité de la matière (Bayard et al., 2018; [JJ10]).

Il est à noté que depuis 2019, le fractionnement a été automatisé afin de réduire le temps de manpulation, le nombre d'étapes et les erreurs de manipulation via l'utilisation de l'ASE 350 (Accelerated Solvent Extractor, Thermofisher). Le développement et le nouveau protocole sont en cours de valorisation. Une déclaration d'invention est un transfert au catalogue analytique de INRAE Transfert Environnement ont été réalisés comme en témoigne l'encadré suivant dédié à ce fait marquant (Fait Marquant 1).

Fait Marquant 1 : Développement de la marque IsBaMO®, déclaration d'invention et transfert au catalogue analytique INRAE Transfert Environnement

Un nouveau développement [JJ24] et l'établissement de base de données via divers projets dans lesquels j'ai participé (cf. Chapitre 1) ont donné lieu à la naissance à IsBaMO® (marque déposée), un indicateur de stabilité et de bioaccessibilité de la MO comme illustré dans la Figure 14. Une déclaration d'invention [DIRV_1] a ensuite permis de transférer la méthode dans le catalogue d'IT-E (INRAE Transfert Environnement, Business Unit de INRAE adossée au LBE) afin de le proposer en prestation analytique.

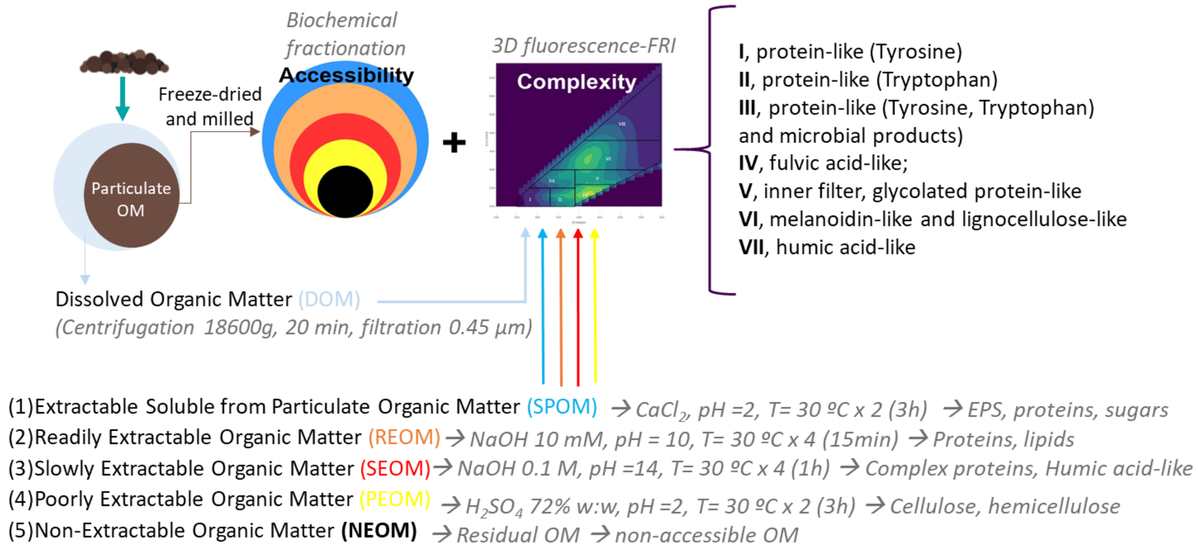

Figure 14 : Protocole schématisé de l'indicateur IsBaMO®

Les résultats proposés dans le cadre de la prestation sont : (i) l'estimation concomittante du potentiel méthane BMP (NmlCH4/gMV) ainsi que le potentiel Carbone minéralisable au sol de l'échantillon via les modèles de régression proposés dans [JJ18] et le positionnement de l'échantillon dans un référentiel issu de la base de données d'échantillons du LBE, suivant les axes complexité (somme pondérée calculée à partir de la taille en DCO de chaque fraction et de son indice de complexité) et accessibilité (somme des fractions extraites en pourcentage de DCO) comme le montre un exemple extrait d'une analyse réalisée sur la Figure 15.

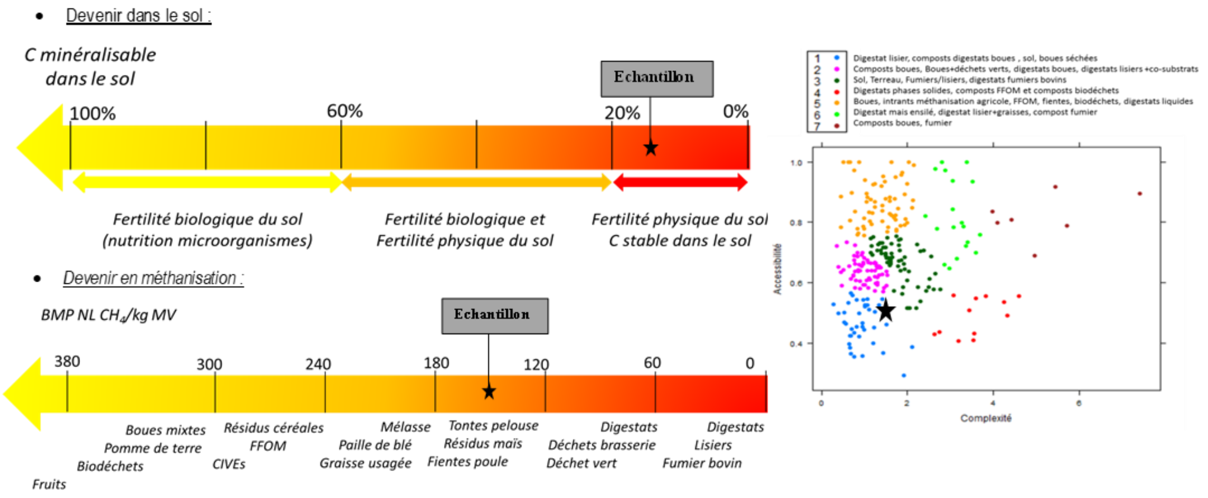

Figure 15 : Résultats proposés suite à l'analyse IsBaMO®

2.3. Devenir des nutriments au sein des procédés de valorisation

Un autre défi relevé a été la caractérisation des nutriments afin de décrire l'accessibilité et la disponibilité de ces derniers pour les plantes. En effet, un des services rendus à définir est celui de la production de fertilisant organique riche en nutriments (NPKS) pouvant substituer totalement ou en partie les engrais chimiques dont la production a un impact environnemental négatif et dont certains voient leur gisement s'appauvrir, comme le phosphore. Les deux macroéléments nutritifs majeurs et essentiels pour la croissance des plantes auxquels je me suis intéressée sont l'azote et le phosphore.
Nécessaire à la croissance des pousses et des feuilles, l'azote est un constituant fondamental des protéines et de la chlorophylle (Unifa). Cet azote est assimilable sous forme minérale (azote ammoniacal ou nitrate). Comme précédemment évoqué, les PROs tels que les digestats sont riches en azote minéral puisque la méthanisation implique la minéralisation de l'azote organique. Toutefois, des enjeux autour du devenir de l'azote existent au sein du procédé et en tant qu'impact environnemental. L'azote organique est minéralisé lors de la méthanisation sous forme d'azote ammoniacal assimilable par les plantes. Or, cette forme d'azote inhibe également la méthanogénèse. C'est pourquoi une meilleure connaissance de la minéralisation de l'azote organique apporterait une gestion meilleure du procédé quant à la production de méthane et de fertilisants. Concernant l'azote minéral apporté dans le sol, une mauvaise gestion du ratio stoechiométrique C/N pourrait mener à une immobilisation de l'azote par les microorganismes du sol, phénomène connu comme « faim d'azote » (Lashermes et al., 2010). De plus, en termes d'impacts environnementaux, des risques de volatilisation de l'azote ammoniacal pendant le stockage des digestats et pendant leur épandage dans certaines conditions (pH, climatiques, type de sol, pratiques agricoles) mènent à une perte azotée et à une pollution participant indirectement aux émissions de GES (Reibel and Leclerc, 2018). De plus, une meilleure connaissance du devenir de l'azote après production par le procédé peut également prévenir d'autres sources d'émissions comme le protoxyde d'azote, puissant GES (équivalent à 264 tonnes de CO_2 produit sur 100 ans) produit lors de la nitrification de l'azote ammonical en autotrophie et lors de la dénitrification ou encore la lixiviation des nitrates produits lors de la nitrification dans le cas d'une mauvaise gestion de l'épandage menant à une eutrophisation des eaux.

Le phosphore quant à lui stimule la floraison et la fructification ainsi que la croissance racinaire, il améliore la résistance au gel et est au cœur du métabolisme énergétique de la plante. Son principal rôle à l'intérieur de la plante est lié au stockage et transfert d'énergie (ATP), à la formation de composés structurels (nucléotides, acides nucléiques, coenzymes, phospholipides, etc…) intervenant dans la croissance des racines, la formation des grains, fruits et fibres, la maturation des fruits et le pouvoir germinatif des semences (Unifa). Une carence en phosphore inhibe la croissance des plantes. Or cet élément voit ses réserves mondiales s'appauvrir ce qui aura un impact important sur la sécurité alimentaire. Ainsi, l'enjeu autour du recyclage du phosphore dans un objectif de bouclage de cycle est de premier plan. Tout comme le nitrate, le phosphore minéral sous forme de phosphate peut être source d'eutrophisation des eaux via la lixiviation si une mauvaise gestion de l'épandage (doses et climat) est réalisée. Concernant le devenir du phosphore dans les procédés, ce dernier est conservé. Lors de la méthanisation, selon les conditions physico-chimiques, une partie peut être précipitée [JJ09] et, selon la composition du digestat, le phosphore peut-être plus ou moins disponible selon s'il est lié avec des sels ou des métaux [JJ07]. En effet, il est en forte intéraction avec la phase solide et forme divers composés minéraux secondaires avec Ca, Fe, Mn, Al. Facilement adsorbable, sa disponibilité est également une problématique à comprendre. De plus, tout comme l'azote minéral, le phosphore peut être immobilisé par l'activité biologique des sols.

Concernant le potassium, ce dernier joue un rôle important dans l'équilibre hydrique des plantes, améliore leur stabilité ainsi que la qualité des fibres et augmente la résistance au froid des cultures. Une carence en potassium perturbe l'équilibre hydrique des végétaux et entraîne le flétrissement des feuilles. Les plantes sont également plus sensibles aux maladies. Toutefois, présent dans la phase soluble des digestats, sa disponibilité n'est pas limitante.
J'ai d'abord commencé à travailler sur l'azote en associant l'IsBaMO® à l'analyse de l'azote organique

et minéral. Concernant le devenir de l'azote en méthanisation, j'ai collaboré à un encadrement de thèse avec l'unité de recherche OPAALE (INRAE). En partageant mon expertise sur l'indicateur IsBaMO®, ce dernier a été utilisé avec succès pour décrire l'accessibilité et la disponibilité de l'azote dans la méthanisation comme publié dans [JJ15]. Dans cette étude, nous avons mis en évidence que l'azote organique contient des fractions de bioaccessibilité très élevée à très faible, comme pour le carbone. Deux groupes de substrats de méthanisation ont été déterminés sur la base des données de biodégradabilité de C et N, séparés par une valeur de leur ratio azote ammoniacal sur azote total de 30%. Ce ratio traduit le degré de minéralisation de l'azote organique. Dans le cas d'un ratio supérieur à 30% (fumiers, lisiers), les biodégradabilités du C et N sont très significativement différentes (i.e. la biodégradabilité de l'azote organique étant bien plus faible) alors que pour un ratio inférieur à 30% (résidus de récolte, déchets agro-industriels non stockés) les biodégradabilités sont similaires et plus élevées. Dans le premier cas, cela veut dire que l'on ne peut prédire correctement la biodégradabilité de l'azote organique à partir de la seule valeur de biodégradabilité du carbone organique. De plus, l'analyse de la bioaccessibilité réalisée via le fractionnement IsBaMO® montre que la bioaccessibilité du C est similaire entre les deux groupes alors que celle de l'azote est très différente, mettant en lumière qu'un degré de minéralisation élevé se traduit par une faible accessibilité de l'azote organique déjà minéralisé avant la méthanisation. La comparaison des fractionnements IsBaMO® et Van Soest (Van Soest, 1963) a été réalisée en parallèle et les résultats ont montré que l'utilisation du fractionnement IsBaMO® était plus adéquat pour décrire la bioaccessibilité de l'azote organique, les extractants Van Soest étant plus agressifs et moins spécifiques. Les fractions azotées issues de IsBaMO® ont ensuite été utilisées pour prédire la biodégradabilité anaérobie de l'azote organique, montrant l'adéquation d'un tel indicateur avec les descriptions des phénomènes observés [JJ15].

Pour aller plus loin, j'ai souhaité apprendre les techniques expérimentales de laboratoire propres aux sciences du sol et tester l'indicateur IsBaMO® pour décrire l'absorption de l'azote et du phosphore par les plantes. Pour cela, j'ai développé une collaboration avec le département d'agronomie de l'Université de Bologne en Italie en 2017 pour travailler sur ce sujet. J'ai ainsi proposé un projet (NutProPlant, cf. Chapitre 1) et ai obtenu deux bourses pour réaliser un séjour dans ce laboratoire, et acquérir également une première expérience internationale. J'ai donc partagé mes connaissances en caractérisation via l'indicateur IsBaMO® développé et l'ai appliqué à l'azote organique et minéral pour décrire l'accessibilité de l'azote contenu dans dix digestats de méthanisation provenant de diverses sources. Mon collègue italien a quant à lui partagé son expertise en termes d'expérimentations via des incubations sur sol (avec et sans plantes) ainsi que son expertise en chimie du phosphore dans le sol, notamment via le protocole d'extractions chimiques séquentielles (Dou et al., 2000; Grigatti et al., 2015) ainsi que des analyses en diffraction aux rayons X afin d'évaluer les phases cristallines des digestats et leur impact sur la disponibilité du phosphore.

Pour la première fois, l'utilisation et la comparaison de ces protocoles (Figure 16) à la fois pour décrire l'accessibilité de la MO, l'azote et le phosphore de dix digestats d'origines diverses ainsi que pour caractériser la disponibilité de ces nutriments pour les plantes ont été réalisées et publiées. Cette étude a mené aux conclusions suivantes [JJ07] :

- Les teneurs en azote et phosphore total ne sont pas suffisantes pour prédire la quantité de nutriments assimilables par la plante : un indicateur de disponibilité de ces nutriments est nécessaire pour parvenir à une gestion contrôlée de la fertilisation ;
- Le devenir de l'azote dans le sol dépend énormément du degré de minéralisation de l'azote organique réalisée pendant les processus de biodégradation de la MO. Ce degré de minéralisation est très fortement corrélé avec le ratio C/N qui représente le caractère fibreux d'un résidu. Plus ce ratio est élevé, plus le risque d'immobilisation de l'azote (faim d'azote) est élevé comme décrit par (Lashermes et al., 2010);
- IsBaMO® est par conséquent adapté à la description du devenir de l'azote organique dans le sol ;

- Le devenir du phosphore dans le sol dépend surtout des conditions physico-chimiques du sol et le phosphore organique est très minoritaire dans les digestats et, de manière générale, dans les résidus organiques ;
- Le phosphore peut être rendu non accessible si certains composés tels que le calcium ou les métaux (fer ou aluminium) sont présents, s'associant avec lui ;
- IsBaMO® et ses extractants ne sont pas adaptés à la description du devenir du phosphore dans le sol contrairement au protocole de Hedley modifié (Grigatti et al., 2015) ;
- La typologie des digestats (reliée à la provenance des intrants) a une influence sur la qualité de la spéciation de N et P et donc sur les résultats obtenus en incubation.

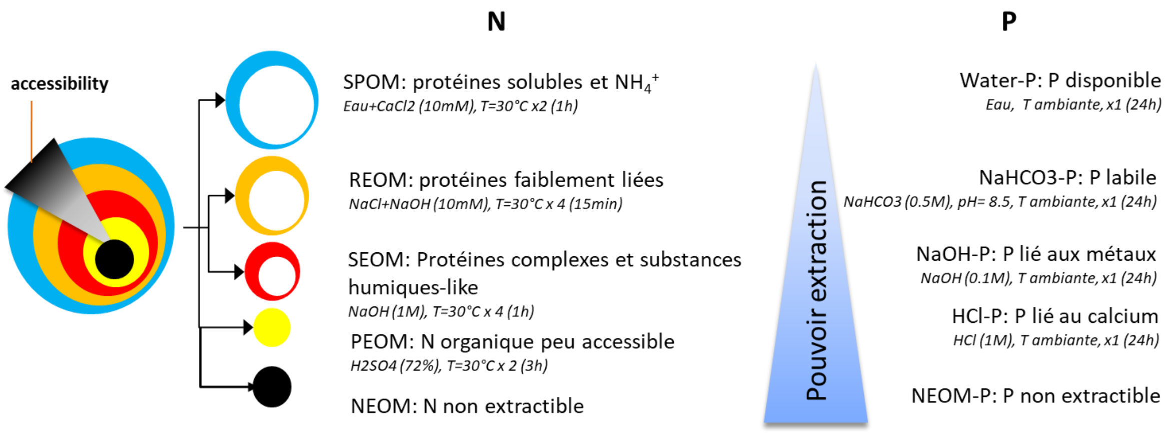

Figure 16 : Protocoles testés pour déterminer l'accessibilité et disponibilité de N et P basés sur [JJ25] pour N et (Grigatti et al., 2015) pour P

Bien que le devenir de la bioaccessibilité de l'azote ait été étudié au même titre que le C et la MO en général au sein des bioprocédés et plus récemment après épandage, le devenir du phosphore n'a été étudié que dans la partie « sol ». De plus, d'autres nutriments intéressants et participant aux antagonsimes/complémentarités entre méthanisation et épandage des produits résiduaires organiques comme le soufre n'ont pas été abordés et ne sont que peu étudiés de manière générale.

3.4. Validation expérimentale de la pertinence des indicateurs

Afin de valider expérimentalement la pertinence des indicateurs de caractérisation développés pour décrire les divers services rendus, plusieurs techniques ont été utilisées. L'axe 2 reviendra notamment sur la pertinence de leur utilisation pour décrire les processus biologiques internes aux bioprocédés. Dans cette section, je parlerai essentiellement de la validation réalisée via (i) des expérimentations dédiées à l'étude de l'hydrolyse de la MO à l'aide du fractionnement IsBaMO® et (ii) le potentiel prédictif de l'IsBaMO® vis-à-vis de réponses biologiques de références à l'aide de modèles statistiques développés. Un fait marquant (Fait Marquant 2) issu de l'étude du suivi des fractions IsBaMO® obtenues sur un fumier mis en incubation dans un sol clôturera ce paragraphe.

Validation expérimentale du concept de Bioaccessibilité et de l'indicateur IsBaMO®

Lors d'une collaboration avec la thèse de Cyrille Charnier (LBE), nous avons mis en place une expérimentation dont le but était d'en apprendre plus sur les mécanismes de l'hydrolyse afin d'améliorer sa modélisation (aspect détaillé dans l'axe 3) et notamment élucider une question récurrente sur le sujet qui n'a jamais trouvé de consensus : l'hydrolyse des fractions constituant la MO d'un résidu organique est-elle séquentielle ou simultanée ?

Pour cela, nous avons utilisé le fractionnement d'IsBaMO® et l'avons appliqué à divers substrats tels qu'une pomme, une paille de blé et une boue de STEP.

Nous avons mis chaque substrat total et dépourvu d'une à plusieurs fractions accessibles à incuber en conditions anaérobies. Les résultats ont montre que, dans le cas de la pomme, la cinétique et le potentiel méthane décroit au fur et à mesure que l'accessibilité décroit et que les fractions accessibles sont enlevées. Ce résultat est observé pour la paille et les boues sur les premières fractions enlevées. Toutefois, pour ces deux substrats, le résidu mis à incuber après l'attaque à la soude (après la fraction SEOM), voit sa cinétique augmenter et son potentiel méthane au même niveau que le potentiel méthane du total (Figure 17). Les hypothèses proposées pour expliquer cela sont que la soude solubilise les barrières « physiques » constituant la paille et les boues, à savoir respectivement une paroi ligneuse et la structure en floc bactérien basée sur des EPS libérant ainsi un matériel biodégradable rendu bioaccessible par l'extraction.
Ainsi, cette expérimentation met en évidence que selon la nature du substrat et sa structure, la biodégradabilité diminue avec la bioaccessibilité dans certains cas (pomme) mais que des fractions peu accessibles peuvent être biodégradables si cette accessibilité est atteinte comme dans le cas des boues et de la paille. Ces deux exemples sont assimilables à la métaphore de la pêche et de la noix de coco abordée en début de chapitre. Ainsi le concept de bioaccessibilité est crucial et permet de comprendre le fonctionnement de la biodégradation des résidus organiques et de le modéliser (axe 3). L'indicateur IsBaMO® a été très utile afin de mettre en évidence ce résultat.
Des questions restent toutefois en suspens concernant la prise en compte de l'impact des réactifs chimiques sur la structure moléculaire des fractions restantes après extraction. Toutefois, comme explicité par la suite, l'IsBaMO® reste avant tout un indicateur permettant de répresenter la matière. Ces résultats font partie d'une publication dédiée à la modélisation des mécanismes de l'hydrolyse détaillée dans l'axe 3.

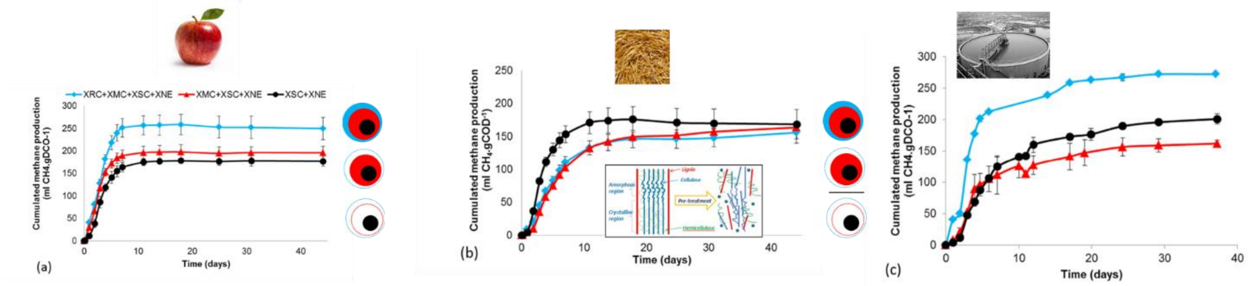

Figure 17 : Résultats obtenus sur l'étude des mécanismes de l'hydrolyse de divers substrats via l'utilisation du fractionnement IsBaMO [JJ10]

Pour aller plus loin et valider l'utilisation de l'IsBaMO®pour décrire le devenir de la MO tout au long d'une filière de valorisation de résidus, une collaboration avec INRAE SAS a été mise en place.
Les résultats issus d'une expérimentation dédiée au suivi de la biodégradation de la MO d'un fumier dans le sol en utilisant l'IsBaMO® est résumée dans le Fait Marquant 2.

<u>Prédiction des réponses biologiques vis-à-vis de l'IsBaMO®</u>
A partir des nombreuses caractérisations réalisées pendant ma thèse, puis mon post-doctorat et plus largement pendant mes sept premières années de recherche à INRAE, j'ai pu établir des bases de données contenant la description des résidus organiques associés souvent aux réponses biologiques que l'on cherche à décrire.

Ainsi, pendant ma thèse, j'ai caractérisé 52 boues de STEP (boues biologiques, primaires et digérées) par la méthode combinée du fractionnement de la bioaccessibilité et de la fluorescence 3D. En parallèle, j'ai effectué des tests de potentiel méthane (tests de biodégradabilité en conditions anaérobie) afin de déterminer le taux de MO biodégradable et en identifiant les courbes de vitesse de production de méthane, j'ai pu également évaluer les fractions rapidement et lentement biodégradables de la MO.

A partir de cette base de données, j'ai réalisé des tests statistiques (tests de régression Partial Least Square, PLS) afin de trouver des corrélations entre les diverses variables et de prédire à la fois la biodégradabilité totale et les fractions rapidement/lentement biodégradables. Des modèles PLS de bonne qualité ont été réalisés et publiés [JJ27], avec des erreurs de prédiction entre 6 et 9%. Le shéma de la Figure 18 résume les résultats obtenus en analysant le poids des variables sur les modèles venant compléter mon questionnement scientique de la Figure 11.

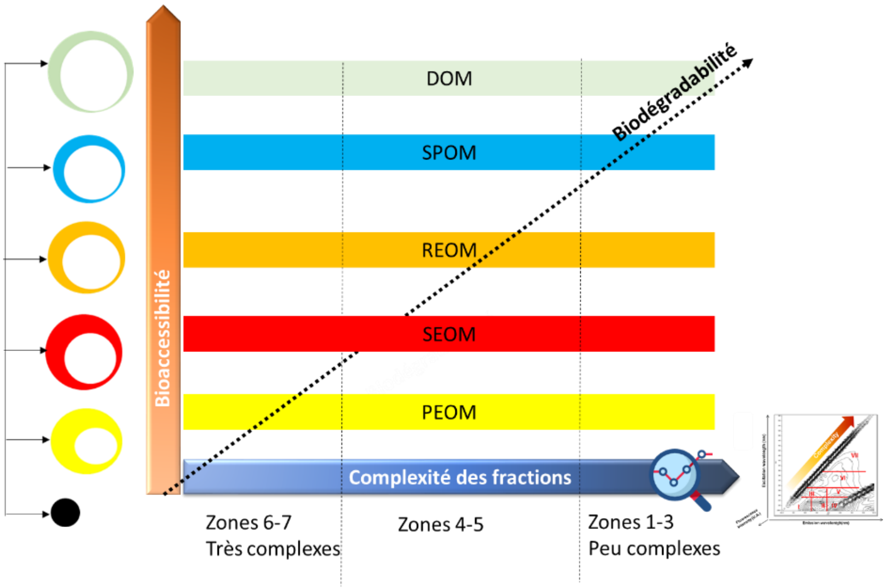

Figure 18 : Variables explicatives pour prédire la cinétique de biodégradabilité anaérobie des boues de STEP (adapté de [JJ27])

Par la suite, en modifiant le protocole et en développant l'indicateur IsBaMO®, une nouvelle base de données s'est constituée. De la même façon qu'auparavant, les réponses biologiques expérimentales associées ont été également obtenues au LBE ou via des collaborations à travers les divers projets abordant la thématique de caractérisation (cf. Chapitre 1).

Ainsi, dans un premier temps, une analyse statistique sur 60 données de caractérisation (bioaccessibilité et complexité) ont mis en évidence que l'IsBaMO® permettait de classer les résidus organiques selon leur nature [JJ25]. En effet, cinq groupes principaux ont été obtenus : (i) les boues et substrats très biodégradables (pommes de terre, résidus alimentaire,…) associés avec une très forte bioaccessibilité et des zones de fluorescence simples, (ii) les digestats de boues, riches en fractions bioaccessibles intermédiaires et des niveaux de fluorescence plus complexes que le premier groupe, (iii) les résidus lignocellulosiques (FFOM, déchets verts, fumiers, …) associés à des fortes fractions en SEOM et PEOM et des zones de fluorescence complexes (lignocellulose-like), (iv) les composts associés à des fractions très faiblement accessibles (PEOM et NEOM) et composés de zones de fluorescence complexes (lignocellulose-like) et (v) les sols et terreau associés avec la plus faible bioaccessibilité, une forte non extractabilité et des zones de fluorescence complexes dans certaines fractions (acides-humique-like). Ces résultats ont montré la pertinence de l'indicateur à représenter la diversité de résidus organiques et à les classer.

Dans un second temps, cette base de données élargie à un large panel de 82 résidus très variés a été utilisée pour prédire le potentiel méthane de ces derniers (gamme située entre 31 à 394 NmL $CH_4.MO^{-1}$), BMP déterminés selon (Angelidaki and Sanders, 2004) et 44 échantillons ont permis la prédiction du carbone organique minéralisable au sol selon la norme XPU 44-163 (gamme située entre 7 et 46% de Carbone organique) à l'aide de modèles PLS [JJ18].

Les modèles ont été testés sur les données avec et sans fluorescence et les résultats montrent que les deux modèles sont beaucoup plus robustes et de bonne qualité avec l'information de la complexité, preuve que seule l'information de la bioaccessibilité n'est pas suffisante (Figure 19).

Enfin, l’analyse de sensibilité des variables sur les modèles a permis d’évaluer statistiquement le poids de ces dernières sur la prédiction du potentiel méthane (BMP) et du carbone minéralisable au sol (C_bio). Certaines molécules contenues dans des fractions ont un effet négatif sur la prédiction du BMP alors qu’elles ont un effet positif sur la prédiction du C_bio. Ainsi, la fraction SEOM a un poids plutôt négatif sur le BMP (notamment les zones de fluorescence complexes 4 à 7) alors qu’elles ont un poids plutôt positif sur la prédiction du C_bio. Certaines molécules sont biodégradables à la fois dans les conditions du BMP et dans dans celles de C_bio comme les fractions très bioaccessibles SPOM et REOM (zones 1-3, protéines-like) et d’autres le sont significativement dans C_bio mais pas dans BMP, comme la fraction PEOM (holocelluloses). Concernant la fraction NEOM (non extractible), cette dernière a un impact significativement négatif sur le C_bio alors que cet impact n’est pas significatif pour le BMP. Ainsi, à partir d’une telle étude, on peut déjà voir apparaître des synergies et antagonismes entre utilisation de la MO pour produire du méthane et utilisation de la MO pour répondre aux besoins de fertilités biologique et physique des sols.

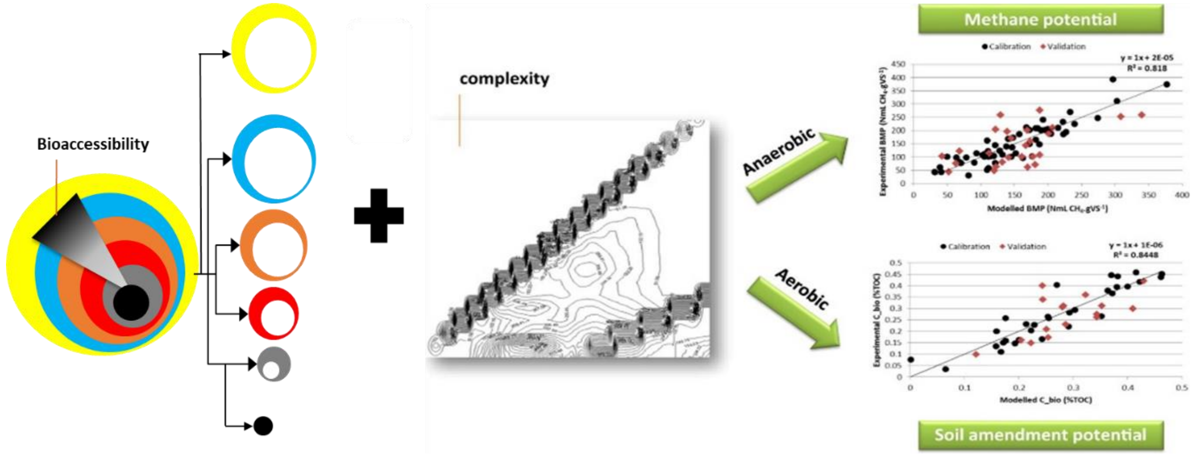

Figure 19 : Graphical abstract adapté sur la prédiction du potentiel méthane et du carbone organique minéralisable au sol à partir de l’IsBaMO® [JJ18]

Plus récemment, cette base de données amendée a été utilisée afin de prédire et comprendre la qualité de la MO des digestats pour l’évaluation de leur stabilité en carbone organique au sol. En effet, à l’aide d’analyses statistiques telles que l’analyse en composantes principales puis la PLS, nous avons mis en évidence dans [JJ04] que (i) la qualité des MO des substrats avait une empreinte statistiquement significative sur la qualité de la MO des digestats associés avec l’identification de quatre groupes de substrats/digestats de même nature, (ii) la prédiction de la qualité de la MO des digestats (bioaccessibilité et fluorescence) à partir de la qualité de la MO des substrats était possible et (iii) l’analyse de ce modèle associé avec le modèle PLS précédemment établi pour prédire la minéralisation du C au sol (C_bio) pouvait mettre en lumière certains leviers d’action pour contrôler la qualité de la MO (aspect approfondi dans la section sur l’axe 2).

Concernant l’azote organique, l’étude réalisée avec [JJ15] a montré que l’IsBaMO® était plus à même de représenter le degré de minéralisation de l’azote organique au sein d’un méthaniseur. En appliquant la même démarche que les validations précédentes, un modèle PLS a été réalisé sur une vingtaine de résidus dont l’azote sur les fractions IsBaMO® a été mesuré afin de prédire la biodégradabilité anaérobie de l’azote organique déterminée expérimentalement via des tests similaires aux tests de potentiel méthane. La qualité du modèle est satisfaisante et se voit augmenter en réalisant un modèle par groupe de degré de minéralisation évalué statistiquement comme évoqué précedemment, diminuant significativement les erreurs de prédiction.

Enfin, les variables ayant un poids significatif sur la prédiction de la biodégradabilité de l’azote organique sont notamment l’azote contenue dans la fraction la plus bioaccessible et dans la fraction qui cible les holocelluloses, moins accessible.

La fraction non extractible n’a aucun poids sur cette prédiction étant donné que cette fraction est censée contenir surtout des molécules comme la lignine.

Toutefois, les fractions extractibles de l'azote organique ne contribuent pas de la même manière que celles du carbone organique comme vu dans [JJ18].
Enfin, les expérimentations décrites précédemment concernant le potentiel prédictif d'IsBaMO® pour décrire la disponibilité de l'azote et du phosphore pour les plantes ont permis d'avoir des données de caractérisation sur 10 digestats d'origines différentes utilisées comme fertilisants pour faire pousser du ryegrass. Des données de quantités d'azote et de phosphore assimilées par les plantes (tissus aériens et racinaires) ont permis de réaliser des tests statistiques afin d'évaluer les corrélations entre caractérisation et réponses de la plante.

Concernant l'azote, une corrélation négative significative entre ratio C/N du digestat et prédiction de l'azote contenue dans les tissus aériens de la plante a été obtenue menant à l'établissement d'un modèle de régression linéaire. Ce ratio déjà très fortement corrélé négativement avec le pourcentage de minéralisation de l'azote dans le sol (sans plante) décrit la limitation de l'azote pour l'activité biologique du sol. En effet, si l'azote est limitant, les microorganismes immobilisent l'azote du sol qui devient indisponible pour la croissance des plantes (Cavalli et al., 2017; de la Fuente et al., 2013; Grigatti et al., 2011; Morvan et al., 2006; [JJ07]). De plus, l'azote soluble minéral est également très corrélé avec l'assimilation de l'azote par les racines, notamment les nitrates contenus dans la fraction soluble, résultat observé également par (Gunnarsson et al., 2010). Les autres fractions issues d'IsBaMO® ont moins d'impacts sur ces prédictions. Toutefois, ces dernières participent au classement des digestats établi selon leurs valeurs de C/N et les caractéristiques des fractions azotées de IsBAMO, notamment les fractions les plus bioaccessibles organiques et inorganiques.

Concernant le phosphore, l'utilisation des extractions chimiques séquentielles (Grigatti et al., 2015) permet de trouver une corrélation entre phosphore disponible chimiquement et phosphore assimilé par les plantes. En effet, le phosphore minéral extrait par de l'eau a un impact positif significatif sur la prédiction du phosphore retrouvé dans les tissus aériens alors que la fraction organique du phosphore extrait par $NaHCO_3$ et NaOH est significativement corrélée avec le phosphore mesuré dans les racines. En effet, le phytate et autres esters de phosphates (phosphore organique) sont extraits dans la fraction NaOH (He et al., 2010). D'après (Gerke, 2015; Lambers et al., 2006), les racines sont susceptibles d'exsuder des phosphatases capables d'hydrolyser le phytate et les formes organiques du phosphore afin de les absorber.

Ainsi, bien qu'utile au classement des digestats, l'ensemble du fractionnement IsBaMO® n'est pas nécessaire à la prédiction de la capacité d'absorbtion de l'azote par les plantes, seule la part de l'azote minéral contenu dans le soluble et le ratio C/N total suffisent. Quant au phosphore, l'IsBaMO® n'est pas pertinent contrairement au fractionnement de Grigatti et al. (2015). Ce dernier devrait être testé pour décrire le devenir du phosphore dans les procédés afin de pouvoir combiner procédés et qualité des produits retournés au sol.

Fait Marquant 2 : Utilisation de l'IsBaMO® pour décrire le devenir de la MO d'un fumier dans le sol, collaboration avec INRAE SAS

Nous avons vu que l'indicateur IsBaMO® était pertinent pour prédire à la fois la biodégradabilité anaérobie du carbone et de l'azote organique. Ce paragraphe est dédié à la validation expérimentale de l'utilisation de cet indicateur pour décrire les cinétiques de biodégradation du carbone et de l'azote dans le sol. Pour ce faire, une collaboration avec INRAE SAS a permis de suivre la biodégradation de la MO au cours d'une incubation sur sol d'un fumier bovin sur plus d'un an. Ce test avait la particularité innovante d'utiliser un litter bag en nylon (porosité de 200 µm) afin d'isoler la matière à dégrader du sol tout en s'assurant que la biodégradabilité du fumier n'était pas perturbée. Ce dernier point a été confirmé en comparant les courbes de cumul de minéralisation du carbone organique entre modalités avec et sans litter bag où il n'y avait pas de différence significative observée.

Des prélèvements de fumier réalisés à plusieurs temps pendant un an ont permis de réaliser des analyses de carbone, azote et de leur bioaccessibilité via IsBaMO®. La Figure 20 présente l'évolution des deux éléments durant le temps d'incubation ainsi que leur pourcentage total de biodégradabilité. Il apparaît que la biodégradabilité de la MO suit l'accessibilité chimique proposée par IsBaMO® étant donné que plus la MO est accessible, plus elle est biodégradée. Concernant la cinétique de biodégradation, il y a encore une fois un gradient de biodégradation cohérent avec IsBaMO® avec une diminution de la vitesse de biodégradation lorsque la matière devient moins extractible (vitesses de biodégradation : SPOM>REOM>SEOM>PEOM>NEOM).

Enfin, ces cinétiques et le degré de biodégradabilité sont toutefois différents entre le carbone organique et l'azote organique mettant en lumière, comme précédemment vu en méthanisation, qu'il faut considérer les deux éléments de manière indépendante.

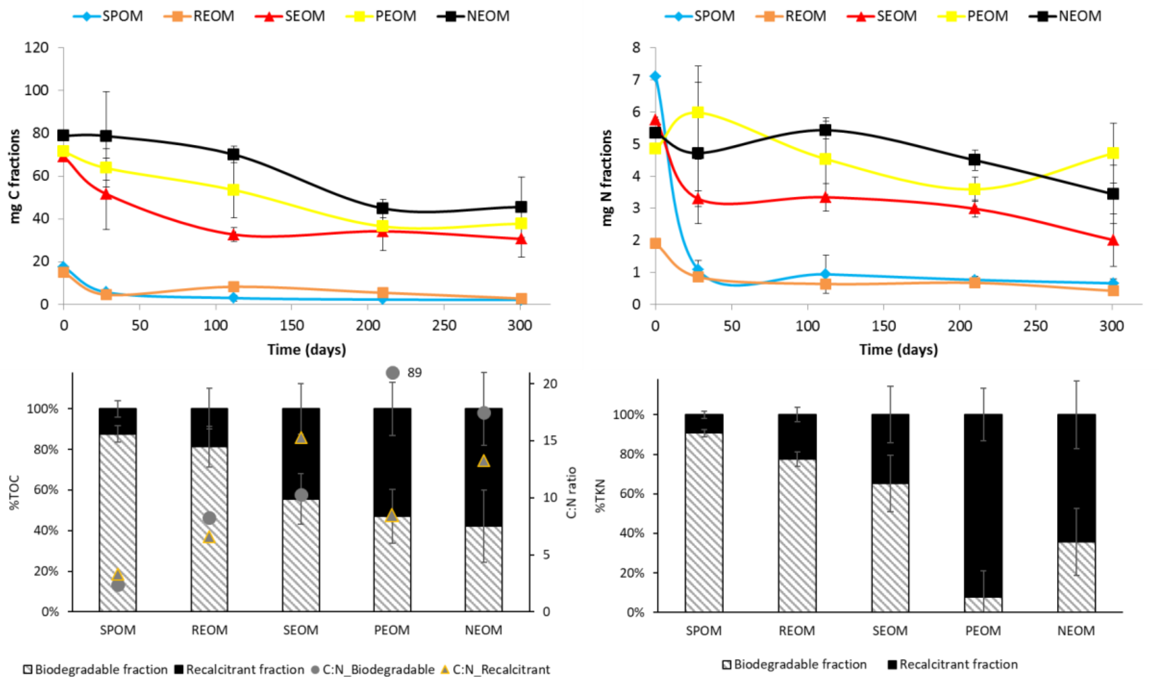

Figure 20 : Evolution du carbone et de l'azote organiques au cours de l'incubation sur sol d'un fumier bovin [JJ32]

2.4. Bilan

L’IsBaMO®, initialement conçu afin de décrire le devenir de la MO pendant la méthanisation des résidus organiques et étendu à son devenir dans le sol, a ensuite été utilisé et associé pour la description d’autres concepts liés à la MO, non développés ici :

- Evaluation des traitements (pré-traitements physico-chimiques) : [JJ16], [JJ06] et [JJ08]
- Devenir des micropolluants [JJ17], [JJ23]
- Survie des pathogènes dans le stockage des digestats [JJ20], [JJ21]

Concernant ces deux derniers volets liés à l’innocuité, j’ai surtout collaboré avec des chercheurs de mon unité (Dominique Patureau pour les micropolluants organiques et Nathalie Wery pour les pathogènes).

J’ai également participé à des études afin d’utiliser la spectroscopie proche infra-rouge pour prédire des variables diverses, que ce soit le potentiel méthane [JJ22] ou encore la qualité agronomique globale des digestats [JJ02]. L’intérêt de cette méthode est qu’elle permet d’analyser rapidement un résidu après lyophilisation et broyage de ce dernier. Toutefois, la complexité n’y est pas révélée et la bioacessibilité manque pour prédire les cinétiques.

Afin de dresser un bilan de mes activités de recherche menées sur l’axe 1 autour de la caractérisation de la matière pour décrire à la fois services rendus, processus biologiques des procédés et variables d’entrées aux modèles associés, leur état d’avancement est présenté dans le Tableau 1.

Jusqu’à présent, ma démarche scientifique a été de baser mon raisonnement à partir des objectifs ciblés pour définir les méthodes de caractérisation. Cela a permis de répondre à plusieurs problématiques liés à cet axe et d’ouvrir de larges perspectives d’utilisation des indicateurs proposés. Toutefois, un des incovénients de la démarche a été de cloisonner mes activités à des techniques analytiques mises en place (fractionnement IsBaMO® et fluorimétrie 3D) et de ne pas avoir une démarche plus prospective en investiguant d’autres méthodes de caractérisation de la matière susceptibles d’apporter un éclairage nouveau. C’est pourquoi, dans le cadre de la thèse de David Fernandez-Dominguez (2020-2023), une revue de la littérature explorant une gamme de techniques spectrales existantes pour mieux identifier la structure de la MO (y compris la spectroscopie infrarouge moyen, la résonance magnétique nucléaire) a été réalisée afin de mettre en lumière les techniques pertinentes et les autres indicateurs existant pour décrire les mécanismes de biodégradation des MO en digestion anaérobie [JJ01]. De plus, les aspects « structure physique » des résidus n’ont été que faiblement abordés via le fractionnement IsBaMO®. En effet, bien qu’utilisable pour décrire une accessibilité chimique (i.e « couches » de fractions plus ou moins accessibles), un des incovénients de cette méthode est l’impact de l’utilisation de réactifs chimiques sur la structure de la matière. Est-elle dénaturée lorsqu’elle est analysée dans les extraits par fluorescence ? Quelles conséquences sur la matière restante ?

La MO extraite étant très complexe, l’identification des molécules est également complexe. Or, si l’on veut aller plus loin dans la connaissance des processus biologiques impliqués dans la biodégradation de la matière, cette identification doit également être élucidée et le choix des méthodes analytiques doit être étendu.

En outre, bien que les indicateurs de bioaccessibilité et de complexité de la matière donnent des éléments de réponses concernant le devenir à court/moyen terme du C (C minéralisable au sol), il manque à définir un « cahier des charges » sur les propriétés spécifiques des besoins des agroécosystèmes issus de ces indicateurs pour réaliser une adéquation optimale entre qualités des PRO et les dits besoins. En effet, quelles propriétés biochimiques seraient souhaitables afin de mieux promouvoir la biodiversité ? La stabilité structurale du sol ? Quelle échelle de temps de biodégradation ? Est-ce que la méthanisation seule ou combinée avec d’autres procédés peut répondre à ces besoins ? Si oui, comment ? De plus, une des perspectives directes de ces travaux est une analyse fine des antagonismes et synergies entre services rendus en étudiant les données générées issues des indicateurs proposées dans cet axe : quel type de MO profite le plus au potentiel méthanogène ? Au carbone minéralisable ? Au carbone stockable ?

Enfin, sur la base de ces développements de caractérisation et d'indicateurs, j'ai pu établir de nouvelles bases de données sur les caractéristiques des résidus organiques et de leurs produits incluant des métadonnées sur les conditions de procédés afin d'évaluer et de modéliser les impacts du traitement sur la qualité des produits pour la rétro-ingénierie.

Tableau 1 : Bilan des travaux sur la caractérisation

Services rendus	Production énergie	Amendement organique	Activateur biologique	Fertilisant	Etat d'avancement/ connaissance
Concept associé	Bioaccessibilité et Biodégradabilité anaérobie	Bioaccessibilité et Stabilité du PRO	Bioccessibilité et Cinétiques biodégradabilité MO sur sol	Accessibilité et disponibilité des nutriments pour les agroécosystèmes	
Carbone	Fractionnement MO : [JJ27], [JJ18], [JJ10] IsBaMO®	Fractionnement MO : [JJ18], [JJ24], IsBaMO®	Fractionnement MO : [JJ24], IsBaMO®	-	
Azote	Fractionnement MO et N : collaboration avec INRAE OPAALE [JJ15]			Fractionnement N : [JJ07], collaboration avec INRAE SAS Rennes [JJ32]	
Phosphore				Fractionnement P : [JJ07]	
K, S					
Identification besoins des agrosystèmes à partir des indicateurs développés	Analyse de sensibilité poussée à mettre en place sur la base des indicateurs et résultats obtenus.				

3. Axe 2 : Comment les procédés de valorisation des résidus impactent la qualité des produits résiduaires organiques ?

Le second axe de recherche que j'ai proposé concerne l'évaluation des leviers d'action qui impactent le fonctionnement des procédés de valorisation des résidus organiques et donc la qualité finale des PRO. Cet axe est donc basé sur une amélioration de la compréhension des processus biologiques des bioprocédés de traitement et également sur une meilleure connaissance des impacts de post-traitements biologiques ou physico-chimiques impliqués dans une filière de valorisation. Mon expertise en génie des procédés, et plus particulièrement en génie des procédés de l'environnement, a débuté lors de mes études d'ingénieur pour se poursuivre pendant ma thèse et lors de mes expériences professionnelles. J'ai notamment aquis de l'expérience sur les procédés impliqués dans le traitement des eaux usées (urbaines et industrielles) ainsi qu'en méthanisation des boues et des FFOM. En arrivant à INRAE, il a fallu relever le défi d'étendre à la fois le spectre des résidus organiques (urbains, agricoles et agro-idustriels) et le spectre des procédés. Dans le cadre d'une filière de méthanisation, cette gamme peut aller de procédés classiques (compostage, séparation de phases, stockage) à des procédés plus innovants existants ou à développer.

3.1. Objectifs et questions de recherche

Si l'on revient à la définition d'ingénierie inverse ou rétro-ingénierie (*« Pratique qui consiste à analyser un produit fini pour connaître la manière dont celui-ci a été conçu ou fabriqué »),* en plus de connaître la composition du produit fini, s'ajoute la notion de connaissance des processus de fabrication. C'est pourquoi, dans ma thématique de recherche, l'ingénierie inverse repose sur un second pilier qui est le génie des procédés.

En reprenant le schéma déjà présenté dans l'axe 1 et en l'adaptant à l'axe 2 (Figure 21), on retrouve les questions de recherche associées au génie des procédés dans le raisonnement de ma thématique de recherche. Afin d'optimiser et concevoir une filière de valorisation, il faut identifier les leviers d'actions sur lesquels jouer afin d'établir des scénarios optimaux.

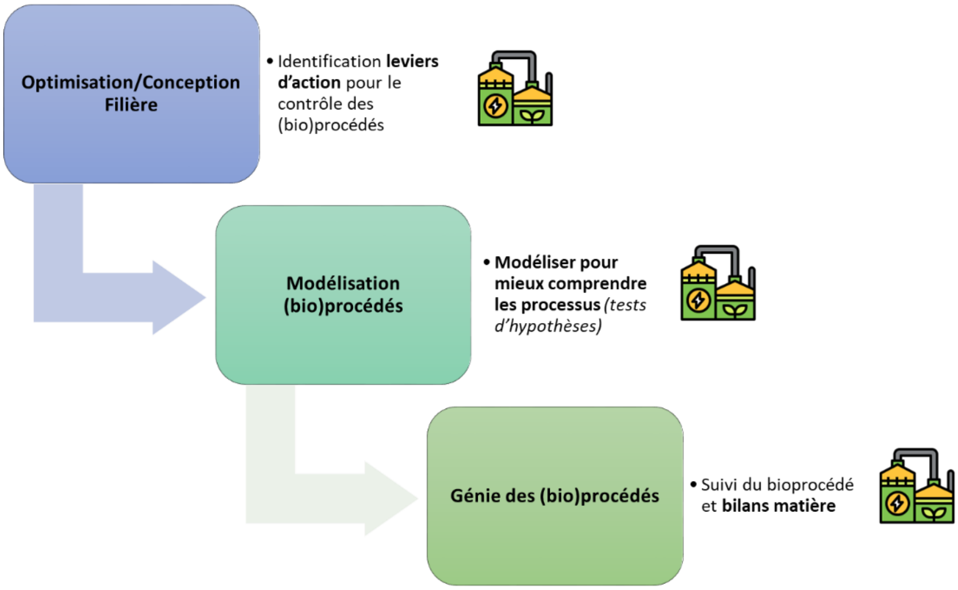

Figure 21 : Questions de recherche associées au Génie Des Procédés au sein de la démarche scientifique de ma thématique de recherche

Dans le cas de la filière de valorisation basée sur la méthanisation, « le challenge du digestat » est au centre de son optimisation. En effet, comme illustré par la Figure 22, la variabilité des intrants croisée avec la variabilité des conditions opératoires et la variabilité des post-traitements entraîne une variabilité de la qualité agronomique des digestats produits.

Dans ma thématique de recherche, je considère ces diverses formes de variabilités comme une opportunité afin de pouvoir contrôler la qualité du produit sortant et ne plus la subir. Toutefois, afin de réaliser cette optimisation, j'ai cherché à répondre aux questions suivantes :

- Est-ce que les différentes formes de variabilité décrites précédemment ont un impact significatif sur la variabilité de la qualité du produit fini ?
- Est-ce que ces impacts sont classifiables ?
- Existe-t'il une hiérarchie en termes d'impacts ?
- Quels sont les mécanismes associés à ces impacts ?
- Est-ce que ces impacts ont un effet sur l'efficacité agronomique des produits ?

Ce paragraphe met en lumière mes recherches effectuées afin de répondre à ces questions.

Figure 22 : Enjeu lié à la variabilité de la qualité des digestats issus de méthanisation

3.2. Evolution matière dans les procédés : variabilité des substrats

A l'aide des outils développés dans l'axe 1, l'étude des procédés tels que la méthanisation, le compostage et d'autres procédés physico-chimiques a été réalisé. Des résultats, que ce soit lors de ma thèse sur les boues d'origine urbaine, lors de mon post-doctorat avec le suivi du continuum méthanisation-compostage ou encore pendant des projets de recherche ont permis de mieux comprendre le devenir de la MO et de proposer des hypothèses quant à son devenir.

Concernant la méthanisation, des suivis de la MO via le fractionnement préliminaire établi lors de ma thèse ont permis de montrer que les boues de STEP étaient biodégradées via plusieurs cinétiques, correspondant à des fractions plus ou moins biodégradables. En effet, un bilan matière effectué sur un réacteur batch montre que la fraction facilement biodégradable est surtout associée aux fractions les plus accessibles, à savoir DOM, SPOM et REOM et que les fractions les moins accessibles participent plutôt à la fraction lentement biodégradable. Cette hypothèse sera confirmée par la suite via une expérimentation dédiée en réacteur continu où la fraction facilement biodégradable des boues de STEP évaluée par identification des cinétiques de production de méthane est associée aux fractions facilement bioaccessibles DOM+SPOM+REOM [JJ27].

Pendant mon post-doctorat puis via les projets de recherches auxquels j'ai pu participé, j'ai récolté des données entrées/sorties de divers méthaniseurs.

Des exemples de bilans matière effectués sur deux types d'intrants contrastés que sont les boues de STEP (riches en protéines) et les fumiers bovins (riches en matière lignocellulosique) sont présentés dans la Figure 23. Comme pendant ma thèse, il apparaît que, globalement, les fractions les plus accessibles sont biodégradées (DOM, SPOM et REOM). Toutefois, les fractions moins accessibles comme SEOM et PEOM ciblant respectivement protéines/substances-humiques/lignocellulose solubles à la soude et holocelluloses n'ont pas la même évolution selon la nature du substrat. En effet, les boues (biologiques) sont plutôt riches en protéines plus ou moins complexes (SEOM) et pauvres en holocelluloses (PEOM) [These_JJ].

En revanche, le fumier bovin voit sa fraction PEOM très riche en holocellulose (fibres biodégradables provenant de la paille composant le fumier) fortement biodégradée alors que la fraction SEOM n'est pas biodégradée.

Ainsi, cette première analyse permet de mettre en évidence que le fractionnement permet de mimer la bioaccessibilité des substrats mais que selon la nature du substrat, les fractions extraites seront plus ou moins biodégradables, d'où l'importance de connaître la qualité de la MO des fractions.
De plus, le temps de séjour de chaque digesteur étudié (25 jours pour les boues et 120 jours pour le fumier) a également un impact sur le degré de biodégradabilité des intrants. En effet, la fraction PEOM, peu accessible, le devient si suffisamment de temps est laissé aux microorganismes afin d'atteindre cette fraction. Ces observations viennent corroborer les expérimentations décrites dans l'axe 1 sur la validation de la bioaccessibilité publiées dans [JJ10].

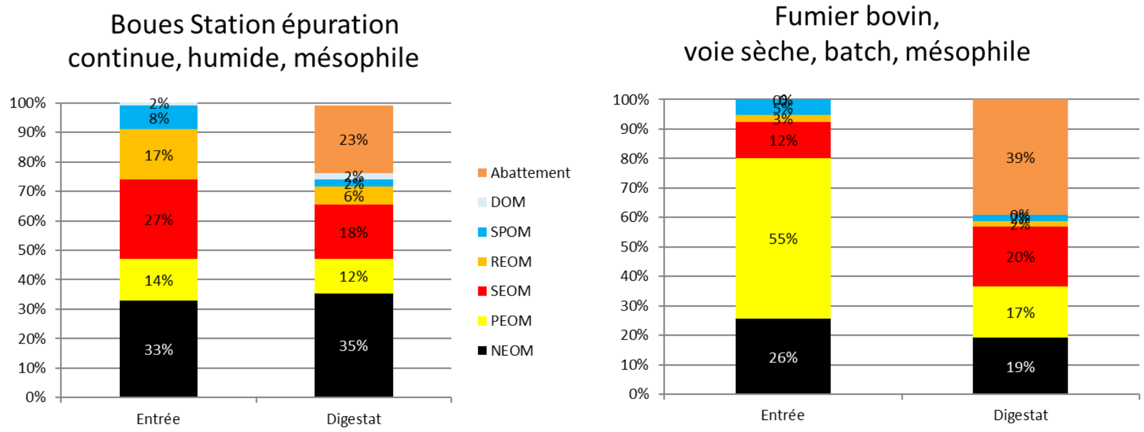

Figure 23 : Bilan matière appliquée au fractionnement IsBaMO aux bornes de deux digesteurs traitant des boues (gauche) et du fumier pailleux (droite)

Ces deux exemples sont assimilables encore une fois à la métaphore de la pêche et noix de coco abordée en début de ce chapitre. Etant donné que la qualité de MO obtenue dans le digestat semble être dépendante de la qualité de l'intrant, on peut supposer que ce dernier peut constituer un levier d'action significatif. Divers travaux ont contribué à illustrer et confirmer cette hypothèse.

Dans le cadre de la thèse de Felipe Guilayn (2016-2019) sous ma co-supervision puis dans le cadre du projet Concept-Dig (2019, ADEME) que j'ai coordonné, une base de données de la qualité agronomique globale[3] des digestats de méthanisation provenant de diverses sources a été établie. Pour la première fois, à l'aide d'outils statistiques (analyses en composantes principales ACP, et classification hiérarchique ascendante, HCA), l'impact des intrants sur la qualité agronomique des digestats a été montré via l'établissement de typologies sur les digestats urbains et agricoles [JJ12] et sur les digestats uniquement d'origine agricole (Concept-Dig, ADEME ; [PO7], [PO10]). En effet, la première étude est basée sur un recensement de plus de 150 digestats issus de données internes du LBE, de SUEZ et de la littérature. Les clusters obtenus sont illustrés dans la Figure 24. L'ACP montre que la MS des digestats liés au type de méthanisation (i.e. voie sèche MS >15% et voie liquide MS <15%) et que les teneurs en MO, C/N (fibres), et nutriments impactent la qualité agronomique des digestats et sont directement liés aux types de substrats majoritaires (Boues STEP, Biodéchets, Fumiers, FFOM…).
Le projet Concept-Dig dont l'objectif était de produire un outil d'aide à la conception de post-traitements des digestats en vue de leur utilisation agronomique a permis de réaliser le même travail sur des digestats d'origine agricole. Une enquête menée auprès de 72 agriculteurs méthaniseurs (Association des Agriculteurs Méthaniseurs de France, AAMF) a généré une base de données de qualité globale de digestats qui a été analysée. Les résultats obtenus montrent là encore que les intrants majoritaires et certains co-substrats ont un impact sur la qualité agronomique des digestats. Huit groupes ont été retenus comme l'illlustre la Figure 25.

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[3] *Matière Sèche (MS) en g/kgMatière Brute, Matière Organique (MO), Carbone Total (CT), Azote Total (NT), Phosphore Total (P2O5), Potassium (K2O), en g/kg MS, ratios C/N, NH4/NT*

Un résultat remarquable a été de différencier le type de l'animal dont provient le résidu organique (i.e. ruminant ou non ruminant, bovin ou porcin) mettant en lumière que la digestion et l'alimentation de l'animal a un effet sur sa déjection, comme dans le cas de la méthanisation en bioréacteur.

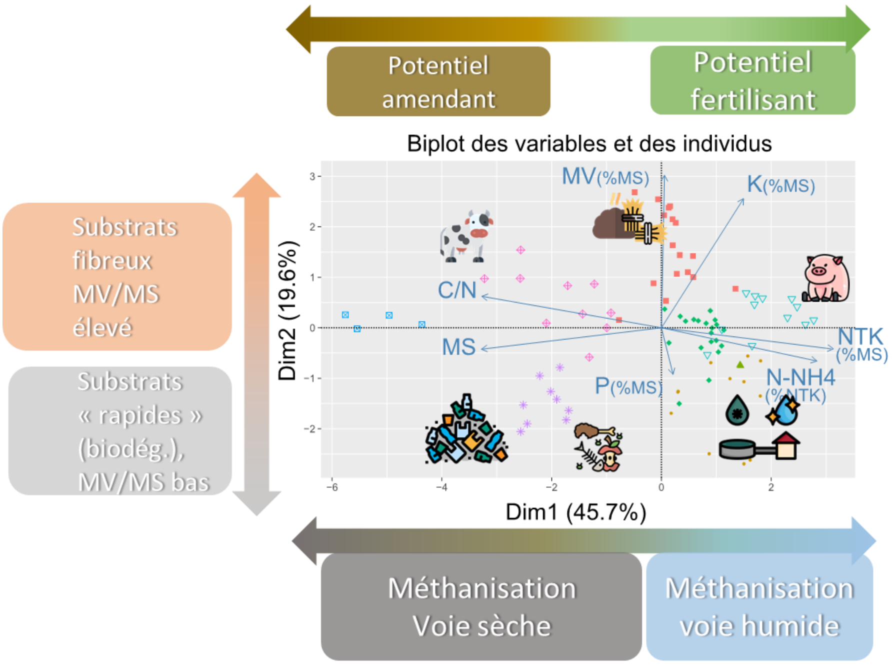
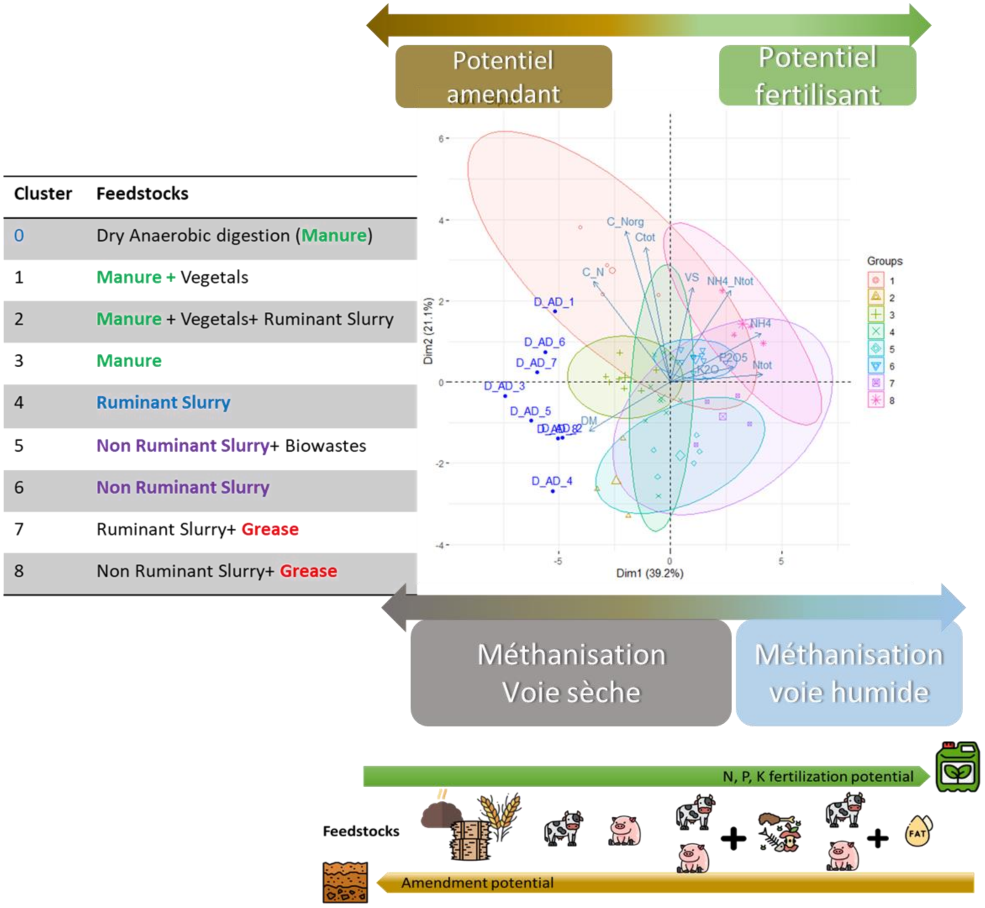


Figure 24 : Etude de la variabilité de la qualité agronomique globale des digestats d'origine urbains et agricoles en fonction des intrants de méthanisation [JJ12]



Cluster	Feedstocks
0	Dry Anaerobic digestion (**Manure**)
1	**Manure** + Vegetals
2	**Manure** + Vegetals+ Ruminant Slurry
3	**Manure**
4	**Ruminant Slurry**
5	**Non Ruminant Slurry**+ Biowastes
6	**Non Ruminant Slurry**
7	Ruminant Slurry+ **Grease**
8	Non Ruminant Slurry+ **Grease**

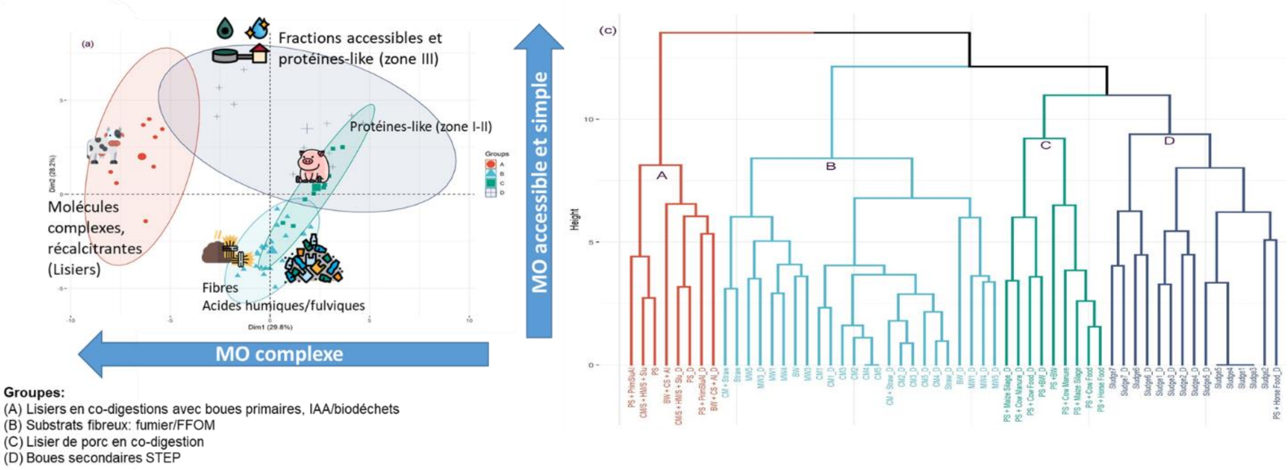
Figure 25 : Etude de la variabilité de la qualité agronomique globale des digestats d'origine agricole en fonction des intrants de méthanisation (Concept-Dig, [PO7], [PO10], [JJ33])

3.3. Evolution matière dans les procédés : variabilité des intrants et mécanismes

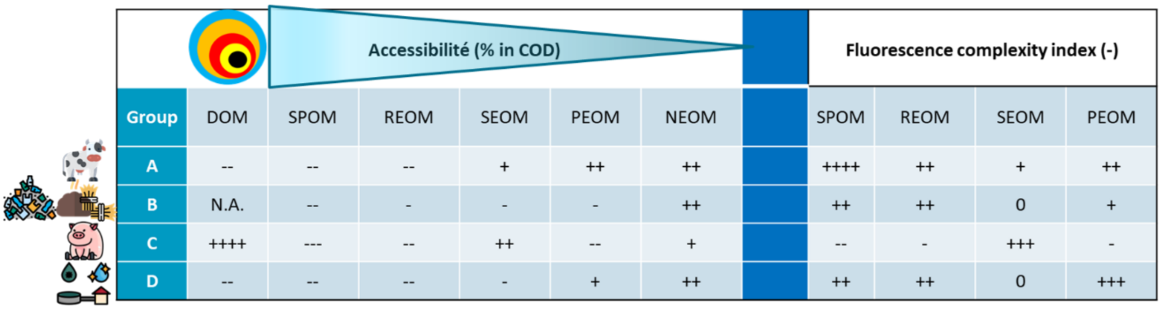
Pour aller plus loin et comprendre le devenir de la MO des intrants pendant la méthanisation et leur impact sur la qualité de la MO des digestats, la base de données générée tout au long des projets de recherche depuis 2013 jusqu'en 2021 concernant la caractérisation fine IsBaMO® (axe 1) a permis de confronter la qualité de la MO des intrants à celle de leurs digestats.

Des analyses statistiques ont permis de mettre en évidence plusieurs résultats importants comme reportés dans la publication [JJ04] : (i) sur une trentaine de couples intrants/digestats, une ACP et un clustering montrent que les digestats sont regroupés avec leurs intrants respectifs, soulignant l'empreinte de la qualité de la MO d'un susbtrat sur la qualité de la MO des digestats, (ii) les groupes déterminés par l'analyse statistique montrent une typologie des couples intrants/digestats par famille de résidus organiques (intrants fibreux fumiers et FFOM), lisiers, boues de STEP et co-digestions diverses), (iii) cette typologie est gouvernée selon les deux axes suivants (déjà définis dans l'axe 1), la complexité et l'accessibilité de la MO et (iv) des grandes tendances d'évolution de la MO et des variabilités significatives permettant d'établir un modèle statistique de prédiction de la qualité de la MO d'un digestat à partir de celle d'un ou plusieurs intrants.

La Figure 26 présente l'ensemble des résultats obtenus dans l'étude publiée [JJ04].



(a)



	Accessibilité (% in COD)							Fluorescence complexity index (-)			
Group	DOM	SPOM	REOM	SEOM	PEOM	NEOM		SPOM	REOM	SEOM	PEOM
A	--	--	--	+	++	++		++++	++	+	++
B	N.A.	--	-	-	-	++		++	++	0	+
C	++++	---	--	++	--	+		--	-	+++	-
D	--	--	--	-	+	++		++	++	0	+++

Evolutions relatives: +: 0 à 25%; ++: 25 à 50%; +++: 50 à 75%; ++++: 75 à 100%; -: 0 à -25%; --: -25 à-50%; ---: -50 à -75%; ----: -75 à -100%. Pourcentage Relatif= (valeur finale digestats- valeur initiale substrats)/ valeur initiale substrats) × 100. N.A. = pas de DOM pour le Groupe B (digestats voie sèche)

(b)

Figure 26 : Etude et prédiction de la variabilité de la MO des digestats en fonction de la qualité de la MO de leurs intrants : analyses statistiques (a) et analyse de l'impact des variables décrivant les intrants sur la qualité des digestats (b) [JJ04] [PO8]

De manière globale, une comparaison relative montre que les fractions particulaires accessibles sont biodégradées avec une augmentation de la complexité de ces fractions dans le digestat final et une augmentation de la fraction non extractible (NEOM) relative aux molécules type lignine-like, humines-like et autres composés non identifiés. Toutefois, selon la catégorie d'intrants, les évolutions de certaines fractions peuvent diverger selon la nature des intrants, venant valider les observations décrites par les bilans matières.

Par exemple, les intrants contenant des fractions plus ou moins ligneuses ont une biodégradation différente selon leur contenu (cas des boues STEP versus le fumier ou FFOM), la fraction SEOM augmenteen quantité et qualité uniquement dans le cas de la méthanisation des lisiers porcins et fumiers bovins et enfin la fraction soluble DOM est en partie biodégradée mais reste élevée avec une haute complexité comme dans le cas du groupe des lisiers porcins.
Ainsi, le modèle PLS établi à partir des variables explicatives issues de l'IsBaMO® a permis d'étudier l'impact de la qualité de la MO des intrants sur la prédiction de la qualité de la MO des digestats montrant que le levier d'action « intrants » est très significatif. L'empreinte du substrat sur le digestat en termes de complexité et de bioaccessibilité apporte des questions supplémentaires à investiguer comme de potentiels transferts de molécules entre fractions provenant de mécanismes comme l'hydrolyse ou encore une complexification de la MO.

3.4. Impact de la variabilité des intrants sur les agroécosystèmes

L'impact des intrants sur la qualité des digestats a été démontré. Pour aller plus loin dans le raisonnement, un résultat remarquable a été obtenu sur l'impact de co-susbtrats sur la qualité agronomique des digestats et leur efficacité au sol. Dans le cadre du projet MAPPED (PIA, ADEME, 2016-2019) et du CDD ingénieur de Bastien Zennaro que j'ai encadré, la caractérisation globale et fine aux bornes de cinq méthaniseurs d'échelle pilote laboratoire a été réalisée afin de caler des modèles de procédés. La particularité de l'alimentation de ces derniers était que l'intrant majoritaire était à chaque fois un lisier porcin (i.e. ration massique > 80%) et que chaque réacteur était alimenté par un co-intrant différent. Les analyses réalisées sur les digestats ont permis ensuite de positionner ces derniers dans la typologie établie dans Concept-Dig (cf. 3.3). Les résultats ont montré que les co-intrants avaient un impact significatif sur la typologie du digestat associé puisque ce dernier était positionné plutôt dans le groupe associé au co-intrant correspondant (fumier, biodéchet, mais ensilé). Seuls les digestats en co-digestion avec la nourriture pour bétail et pour chevaux sont intermédiaires et restent dans le groupe « lisier porcin ». Cela a été aussi confirmé en termes de qualité de la MO des digestats (IsBaMO®) puisque ces échantillons ont été utilisés également dans l'étude de [JJ04].
Puis, en collaboration avec INRAE ECOSYS, des tests agronomiques de minéralisation C et N au sol des cinq produits ont été réalisés. La Figure 27 présente les résultats obtenus concernant l'azote disponible et le potentiel de stockage du C après 91 jours d'incubation. Il apparaît clairement que selon le type de co-substrat, le devenir agronomique du digestat au sol est différent (Figure 27). En effet, l'apport d'un fumier augmente le potentiel de stockage du C dans le sol mais aura un potentiel fertilisant azoté limité contrairement à l'apport d'un biodéchet comme le co-substrat.

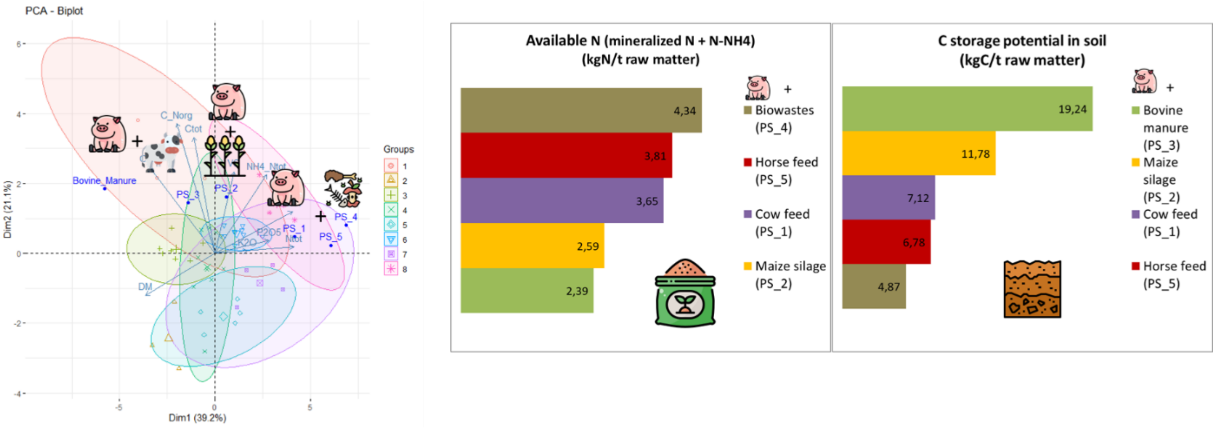


Figure 27 : Validation de la typologie Concept-Dig et impact de la recette entrante sur la qualité agronomique d'un digestat [PO7] [JJ33]

Ainsi, la typologie des digestats associée aux intrants semble impacter les effets agronomiques de ces derniers lors de leur épandage.

3.5. Impact de la qualité des digestats et conditions opératoires

Le temps de séjour hydraulique, la température, l'humidité (i.e. voie sèche, voie humide) et la configuration du procédé (batch type garage, piston, infiniment mélangé continu) sont les principales conditions opératoires de la méthanisation. Concernant l'humidité directement corrélée avec la MS, nous avons vu précédemment qu'elle jouait un rôle important dans la classification des digestats. Toutefois, la voie sèche ou humide est bien souvent dépendante du type d'intrant traité (voie sèche : fumiers, FFOM versus voie liquide : boues, lisiers, intrants liquides). En effet, dans le projet Concept-Dig, les digestats issus de la voie sèche (et alimentés majoritairement par des fumiers bovins) se retrouvent regroupés ensemble. Ce constat est également valable pour le type de réacteur qui ne montre pas d'impact significatif sur la qualité des digestats (réacteur batch garage corrélé avec la voie sèche et les fumiers, réacteur piston corrélé avec la voie sèche et FFOM puis réacteur continu infiniment mélangé corrélé avec la voie humide).
En outre, nous avons montré dans l'étude de [JJ04] qu'en ajoutant les conditions opératoires (temps de séjour et température) au modèle de prédiction de la qualité de la MO des digestats, les modèles statistiques n'étaient pas améliorés et que le poids de ces variables n'étaient pas statistiquement significatifs. De la même façon, lors des études statistiques réalisées dans la thèse de Felipe Guilayn [JJ12] et dans le projet Concept-Dig [PO7] [JJ33], l'ajout de ces variables à celles de caractérisations des digestats n'a pas permis d'améliorer le pourcentage de variance expliqué.

Toutefois, ces conclusions sont à nuancer. En effet, comme précédemment évoqué, les conditions de températures et de temps de séjour sont très corrélées avec le type d'intrant (par exemple, les boues sont souvent traitées en mésophilie avec des temps de séjour allant de 15 à 30 jours alors que des méthaniseurs traitant du fumier ont des temps de séjour supérieur à 100 jours et ceux traitant les FFOM sont opérés en thermophilie). De plus, dans l'étude [JJ04], le modèle statistique suppose que le temps de séjour est suffisant pour que la MO biodégradable le soit dans le méthaniseur. En effet, une des hypothèses sur l'impact du temps de séjour sur la qualité de la MO des digestats est que plus ce dernier est élevé, plus la MO se stabilise et se complexifie.
Ainsi, afin d'aller plus loin, une décorrélation entre conditions opératoires et types d'intrant doit être prise en compte via des tests réalisées sous conditions opératoires diversifiées.

3.6. Variabilité de la qualité des produits et impacts des post-traitements

Le second levier d'action identifié dans la filière méthanisation est la variabilité de post-traitements potentiels, plutôt classiques comme la séparation de phases, le compostage de la phase solide, le stockage des diverses phases (brut, liquide, solide) et moins classiques comme des procédés thermo-chimiques ou la pyrolyse.
Au cours de la thèse de Felipe Guilayn [JJ13] et dans le projet Concept-Dig (ADEME, 2019), des études ont été réalisées sur la séparation de phases et l'impact de ce procédé sur la qualité agronomique des digestats. Un premier constat est que la séparation de phases, quel que soit le procédé, permet d'obtenir deux produits, une phase liquide à potentiel fertilisant et une phase solide à potentiel amendant comme illustré par l'ACP réalisée lors du projet Concept-Dig (Figure 28).

Dans l'étude de [JJ13], nous avons étudié le bilan matière du procédé à partir de données internes au LBE, à Suez et de la littérature et sommes arrivés à l'élaboration d'indicateurs d'efficacité permettant de prédire les concentrations en éléments majeurs selon le type de procédé associé à son efficacité de séparation via deux groupes : les procédés à faible efficacité (presse à vis) et les procédés à forte efficacité (centrifugation). La Figure 29 présente les résultats obtenus dans l'étude sur l'élaboration de ces indicateurs ainsi que les répartitions moyennes des concentrations en éléments majeurs dans les phases liquides et solides obtenus dans chacun des groupes. Ces indicateurs et hypothèses ont par la suite été validés en utilisant des bilans matière appliqués à des cas réels sur sites [PO7], [JJ33]. La répartition des éléments est significativement impactée selon le type de séparateur. Dans les deux cas, la phase solide est plus concentrée en MO qu'en nutriments.

Toutefois, dans le cas d'un séparateur de type presse à vis, la fraction liquide, en moyenne, est proche de la composition du digestat brut initial. Dans le cas d'une centrifugeuse, la séparation est plus performante et la récupération de matière dans la phase solide augmente. Non seulement la MO et l'azote organique augmente mais la distribution du P est différente. Plutôt dans la phase liquide dans le cas d'une presse à vis, le P se retrouve dans la phase solide après une centrifugation.

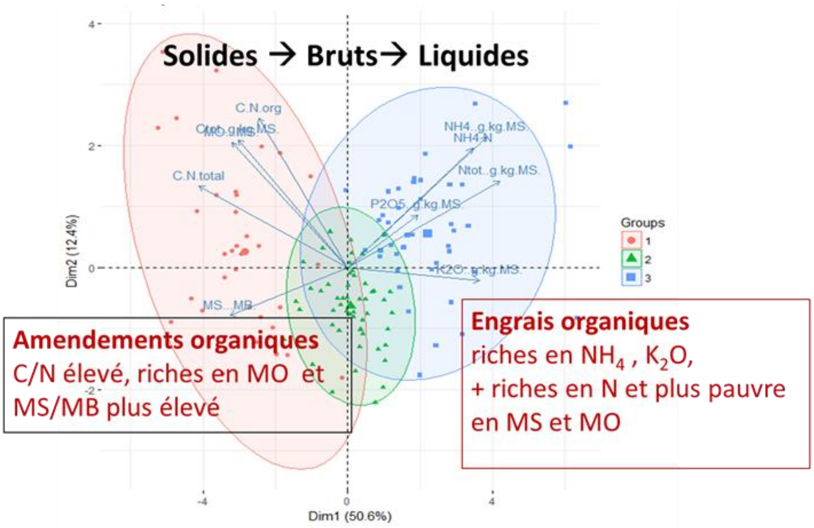


Figure 28 : Analyse ACP sur les digestats bruts, solides, liquides inventoriés dans le projet Concept-Dig [JJ33] [PO7], [PO10], [PO19]

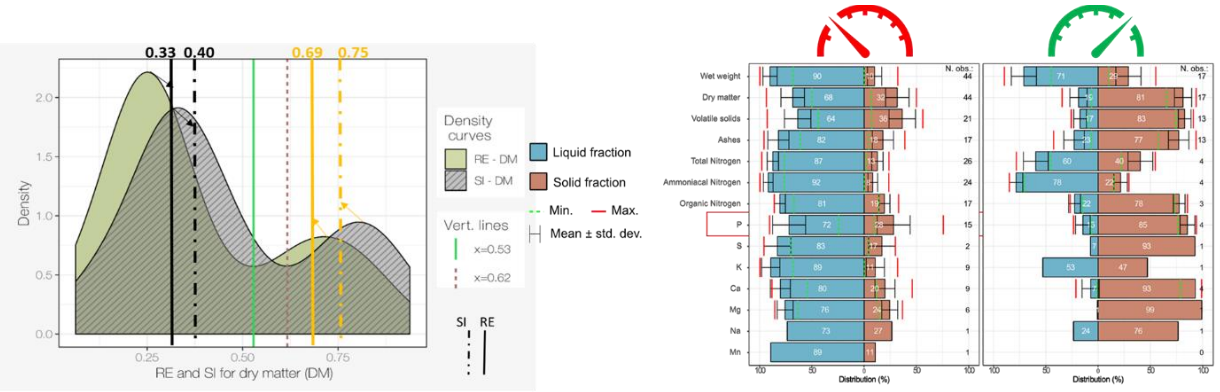


Figure 29 : Distribution des indicateurs d'efficacités de séparation de phases développés dans [JJ13] (gauche) et répartition des éléments selon l'efficacité (faible et élevée) du séparateur de phases [JJ13] (droite)

Des analyses statistiques ont été également réalisées sur les données de qualité agronomique globale des digestats issus de la phase liquide et de la phase solide après séparation de phases.

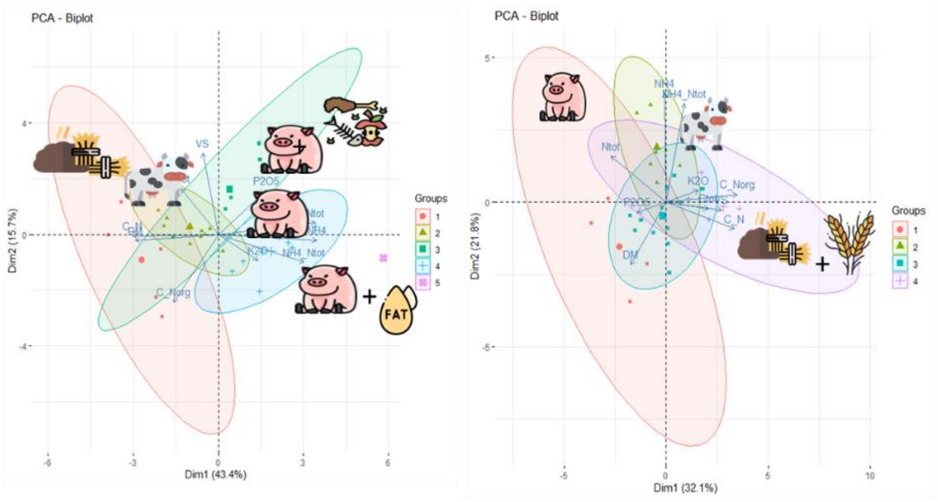
Il apparaît que l'empreinte des intrants subsiste, avec des clusters de digestats associés aux intrants majoritaires (Figure 30 : *Analyses ACP réalisées sur les digestats liquides et solides dans le cadre du projet Concept-Dig [JJ33] [PO7]*).

J'ai également étudié d'autres post-traitements tels que le stockage et le compostage des digestats. Concernant le stockage des phases liquides et brutes des digestats, l'évolution de la MO globale est très faible avec des abattements inférieurs à 6% mais l'accessibilité peut être impactée.

L'évolution de l'azote montre des pertes d'azote ammoniacal par volatilisation pouvant aller jusqu'à 80% sans couverture du stockage. Concernant le stockage de la phase solide, ce dernier était proche d'un compostage de cette même phase avec une forte biodégradation de la MO.

Le compostage et le stockage de phases solides augmente l'humification et le potentiel amendant de la MO [JJ20] [JJ21].

Remarque : similairement au procédé de méthanisation, des bilans matières aux bornes du co-compostage de boues digérées avec des déchets verts (projet Risq-PRO, ONEMA) ou de compostage de digestats de marcs de raisin (Biogaz-Rio) montrent que les fractions de l'IsBaMO® décrivent avec pertinence l'évolution de la MO pendant le compostage [JJ34].



Phases liquides

Phases solides

Figure 30 : Analyses ACP réalisées sur les digestats liquides et solides dans le cadre du projet Concept-Dig [JJ33] [PO7]

Dans le cadre de la thèse de Felipe Guilayn, une revue des traitements des digestats a été réalisée [JJ09] et propose plusieurs voies alternatives de valorisation des digestats (Fait Marquant 3). D'autres procédés moins classiques ont été évalués dans le cadre du projet Concept-Dig, non développés ici tels que des procédés thermochimiques (ajout de base ou acide à température ambiante à haute, 133°C) ou la pyrolyse.

Fait marquant 3 : Développement de procédés innovants à partir d'hypothèses issus de la caractérisation de la matière

Une autre partie de mes travaux de recherche a été dédiée à utiliser la connaissance de la matière afin de proposer des traitements innovants pour extraire ou concentrer certaines molécules et éléments d'intérêt. Ce fut le cas lors de la thèse de Felipe Guilayn (2016-2019) sur les procédés de valorisation des digestats de méthanisation urbaine. Grâce à une étroite collaboration avec SUEZ et après avoir réalisé un état de l'art sur les traitements des digestats [JJ09], nous avons proposé deux voies de valorisation alternatives à l'épandage direct des digestats d'origine urbaine. En effet, les coûts de transport et la réglementation sont des freins majeurs à leur utilisation en agronomie sans post-traitement hygiénisant ou capable de réduire les volumes des digestats. Dans ce contexte, nous avons investigué d'autres voies de valorisation en étudiant les caractéristiques des digestats. Comme décrit précédemment, les digestats contiennent de la MO complexe comme des substances humiques, entre autres, ayant un potentiel biostimulant et biosurfactant [JJ11].

D'un autre côté, les digestats contiennent une part importante d'azote ammoniacal disponible pour les plantes mais potentiellement volatilisable au pH de ces derniers. L'hygiénisation et la réduction du volume des produits finis font partie également du cahier des charges établi pour une valorisation optimale. Ainsi, les deux voies retenues sont (i) le fractionnement qui consiste en l'extraction de molécules type substances humiques afin de proposer ce produit comme biostimulant et (ii) la conservation consistant en la concentration des éléments. Lors de la thèse, la preuve de concept a été réalisée notamment sur le fractionnement sur plusieurs types de digestats d'origine urbaine : boues, FFOM, biodéchets, territorial et fumier afin d'avoir une référence d'origine agricole.

En effet, inspiré du fractionnement IsBaMO® et de tests similaires sur les composts et autres résidus organiques, nous avons proposé d'extraire les substances humiques avec de la potasse (pH 12). En parallèle, le pH basique favorisant la volatilisation de l'azote ammoniacal, l'idée est de stripper ce dernier et de le récupérer dans un piège acide afin de produire des sels d'ammonium potentiellement utilisables et commercialisables comme engrais. Une caractérisation poussée des substances humiques a été réalisée via des fractionnements sur résines afin de déterminer les acides fulviques, humiques, hydrophobes et hydrophiles ainsi que la caractérisation spectrale en fluorimétrie qui a montré la présence de substances humiques similaires à des références commerciales de biostimulants existant sur le marché. Le potentiel biostimulant a ensuite été testé et validé via des tests en hydroponie [JJ11] toujours en comparaison d'une référence commerciale, ainsi que le potentiel surfactant afin de dépolluer des sols riches en hydrocarbures [JJ11]. Concernant la voie dite de « conservation », des premiers tests ont été réalisés, notamment en termes d'acidification, d'ajout d'additifs ou non et de température de séchage à faible température (70°C) avec toutefois un manque de conclusion sur l'efficacité agronomique du produit final. Il est à noter que la typologie des digestats semble avoir une influence sur les résultats obtenus et l'idée est de proposer une voie plutôt qu'une autre selon le type de digestat et selon certains indicateurs.

Ces premiers résultats encourageants ont permis de proposer une nouvelle étude en collaboration avec SUEZ via le projet Biogaz-Rio (2020-2023). Cette étude a pour objectif de monter en échelle et de réaliser un réacteur pilote afin de tester les deux voies de valorisation et l'efficacité agronomique des produits finis dans les deux cas sur trois types de digestats urbains (FFOM, territorial et biodéchets).

Cet exemple de conception de procédé à partir de la connaissance de la matière pour sa valorisation montre que l'on peut aller vers des services rendus non anticipés au départ et développer des procédés intéressants et innovants.

3.7. Variabilité des produits : hiérarchie des leviers d'actions et perspectives

Un bilan des activités de recherche liées à l'axe 2 est dressé comme le résume le Tableau 2.
Ainsi, à l'aide des différents projets et thèses cités, mes travaux ont permis de mettre en évidence que la variabilité de la qualité des digestats dépend majoritairement de la nature des intrants, des post-traitements appliqués et de certaines conditions des procédés (voie sèche vs voie humide).
Les post-traitements sont un levier d'action significatifs mais nous avons montré que l'empreinte des intrants demeure. Ces variabilités peuvent être utilisées comme leviers d'action afin de produire des produits en adéquation avec les agroécosystèmes, via l'ingénierie inverse. Toutefois, il reste encore à investiguer certains points comme la variabilité des conditions opératoires afin de les décorréler des intrants.
Ces travaux ont aussi mis en lumière des verrous à lever tels les mécanismes de complexification de la MO pendant le bioprocédé, le devenir des nutriments pendant les procédés, la hiérarchie des leviers d'action et enfin les liens entre typologie des produits établie lors de ces études et typologie des effets sur les agroécosystèmes. En effet, l'hypothèse sous-jacente à l'ensemble de la question de recherche citée ici est que la variabilité de la qualité agronomique des digestats a un impact sur les agroécosystèmes. Même si des premiers éléments de réponses ont pu être donnés via les études de [JJ07] (besoins N et P des plantes et digestats) et du projet MAPPED [PO7] et [JJ33], la question demeure : « la typologie des impacts sur les agroécosystèmes associés à l'épandage des digestats correspond-telle à la typologie des digestats ? ».

A partir des indicateurs, de la connaissance de la matière et son devenir dans les procédés, la modélisation de ces derniers est un moyen de prédire des performances associées à des services rendus, de tester des hypothèses sur les mécanismes du devenir de la matière et de proposer des scenarios afin d'optimiser le design d'une filière de valorisation.

Tableau 2 : Bilan des travaux sur l'Axe 2

Questions de recherche	Principaux résultats	Travaux associés	Supervision thèses, projets, ingénieurs	Etat d'avancement /connaissance
Variabilités des substrats comme levier d'action	Typologie des digestats (qualité agronomique globale et IsBaMO®) Empreinte de la qualité de la MO des substrats sur la MO des digestats Prédiction de la qualité MO des digestats à partir de la qualité MO des substrats	[JJ12] [JJ04] [PO7] [JJ33]	-Thèse CIFRE SUEZ Felipe Guilayn (2016-2019) -Thèse David Fernandez (2020-2023) - Encadrement d'un CDD ingénieur dans le cadre du projet MAPPED (2016-2019) - Coordination du projet Concept-Dig (2016-2019) - Coordination du projet Ferti-Dig (2021-2024)	
Variabilités des conditions opératoires comme levier d'action	Impact de l'humidité sur la classification des digestats Pas d'impacts du temps de séjour, et température sur les variables étudiées	[PO7] [JJ33] [JJ04]	- Coordination du projet Concept-Dig (2016-2019) - Thèse David Fernandez (2020-2023)	

	Mise en évidence d'une corrélation entre conditions opératoires et types de substrats Besoin de décorreler pour aller plus loin			
Variabilités des post-traitements comme levier d'action	Séparation de phases permet d'obtenir deux produits de qualité agronomique et MO différente (potentiel fertiliant et potentiel amendant) Compostage et stockage permettent la stabilisation de la MO Stockage sur digestats liquides et bruts : effet sur l'azote significatif selon conditions Conception de post-traitements innovants à partir de la connaissance de la matière	[JJ13] [JJ33] [JJ20] [JJ21]	-Thèse CIFRE SUEZ Felipe Guilayn (2016-2019) - Coordination du projet Concept-Dig (2016-2019) - Coordination de l'étude sur la valorisation des digestats dans le projet Biogaz-Rio (2020 – 2022) - Encadrement Assistant ingénieur et ingénieur CDD (Biogaz-Rio)	
Hiérarchie des leviers d'actions	Empreinte des substrats sur la qualité des digestats traités et non traités Effet du traitement semble dépendre de ce dernier et de la typologie du digestat Question encore à investiguer, notamment avec l'ajout de nouvelles études sur les conditions opératoires	[JJ04] [JJ12] [JJ13] [JJ20] [JJ21] [JJ33]	-Thèse CIFRE SUEZ Felipe Guilayn (2016-2019) -Thèse David Fernandez (2020-2023) - Encadrement d'un CDD ingénieur dans le cadre du projet MAPPED (2016-2019) - Coordination du projet Concept-Dig (2016-2019) - Coordination du projet Ferti-Dig (2021-2024) Coordination de l'étude sur la valorisation des digestats dans le projet Biogaz-Rio (2020-2022) - Encadrement Assistant ingénieur et ingénieur CDD (Biogaz-Rio)	

Mécanismes -MO -Nutriments	Evolution de la MO dans les procédés : bilans matières et pertinences de l'indicateur IsBaMO® Bioaccessibilité, Hydrolyse et mécanismes séquentiel versus simultanné (décrit dans Axe 1) Evolution N organique différente du C (décrit dans Axe 1) A investiguer : mécanismes impliquant une complexification de la MO ; autres nutriments (P et S)	[JJ04] [JJ07] [JJ10] [JJ15]	-Thèse David Fernandez (2020-2023) - Collaboration thèse Younès Bareha (2018) - Collaboration thèse Cyrille Charnier (2017)	
Typologie des produits versus Typologie des effets agronomiques	Typologie des digestats impactée par l'ajout de co-susbtrats induisant des effets agronomiques variables A investiguer : typologie des effets sur les agroécosystèmes en adéquation avec la typologie des produits ?	[JJ04] [JJ33]	-Thèse David Fernandez (2020-2023) - Encadrement d'un CDD ingénieur dans le cadre du projet MAPPED (2016-2019) - Coordination du projet Ferti-Dig (2021-2024)	

4. Axe 3 : Comment réaliser l'ingénierie inverse dans un système de Bioraffinerie Environnementale dédié à la double valorisation énergétique et agronomique sous contrainte environnementale ?

Dans le cadre de ma thématique de recherche, mon ambition est de réaliser une optimisation multi-objectif du système de production d'énergie et d'engrais organiques en fonction des besoins des agroécosystèmes, sous contraintes environnementales via une logique d'ingénierie inverse. Mon idée principale est de proposer des outils mathématiques capables de formaliser le système de bioraffinerie environnementale pour généraliser et produire des scénarios optimaux *in silico*. Ce concept étant complètement nouveau dans ce domaine au début de ma carrière à INRAE, j'ai dû faire face à plusieurs défis concernant la modélisation, la méthodologie d'optimisation multi-objectif et la description des critères d'optimisation. Dans un premier temps et jusqu'à aujourd'hui, j'ai relevé le défi de modéliser la bioraffinerie environnementale et la combinaison des procédés afin de les coupler avec des modèles sol/culture utilisés pour simuler les besoins et contraintes des agroécosystèmes.

4.1. Objectifs et questions de recherche

Comme pour les deux premiers axes de recherche, la Figure 31 présente la place de la modélisation dans ma thématique de recherche. Pour atteindre l'objectif d'optimisation et de conception de filière, la modélisation est un outil puissant capable d'être utilisé pour prédire les performances d'un procédé, sa cinétique et ses limites. La stratégie classique suivie a été d'utiliser des données expérimentales issues des suivis des procédés en tant que données de calage et de validation des modèles. Ces modèles représentant les procédés sont ensuite combinés afin de simuler une filière de valorisation. Enfin, une étude de scénarios basée sur une optimisation multi-objectif selon des critères associés aux services rendus, limitée par des contraintes à définir et se basant sur des leviers d'actions est réalisée.

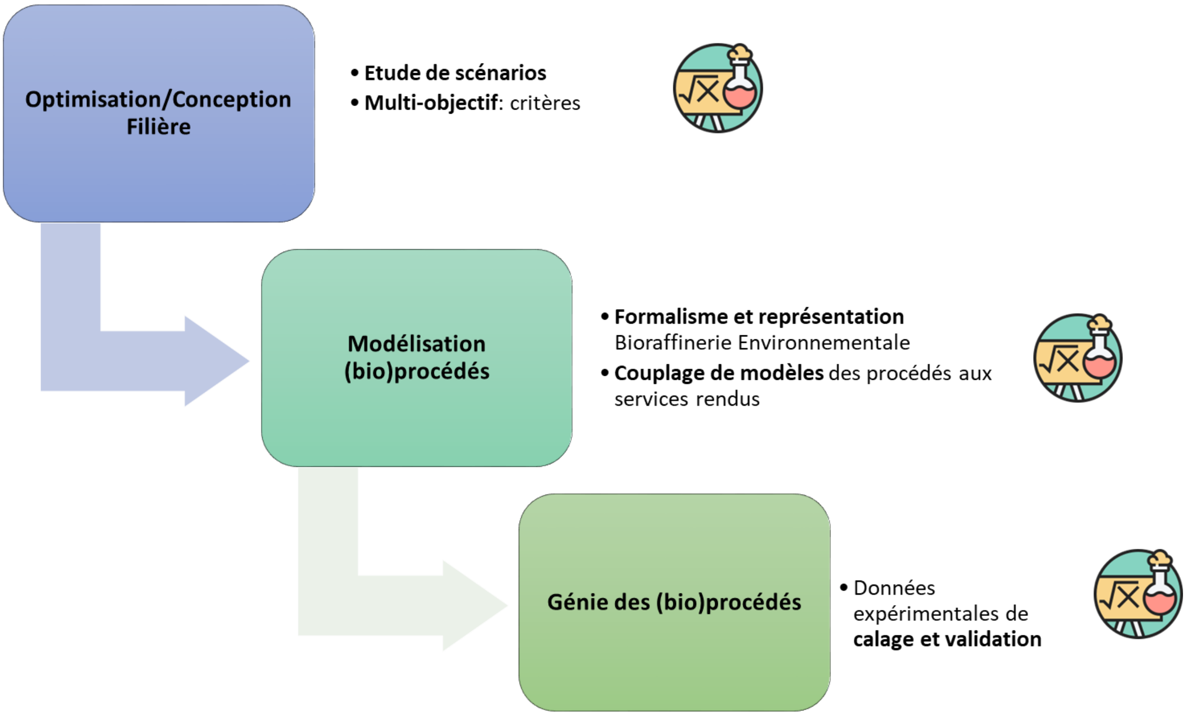


Figure 31 : Place de la modélisation au sein de la démarche scientifique de ma thématique de recherche

En définissant ces objectifs, j'ai identifié des verrous et questions de recherche relatifs à cet axe :

- Les modèles des procédés existants sont-ils suffisamment descriptifs pour prédire les services rendus ciblés ? Si non, comment les améliorer ?

- Les modèles des procédés existants ou développés peuvent-ils être combinés entre eux et avec des modèles agronomiques afin de simuler une filière entière, de l'intrant au retour au sol ? Si non, quelle stratégie mettre en place et quels verrous associés aux couplages ?
- Quel degré de complexité nécessite les modèles de procédés pour répondre aux enjeux de l'optimisation multi-objectif ?
- Quels critères et quelle méthodologie d'optimisation utiliser lors de cet exercice ?

Comme décrit par la Figure 32, à partir d'expérimentations avec des procédés (suivi fonctionnement, expérimentations ciblées dans des conditions opératoires définies, plan d'expériences), des analyses globales fines (i.e. indicateurs de l'axe 1) et des bilans matières réalisés (axe 2), des bases de données contenant à la fois des données analytiques et des métadonnées (i.e. conditions opératoires, données descriptives de l'expérience) ont été établies. L'étape suivante est l'interprétation des résultats via la proposition d'hypothèses explicatives des phénomènes observés et mesurés. Dans cette étape, l'objectif est d'analyser la base de données ou les résultats d'une expérimentation ciblée pour répondre à une question précise afin d'aller jusqu'à la proposition de processus explicatifs et l'identification de leviers d'action sur lesquels s'appuyer pour piloter le procédé. Enfin, l'étape de modélisation est réalisée afin de mettre en équations mathématiques les hypothèses émises à l'étape précédente, de caler ce modèle sur la base de données générées et de le valider grâce à des données externes à celles utilisées en calibration. Si la validation n'aboutit pas, il est nécessaire de revenir aux étapes de calage et de mise en équation, voire à l'étape précédente d'hypothèses et d'interprétation. Il est également possible de concevoir des expérimentations dédiées pour répondre à une hypothèse formulée.

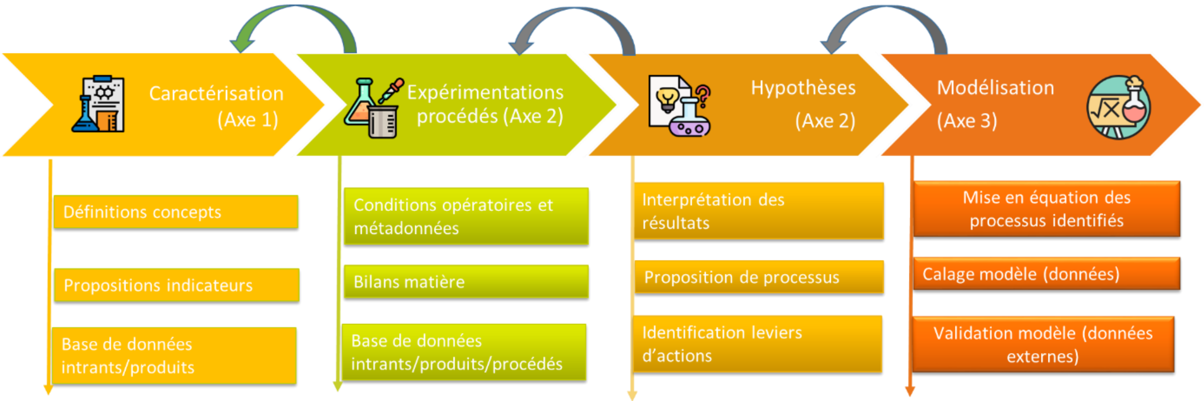


Figure 32 : Démarche scientifique suivie afin de définir un modèle de procédé

Ainsi dans les paragraphes suivants, j'illustrerai des exemples d'études associées à la résolution de certaines questions de recherche posées précédemment : le couplage des modèles pour simuler la filière dans son ensemble et les services rendus associés, le degré de compléxité des modèles et les travaux en cours sur l'optimisation multi-objectif.

4.2. Modélisation des procédés : de la complexité des modèles au défi du couplage

Il existe plusieurs types de modèles selon l'objectif fixé de modélisation. En effet, l'objectif de modélisation est de comprendre les processus biologiques afin de tester des hypothèses ou des scénarios précis, les modèles mécanistiques sont plus appropriés, tels que le modèle ADM1 (Bastone et al., 2002) pour la méthanisation. Au contraire, si l'objectif est de prédire une performance ou un service rendu à partir d'éléments intrants connus, sans prendre en compte les processus biologiques impliqués afin de gagner en temps de calcul et en s'affranchissant de la connaissance des paramètres cinétiques, la modélisation dite « boîte noire » basée sur des modèles statistiques est le choix le plus judicieux (exemple des modèles PLS de [JJ18] pour prédire le potentiel méthane et le carbone minéralisable au sol de divers résidus organiques à partir de données de caractérisation).

Dans ce paragraphe, je vais présenter mes travaux de recherche réalisées sur l'utilisation de ces deux types de modélisation afin de simuler une filière de traitement, à savoir des substrats en méthanisation au retour au sol des digestats traités ou non.

Modèles mécanistiques : mieux comprendre les processus biologiques

La modélisation d'une filière de traitements suppose que les modèles de chaque procédé puissent être combinés entre eux. Or, dans la littérature, les modèles de procédés tels que celui pour la digestion anaérobie (ADM1, Batstone et al., 2002) ou encore le compostage (Sole-Mauri et al., 2007 ; Zhang et al., 2012 ; Denès et al., 2015 ; Oudart et al., 2015) ne sont pas combinables étant donné que les variables d'entrées et sorties des modèles sont différentes. De même, les variables utilisées dans les modèles de dégradation de la MO dans les sols (CANTIS, Garnier et al., 2003, STICS résidus, Nicolardot et al., 2001) ne sont pas basées sur les mêmes définitions de variables étant donné que chaque modèle a été réfléchi indépendamment pour des objectifs différents, comme illustrés par la Figure 33.

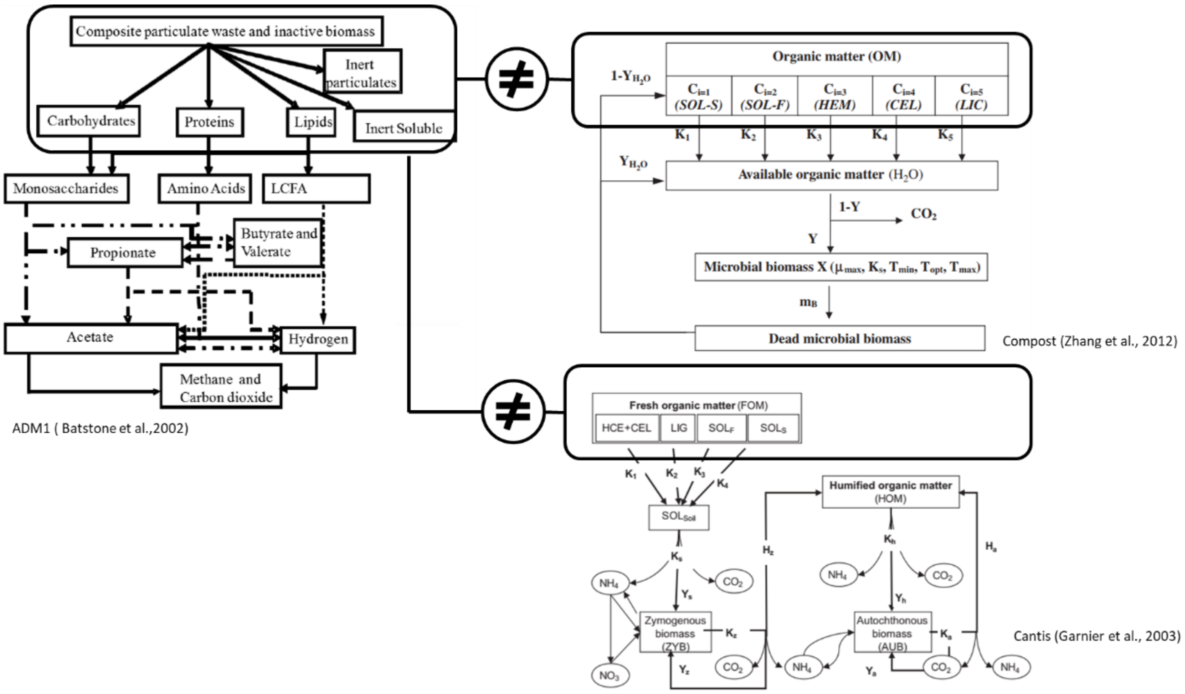


Figure 33 : Illustration de la problématique du couplage des modèles

Cette problématique a été longuement traitée dans le cas de la modélisation d'une STEP (couplage boues activées et méthanisation des boues). Deux réponses ont été apportées : (i) la mise en place d'une interface de transformation des variables, basées sur des hypothèses plus ou moins vérifiées et vérifiables (Jeppsson et al., 2007) et (ii) l'utilisation des mêmes variables tout au long des modèles, quitte à ne pas en utiliser certaines selon les procédés (Grau et al., 2007). Durant mon expérience dans le domaine de la modélisation de station d'épuration, j'ai été confrontée à la première option. Cette dernière suppose des hypothèses non justifiées comme par exemple que les variables représentant la MO non biodégradable restent les mêmes que ce soit dans le procédé boues activées ou dans le procédé de méthanisation. Cette option n'est pas non plus optimale pour coupler méthanisation/compostage et sol étant donné que les processus biologiques impliqués ne sont pas les mêmes et n'impliquent pas les mêmes communautés biologiques (bactéries et archae anaérobies en méthanisation versus bactéries aérobies, actinomycètes, champignons en compostage et sol).

De plus, dans les travaux de [JJ18], j'ai montré que la biodégradabilité d'un résidu au cours de la méthanisation n'était pas la même que celle mesurée avec du sol.

Ainsi, j'ai privilégié la seconde option, à savoir l'utilisation de mêmes variables tout au long du continuum procédés-sol.

J'ai donc modifié le modèle mécanistique de digestion anaérobie ADM1 sur les variables d'entrée et ai introduit le concept de bioaccessibilité (en utilisant le fractionnement issu d'IsBaMO®) en challengeant la modélisation classique de l'hydrolyse (Fait Marquant 4 basé sur [JJ10].
Ensuite, en combinant les modèles de compostage de (Oudart et al., 2015) et (Sole-Mauri et al., 2007), j'ai proposé un modèle de compostage en prenant comme variables d'entrées les variables de sortie de l'ADM1. Enfin, j'ai modifié le modèle de sol CANTIS (Garnier et al., 2003) en remplaçant les fractions Van Soest initialement utilisées pour prédire la biodégradation de résidus organiques au sol par les fractions IsBaMO®.
Afin de caler et valider les modèles, j'ai mis en place expérimentalement une filière de valorisation d'un mélange afin d'avoir un substrat ayant différentes fractions IsBaMO® à différentes complexités (boues activées épaissies, terreau et biscotte). Après avoir opéré un méthaniseur à l'échelle laboratoire (5L) à l'aide de la supervision d'un stage (Camilla Carvajal, 2015), une étape de compostage a été réalisée en laboratoire avant incubation sur sol.

Un suivi fin des procédés en termes de MO, fractionnement IsBaMO® et variables d'intérêt pour les modèles (production de biogaz, métabolites intermédiaires, pH, MS, azote et carbone minéralisés par l'incubation sur sol) a permis de caler les modèles. Il est à noter que des perturbations (charge et forme de MO) ont été opérées sur le méthaniseur afin de générer des données de validation pour le modèle ADM1. Les résultats ont montré qu'une bonne connaissance de la MO permet de bien simuler les expérimentations. Les paramètres cinétiques ont été calés par fraction et le devenir des fractions pendant la méthanisation (procédé continu) et le compostage (procédé batch) a été un succès comme le montre la Figure 34. Toutefois, concernant le devenir au sol de ces fractions, la seule variable à caler était la cinétique de production de carbone organique minéralisé, générant un problème d'identifiabilité des paramètres de dégradation des fractions. En effet, un suivi de la MO biodégradée dans le sol manque afin d'aller plus loin sur ce calage. L'étude avec INRAE SAS sur la dégradation en litter bag de fumier bovin (Fait Marquant 2) nous donnera l'idée de réaliser une étude dédiée sur le devenir de la MO des digestats au sol en utilisant ce procédé expérimental intéressant (cf. axe 3). L'ensemble de ce travail a été valorisé via des communications en conférences internationales [PO25], [PO26], [PO31][JJ34].

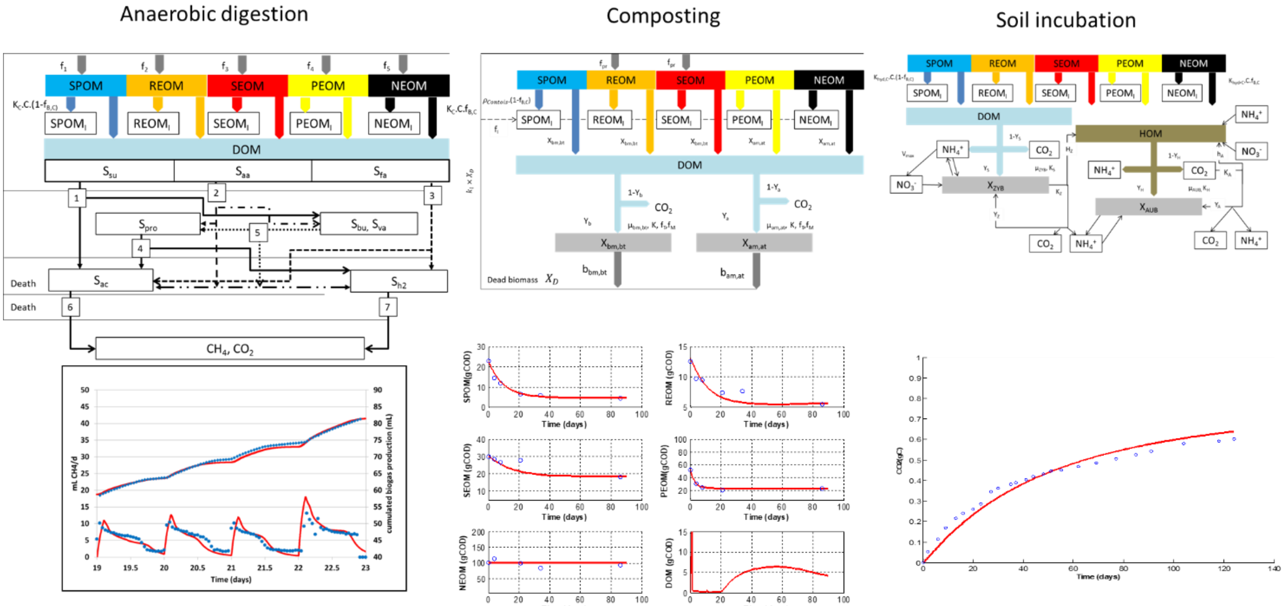


Figure 34 : Résultats obtenus sur la modélisation de la filière de méthanisation des boues [PO25] [PO26] [JJ34]

Concernant les modèles et les besoins des agroécosystèmes, je collabore avec des experts en agronomie à INRAE (ECOSYS, EMMAH, SAS) et à l'étranger (Université de Bologne) pour pouvoir combiner des modèles de bioraffinerie environnementale avec des modèles d'agroécosystèmes. Cependant, mon expertise en matière de modélisation était principalement axée sur le devenir de la MO et le carbone tout au long de cette filière. La prise en compte des nutriments était nouvelle et, comme pour l'axe 1, il a fallu développer des collaborations impliquant mon travail sur des modèles pour inclure ces éléments. Comme présenté et décrit dans l'axe 1, l'utilisation des fractions IsBaMO® pour décrire le devenir de l'azote organique a été démontrée comme pertinente par l'étude réalisée avec [JJ15]. La modélisation du devenir de l'azote organique et minéral dans la digestion anaérobie a été proposée en collaboration avec OPAALE (INRAE) mais des travaux sont encore nécessaires pour d'autres nutriments, ce qui pose de nouveaux défis pour les recherches futures.

Fait marquant 4 : Modélisation des procédés : challenger la modélisation de l'hydrolyse

Dans l'axe 1, j'ai abordé la validation expérimentale du concept de bioaccessibilité dont le but était d'en apprendre plus sur les mécanismes de l'hydrolyse afin d'améliorer sa modélisation et notamment élucider une question récurrente sur un sujet qui n'a jamais trouvé de consensus : l'hydrolyse des fractions constituant la MO d'un résidu organique est-elle séquentielle ou simultanée ? En effet, l'hydrolyse est l'étape limitante des processus biologiques visant à biodégrader la MO complexe contenue dans la plupart des résidus à valoriser [JJ27]. Dans la littérature, que ce soit pour les procédés anaérobies ou aérobies, il n'y a pas de consensus sur comment la modéliser. Selon les auteurs, certains utilisent plusieurs fractions pour décrire la matière (i.e. facilement et lentement biodégradables, tailles des particules), des cinétiques différentes (premier ordre, Contois) et lorsque plusieurs fractions sont condidérées, certains auteurs proposent de décrire l'hydrolyse de la matière comme étant séquentielle et d'autres comme étant simultanée [JJ10]. Ainsi, afin de challenger l'hypothèse commune de l'hydrolyse simultanée, et en se basant sur les hypothèses suivantes (i) la bioacessibilité gouverne l'hydrolyse et (ii) cette dernière est séquentielle, nous avons construit une série d'expérimentations de biodégradation anaérobie sur plusieurs substrats, comme décrit dans l'axe 1.

En se basant sur la bioaccessibilité décrite par le fractionnement issu d'IsBaMO®, nous pouvons décrire chaque concept comme illustré par la Figure 35. Dans le cas du concept simultané, toutes les fractions sont « attaquées » en même temps par les microorganismes alors que dans le cas du concept séquentiel, la fraction la plus bioaccessible est d'abord hydrolysée jusqu'à un certain seuil permettant de libérer et donner accès à la fraction bioaccessible suivante et ainsi de suite. Nous avons modifié le modèle ADM1 et y avons introduit des nouvelles fractions hydrolysables suivant une cinétique de Contois (décrit dans le paragraphe suivant).

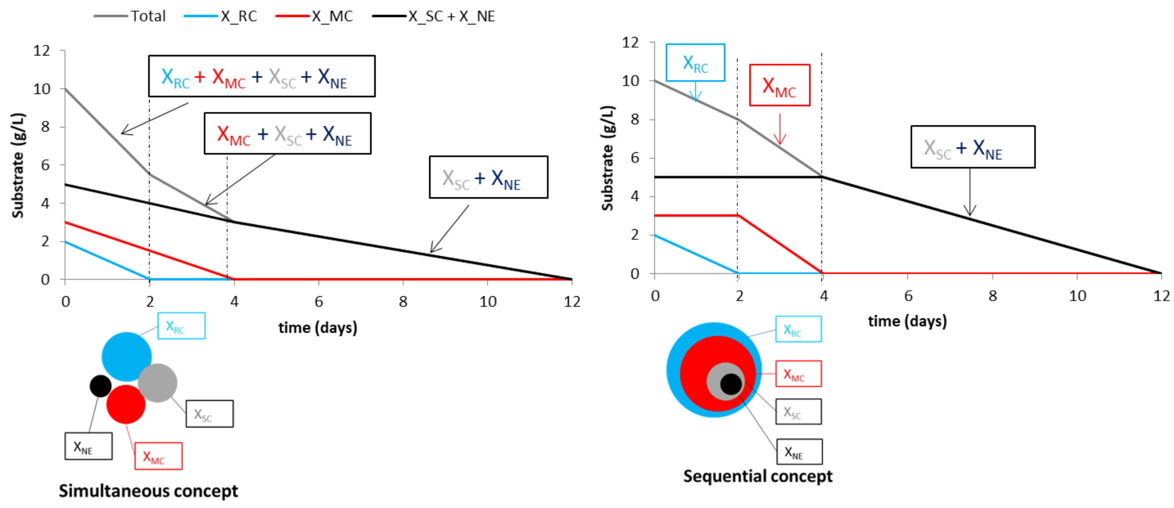


Figure 35 : Définition des concepts de biodégradation simultanée et séquentielle à partir des fractions IsBaMO®[JJ10]

Nous avons ainsi mis à incuber en anaérobiose plusieurs substrats afin de mesurer la cinétique de production de méthane, et pour trois d'entre eux, nous les avons incubés sous leur forme totale puis « déshabillée », à savoir en enlevant progressivement leurs fractions bioaccessibles via le protocole IsBaMO®. Afin de modéliser le concept « séquentiel », nous avons introduit une fonction à une cinétique de Contois décrivant l'hydrolyse de chaque fraction :

$\rho_i = K_{kyd}\_S_i \times \frac{S_i/X_i}{K_{S_i}+S_i/X_i} \times X_i \times F_{accessibility}(S_i)$, avec $F_{accessibility}(S_i) = \frac{1}{1+ S_i/K_{I\_S_i}}$

avec $K_{hyd}\_S_i$ (j^{-1}) le taux de croissance des bactéries hydrolytiques X_i (kg DCO/m^3)pour les fractions S_i (kg DCO/m^3), KS le taux de demi-saturation des bactéries X_i et $K_I\_S_i$, la concentration seuil en S_i permettant de passer à al dégradation de la fraction suivant.

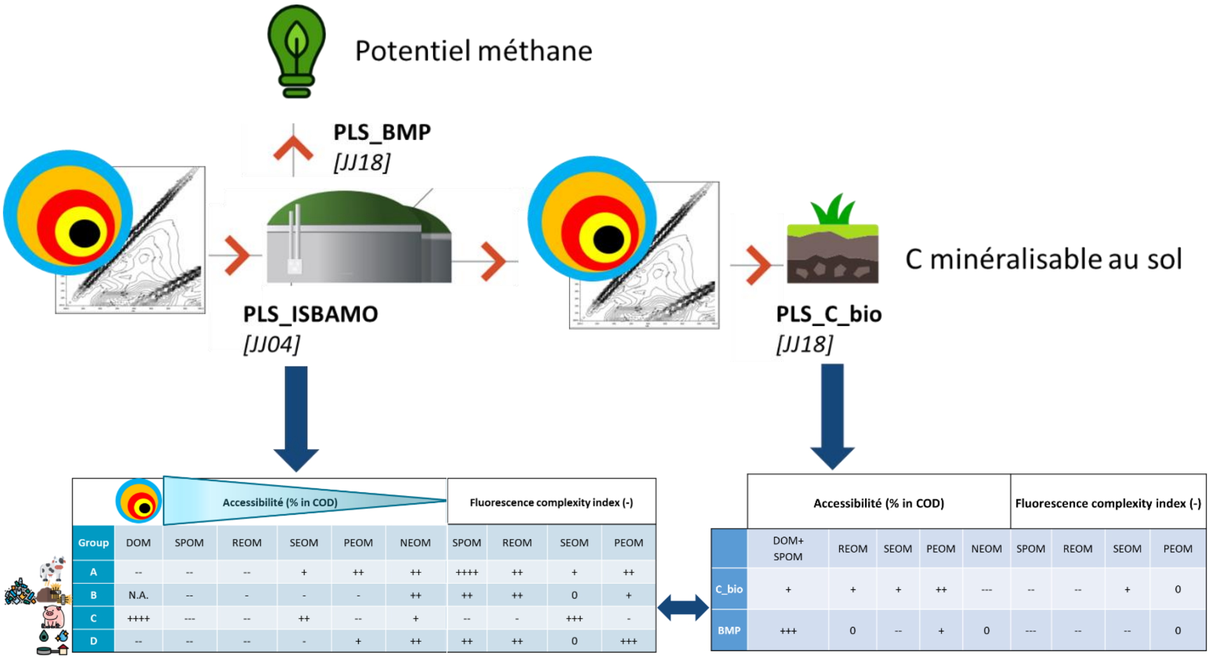
Lorsque l'on veut calculer la biodégradation de chaque fraction par différence, il s'avère qu'en se basant sur l'équation dite « simultanée », les cinétiques de biodégradation de certaines fractions des boues et de la paille sont négatives mettant en évidence que la considération du concept simultanée ne convient pas dans ces cas. Les résultats du calage des modèles « simultané » versus « séquentiel » en utilisant la fonction $F_{accessibility}$ montrent que les erreurs sont siginficativement plus faibles en utilisant le concept séquentiel. Toutefois si l'on étend ce raisonnement à la considération de co-digestion de plusieurs susbtrats, la question reste posée : comment se déroule l'hydrolyse ? Y a-t-il à la fois des processus de dégradation séquentielle et simultanée comme avancé par Morgenroth et al. (2002) ? Il est effectivement à noter que la modélisation mécanistique de la co-digestion reste encore un verrou scientifique dans le domaine de la modélisation de la digestion anaérobie.

Ces résultats et reflexions fondamentales ont été discutées, partagées puis co-publiées avec des grands experts en modélisation des procédés de valorisation des résidus dans [JJ10].

<u>Modèles statistiques boîtes noires : prédire la composition de la matière et son devenir au sol</u>

Dans un objectif plutôt opérationnel, les modèles statistiques permettent de prédire des grandeurs d'intérêts en utilisant bien souvent des données facilement disponibles comme des caractéristiques d'intrants. Pour ce qui est de la prédiction à la fois de l'intérêt agronomique et énergétique de la filière de méthanisation, comme décrit dans l'axe 1 puis 2, les indicateurs issus de la méthode de caractéristaion IsBaMO® ont permis de générer une base de données sur les résidus organiques afin de prédire les potentiels méthane et carbone minéralisable comme décrit dans [JJ18]. Ces mêmes données ont été également utilisées dans un modèle statistique afin de prédire la qualité de la MO des digestats (indicateurs IsBaMO®) à partir de celle des intrants [JJ04]. Ainsi, à partir de ces deux travaux, le couplage de modèles pour prédire le potentiel méthane, la qualité du digestat et, donc le carbone minéralisable au sol via le modèle C_bio peut être établi comme proposé par le schéma de la Figure 36.

Comme explicité dans l'axe 1 puis 2, la combinaison de ces deux travaux permet également de chercher les antagonismes et synergies existants entre les modèles, à partir du poids des variables et l'analyse de leur sensibilité afin d'optimiser le système. Par exemple, pour produire à la fois du méthane et avoir un potentiel stockage C au sol important, avoir un substrat riche en DOM+SPOM avec des zones de fluorescence riches en protéines-like générant un digestat riche en SEOM complexe serait une solution. Il faudrait donc voir dans la liste des intrants et co-intrants du modèle PLS_IsBaMO®, quelle combinaison serait optimale. Cette analyse poussée du modèle intégré de filière fait partie de perspectives à court terme.



	Accessibilité (% in COD)					Fluorescence complexity index (-)			
Group	DOM	SPOM	REOM	SEOM	PEOM	NEOM	SPOM	REOM	SEOM	PEOM
A	--	--	--	+	++	++	++++	++	+	++
B	N.A.	--	-	-	-	++	++	++	0	+
C	++++	---	--	++	--	+	--	-	+++	-
D	--	--	--	-	+	++	++	++	0	+++

	Accessibilité (% in COD)					Fluorescence complexity index (-)			
	DOM+ SPOM	REOM	SEOM	PEOM	NEOM	SPOM	REOM	SEOM	PEOM
C_bio	+	+	+	++	---	--	--	+	0
BMP	+++	0	--	+	0	---	--	--	0

Figure 37 : Schématisation de la modélisation filière de méthanisation à partir des modèles PLS obtenus pour la production de potentiel méthane [JJ18], de la qualité des digestats [JJ04] et du carbone minéralisable au sol [JJ18]

Enfin, dans le cadre du projet Concept-Dig, après avoir mis en évidence l'impact des intrants sur la qualité des digestats, nous avons désiré utilisé les rations massiques des intrants comme variables explicatives d'un modèle PLS de prédiction de la qualité agronomique des digestats. Les intrants ont été répartis en 10 catégories (Fumier, lisier non ruminant, lisier ruminant, ensilages, résidus de cultures, CIVEs, végétaux, biodéchets, déchets graisseux, autres déchets). Des modèles PLS ont été établis sur un jeu de données de 50 digestats afin de prédire les 10 paramètres définissant leur composition (MS, MO, Ctotal, Ntotal, C/N, C/Norg, P_2O_5, K_2O, NH_4, NH_4/Ntotal). Les résultats sont illustrés par la Figure 38 : Schématisation de la prédiction de la qualité agronomique des digestats obtenu dans le projet Concept-Dig [PO7], [JJ33]. A partir de la composition prédite et à l'aide d'un calcul de distance euclidienne, le type de digestat peut être estimé ainsi que les propriétés agronomiques associées [PO7] [JJ33].

Ainsi, le modèle permet de prédire la composition globale agronomique des digestats, leur typologie et associer un potentiel amendant et fertilisant. Il permet de mettre également en évidence l'impact de l'ajout d'un intrant sur la composition du digestat et son devenir au sol. Ces modèles et calculs font parti de l'outil web Concept-Dig décrit par le Fait Marquant 5.

Toutefois, ces modèles statistiques ont des limites. En plus du nombre limité d'observations, certains types de digestats ne sont pas bien représentés (notamment les digestats issus de nouveaux intrants de plus en plus utilisés en méthanisation, à savoir les digestats de CIVEs ou certains digestats en co-digestion avec des biodéchets et effluents graisseux). En perspectives, le projet Ferti-Dig que je co-coordonne avec la chambre d'agriculture de Bretagne va permettre de palier ces manques avec plus de 810 digestats inventoriés dans une base de données dont 459 digestats bruts sur lesquels nous allons pouvoir travailler afin de réaliser une mise à jour des modèles.

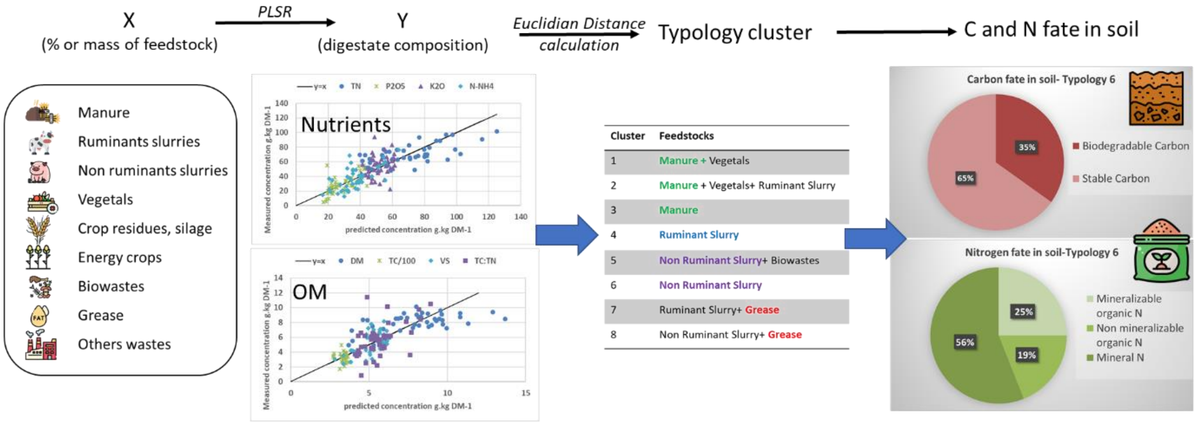


Figure 38 : Schématisation de la prédiction de la qualité agronomique des digestats obtenu dans le projet Concept-Dig [PO7], [JJ33].

Fait marquant 5: Modélisation des procédés pour le développement d'outil d'aide à la décision

L'outil développé dans le cadre du projet Concept-Dig est un autre fait marquant de mes travaux de recherche. Les variables d'entrée étaient la recette type de matière première de la digestion anaérobie pour prédire la valeur agronomique globale (composition en C, N, P, K) des digestats ainsi que leur valeur amendante/potentielle en fonction de leur typologie. Dans cet outil web, des bilans massiques basés sur des indicateurs validés (séparation de phase selon le type de procédé basés sur [JJ13]) et des valeurs d'abattement expérimentales ont permis de prédire la qualité des digestats traités après séparation des phases et stockage/compostage. L'outil a plusieurs entrées : (i) en renseignant la recette en pourcentages massiques des 10 catégories d'intrants possibles, (ii) en renseignant en tonnage la recette des intrants, (iii) en renseignant directement les analyses connues d'un digestat.

Dans les deux premiers cas, des modèles statistiques permettent de prédire 10 paramètres physico-chimiques définissant la composition agronomique globale des digestats (MS, MO, Ctotal, Ntotal, C/N, C/Norg, P_2O_5, K_2O, NH_4, NH_4/Ntotal).

Dans les trois cas, la typologie associée est prédite et donne des caractéristiques agronomiques associée à cette typologie (potentiel amendant, carbone labile et stable, azote disponible, azote organique minéralisable ou peu minéralisable). Ces indications proviennent des données disponibles moyennes inventoriées pour chaque typologie de digestats.

Ensuite, quelle que soit l'entrée choisie, l'utilisateur peut créer des scénarios de traitement des digestats (séparation de phases par presse à vis ou centrifugation, stockage des digestats bruts, liquide et solide).

Cet outil permet de mieux prédire la qualité des digestats en fonction des intrants et des post-traitements afin de proposer une utilisation *ad hoc* dans les sols agricoles. C'est la première fois qu'un tel outil est proposé pour la gestion des digestats et mis à la disposition des propriétaires de digesteurs (agriculteurs) et des instituts techniques.

Ce web tool est disponible sur le site web de la plateforme GRDF sur la méthanisation (https://projet-methanisation.grdf.fr) et sera mis à jour via le projet Ferti-Dig en 2023/2024.

4.3. Optimisation multi-objectif d'une filière de méthanisation

L'ingénierie inverse au coeur de ma recherche repose sur une démarche d'optimisation multi-objectifs dont les variables cibles sont les services rendus de la bioraffinerie environnementale, les paramètres à optimiser sont les leviers d'action ayant un impact significatif sur lesdits services rendus et les contraintes sont définies selon les impacts environnementaux déterminés.

Dans le cas de la filière méthanisation, les services rendus sont notamment la production d'énergie, les services agronomiques du digestats (qualité globale et fine du C, N, P, K) selon les besoins sols/plantes, les contraintes sont associées aux impacts environnementaux (limitation volatilisation azote ammoniacal, lixiviation nitrates, emissions gaz à effet de serre) auxquelles on peut ajouter des contraintes réglementaires (seuils en termes de concentrations de certains éléments par exemple),

des contraintes d'innocuité (seuils en micropolluants) et enfin des contraintes territoriales comme le gisement des substrats disponibles.
Les paramètres à optimiser sont les paramètres identifiés comme leviers d'action dans l'axe 2, à savoir le type de substrats et leur qualité, la recette entrante (ration de chaque substrat), les conditions opératoires du procédé de méthanisation et de ses post-traitements.

Toutefois, il s'agit-là d'un objectif ambitieux qui nécessite au préalable de pouvoir modéliser la filière de traitement selon le degré d'optimisation souhaité et des variables à calculer, d'avoir les informations en besoins des agroécosystèmes et de trouver l'algorithme d'optimisation approprié.
En effet, actuellement, la modélisation de la filière n'intégre pas les calculs des impacts environnementaux, et, malgré un premier effort sur le couplage des modèles, elle n'inclut pas l'utilisation des données simulées pendant les procédés pour calculer le devenir des éléments à la fois au sol et dans la plante.
Afin d'apporter des réponses à ces problématiques, je co-dirige la thèse de Margaud Pereme (2020-2023) sur l'optimisation multi-objectif pour l'éco-conception d'une filière de valorisation des résidus organiques via la méthanisation. Les questions de recherche associées sont : comment coupler des modèles pour une représentation globale de la filière de méthanisation, des procédés au sol/plante en considérant les impacts environnementaux ? Comment trouver un compromis entre les objectifs parfois contradictoires de la filière méthanisation via l'utilisation d'un algorithme d'optimisation multi-objectif ?

Concernant la première question, la méthodologie utilisée pour le couplage des modèles a consisté à sélectionner et developper des modèles capables de représenter les variables d'intérêt, de réaliser le dit couplage et de coupler le modèle mécanistique de filière à une analyse de cycle de vie pour simuler les impacts environnementaux. Comme explicité en introduction de l'axe 3, la stratégie retenue afin de réaliser le couplage de modèles mécanistiques est d'utiliser les mêmes variables d'état tout au long de la filière. Etant donné les recherches et résultats obtenus, nous avons opté pour le modèle basé sur ADM1 et modifié en considérant les fractions IsBaMO® comme variables d'état, comme expliqué dans le paragraphe précédent [JJ34] en intégrant le devenir de l'azote organique (Bareha et al., 2018). Les post-traitements (séparation de phase et stockage) ont été basés sur des bilans matière comme décrit dans [JJ13] et (Bareha et al., 2021).

Toutefois, les modèles de sol et cultures de la littérature ne correspondaient pas à l'objectif de modélisation de la filière. L'objectif de ce modèle n'est pas de « challenger » les modèles existants de sol (CANTIS, STICS, etc…) mais de pouvoir utiliser les variables décrivant les sorties du modèle de méthanisation afin d'évaluer les services rendus agronomiques associés via le devenir du digestat après épandage au sol et son adéquation avec les besoins d'une culture (N). Ainsi un modèle de sol/culture a été développé afin de décrire la biodégradation de la MO (C et N) des fractions IsBaMO® (Figure 39). Ce modèle a ensuite été associé à des modules de calcul pour la volatilisation du NH_3, la lixiviation des nitrates, la prise en compte de l'humidité relative du sol en fonction du climat et enfin la croissance de la plante (AquaCrop, FAO).

Dans le cadre de ce travail, une expérimentation en incubation sur sol de deux digestats de caractéristiques différentes (i.e. C/N et nature d'intrant) a été mise en place et suivie en mode litter bag pour suivre la biodégradation de la MO des digestats. Les résultats ont permis de rédiger une publication en cours de relecture [JJ35] et de montrer que le fractionnement de la MO et de l'azote est capable de décrire les cinétiques de biodégradation des digestats dans le sol mais que la connaissance préalable de la complexité et du taux de biodégradation de chaque fraction dans le sol est nécessaire à l'utilisation du modèle.

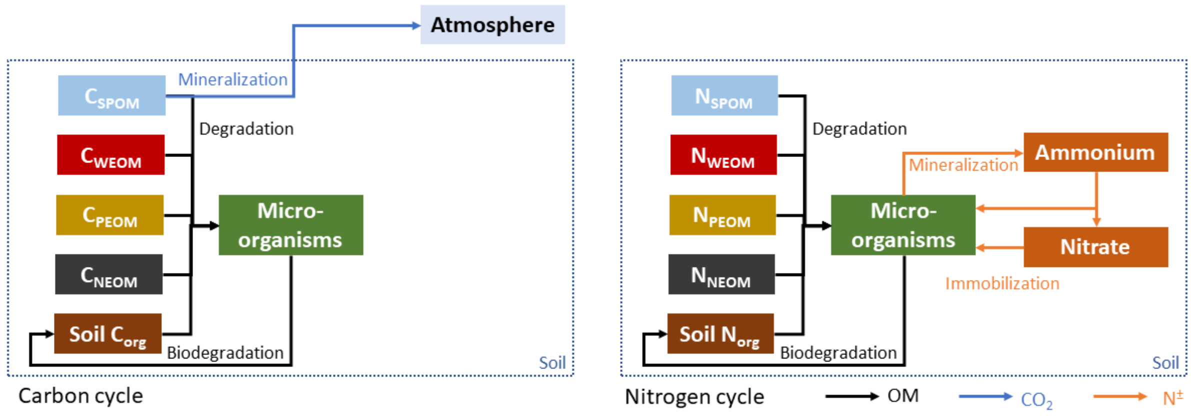


Figure 39 : Modèle de biodégradation de la MO exogène provenant des digestats dans le sol développé dans le cadre de la thèse de Margaud Pereme [JJ35]

Ainsi, une plateforme de simulation de la filière comprenant tous les procédés unitaires jusqu'au compartiment sol et plante a été codée sous Python (Figure 40). La prochaine étape consiste en l'ajout et la combinaison des simulations générées par la plateforme avec les calculs d'impact environnementaux comme réalisés dans les Analyses de Cycle de Vie (ACV). Cette étape cruciale permettra également d'avoir une idée plus précise de la sensibilité des dynamiques et leviers d'actions identifiés « procédés » sur l'analyse environnementale et d'identifier les variables d'état manquantes à modéliser.

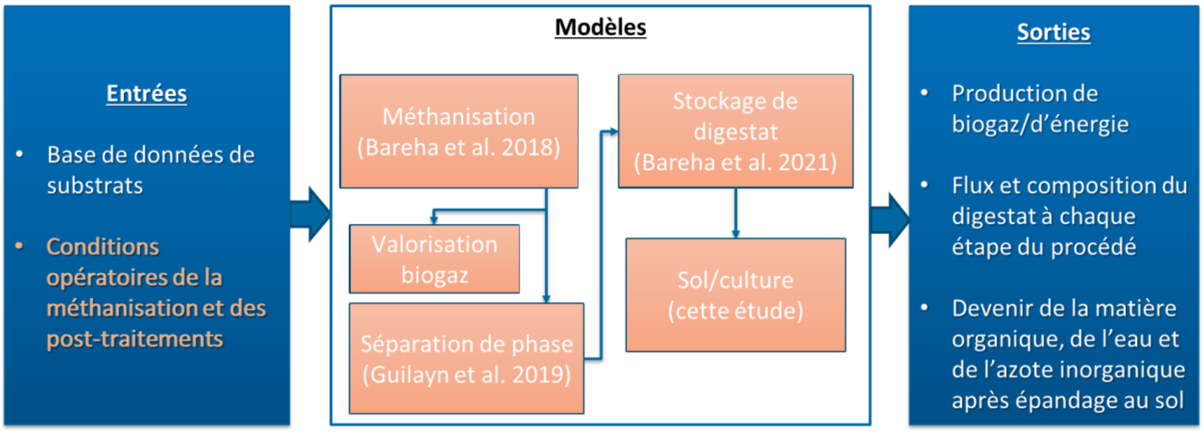


Figure 40 : Plateforme de modélisation de la filière de méthanisation développée sous Python dans le cadre de la thèse de Margaud Pereme [PO9]

Enfin, la dernière étape de ce travail ambitieux sera d'appliquer un algorithme d'optimisation multi-objectifs afin de générer divers scenarios optimaux de cas de filières de méthanisation et d'identifier les leviers d'actions les plus impactants.

4.4. Bilan

Comme pour les axes 1 et 2, le Tableau 3 présente l'état d'avancement des questions de recherche et des travaux associés. Il apparaît que l'état d'avancement est moindre que les axes 1 et 2 étant donné que ces deux derniers alimentent l'axe 3.

Tableau 3 : Bilan des travaux sur la modélisation et l'optimisation de la filière

Questions de recherche	Principaux résultats	Travaux associés	Supervision thèses, projets, ingénieurs	Etat d'avancement/ connaissance
Modélisation et simulation des services rendus	Modèle mécanistique de la méthanisation mis à jour sur la MO Modèle mécanistique proposé pour le compostage Modèles statistiques pour prédire le potentiel méthane, la qualité du digestat et in fine le devenir du carbone au sol Modèle statistique pour prédire la qualité globale agronomique et la typologie associée (propriétés moyennes en C et N de la typologie) à partir de la ration entrante Modèles bilans matières pour la séparation de phases et le stockage (Axe 2)	[PO31], [JJ10] [PO25], [PO26], [JJ34] [JJ04] [JJ18] Outil web Concept-Dig [JJ13], [PO7], [JJ33]	-Thèse CIFRE SUEZ Felipe Guilayn (2016-2019) -Thèse David Fernandez (2020-2023) - Coordination du projet Concept-Dig - Coordination du projet Ferti-Dig	
Couplage des modèles	Couplage modèles mécanistiques méthanisation, compostage et Cantis (MO, C) Couplage modèles statistiques (PLS) Couplage modèles mécanistiques et ACV (en cours)	[PO25], [PO26] [JJ04] [PO4]	- Projet Risq-Pro (post-doctorat Julie Jimenez puis Quentin Aemig) - Coordination du projet Concept-Dig - Thèse David Fernandez (2020-2023) - Thèse Margaud Pereme (2020-2023)	
Compléxité des modèles et objectifs	Utilisation de différents types de modèles (mécanistiques et statistiques) pour décrire une filière de méthanisation selon les objectifs	[JJ10], [JJ34] [JJ18] |JJ04] [PO7], [JJ33] Outil web Concept-Dig en ligne	- Thèse David Fernandez (2020-2023) - Coordination du projet Concept-Dig	
Optimisation multi-objectifs	Travaux en cours sur l'utilisation d'algorithmes d'optimisation (algorithmes génétiques)	[PO4]	Thèse Margaud Pereme (2020-2023)	

La modélisation de la filière méthanisation couplant modèles de procédés et devenir de la matière au sol a été réalisée avec succès, que ce soit en considérant des modèles statistiques ou mécanistiques. Dans les deux cas, la stratégie d'utilisation des mêmes variables d'état a été utitilisée et permet non

seulement de suivre le devenir des éléments au fil des procédés jusqu'au système sol/culture mais également d'identifier des variables d'intérêt, source d'antagonismes ou de synergies pour la prédiction des services rendus.
Toutefois, dans les deux méthodes de modélisation, orientées selon l'objectif de recherche poursuivi, des données sont nécessaires. Dans le cas des modèles statistiques, ces derniers sont groumands en données d'apprentissage et les limites en termes de précision de prédiction (extrapolation) et de de robustesse en sont impactées. Dans le cas des modèles mécanistiques, l'utilisation du fractionnement IsBaMO® dans le modèle de méthanisation requiert de réaliser cette caractérisation et d'associer la biodégradabilité de chaque fraction C et N aux indicateurs de fluorescence 3D. De même, bien que la plateforme de modélisation filière génère le fractionnement IsBaMO® C et N des digestats, la connaissance de la biodégradabilité de chaque fraction n'est pas prédite alors qu'elle est nécessaire pour décrire la biodégradation des digestats dans le modèle de sol/culture. Ce type de données serait intéressant à compiler afin de proposer des valeurs de référence dans la plateforme de modélisation.

Pour aller plus loin, en termes de verrous encore non élucidés, la modélisation de la co-digestion dans tous les modèles évoqués (sauf dans le cas de l'outil Concept-Dig) n'est pas prise en compte. En effet, un seul substrat est considéré dans les modèles mécanistiques et PLS utilisant IsBaMO® comme variables d'état et explicatives respectivement. Actuellement, la prise en compte de la co-digestion réside dans une somme pondérée des caractéristiques des intrants selon le pourcentage de ration massique de ces derniers et notamment de leur biodégradabilité, sans tenir compte de possibles synergies ou interactions entre substrats comme reporté dans la littérature où il n'y a pas de consensus sur le sujet.
De plus, le manque de simulation des impacts environnementaux dans les modèles de filière développés peut être comblée par un couplage avec de l'analyse de cycle de vie (ACV). Cet exercice pourra nous aiguiller sur les variables sensibles à modéliser et les variables manquantes à considérer.

Enfin, l'optimisation multi-objectif issue de la thèse de Margaud Pereme affinera les perspectives de recherche au niveau des leviers d'actions et du niveau de connaissance en terme de biodégradation de la matière à aller chercher et donc à modéliser, cohérent avec les impacts environnementaux et le devenir du C et N des digestats dans le sol.

Chapitre 3. Perspectives de recherche

S’appuyant sur les régulations naturelles, la conception de (Altieri, 2002) sur l’agroécologie repose sur deux piliers fondamentaux : l'amélioration de la qualité du sol et de son activité biologique, au moyen d'apports importants de MO, et le renforcement de la biodiversité végétale de surface afin d'améliorer les habitats qui abritent des biotes bénéfiques. Ces deux piliers assurent la santé des cultures et de l'ensemble de l'agroécosystème. (Jeuffroy, 2022) précise que baser la construction des modes de production sur l’agroécologie nécessite de (i) diminuer les impacts environnementaux en réduisant voire éliminant les intrants chimiques, (ii) renforcer la santé des agroécosystèmes (contrôle naturel des bioagresseurs), (iii) favoriser l’agrobiodiversité et les synergies entre les composantes de l’agroécosystème, (iv) améliorer la conservation et régénération des sols, de la ressource en eau et de la biodiversité et (v) optimiser le métabolisme des agroécosystèmes en organisant le recylage des nutriments. Il est aisément identifiable que la méthanisation peut constituer, en théorie, un des leviers d’action significatifs et contribuer à la transition agroécologique. En effet, ce procédé, s’il est maîtrisé et optimisé, peut permettre de recycler des résidus organiques, de transformer les matières en produits pouvant répondre aux besoins des agroécosystèmes tout en générant une production de gaz vert participant à répondre à l’enjeu du changement climatique, et, *in fine,* de boucler les cycles géochimiques dans un souci de bioéconomie circulaire. La variabilité des résidus organiques et des traitements, croisés à la variabilité des agrosécosystèmes, est une opportunité qui confère à l’outil « méthanisation » un potentiel de flexibilité et d’adaptabilité à l’évolution des pratiques et à la diversité de situations territoriales. Théoriquement, cette adéquation entre agroécologie et valorisation des résidus organiques via la méthanisation a un très fort potentiel. Toutefois, ce potentiel peut être atteint à conditions de maîtriser les impacts et effets de cette pratique sur les agroécosystèmes et l’environnement. Cette optimisation passe nécessairement par la gestion de compromis entre agroécologie et production d’énergie pouvant générer des antagonismes entre ces services rendus. Comme le souligne (Jeuffroy, 2022), l’agroécologie doit être raisonnée via une vision systémique en prenant en compte diverses disciplines afin d’être optimisée. Tout au long de mon mémoire, j’ai voulu répondre à diverses questions de recherches liées à cette problématique en me focalisant sur la filière méthanisation. J’ai proposé un changement de paradigme en promouvant une conception optimale de filière en réalisant de l’ingénierie inverse, c’est-à-dire en partant des besoins des agroécosystèmes et des services rendus par la filière de valorisation. A partir de cette ligne directrice, j’ai décliné ma problématique de recherche en trois axes : (i) caractérisation via le développement d’indicateurs, (ii) procédés et identification des leviers d’actions et enfin (iii) modélisation et optimisation. Suite au bilan décrit dans le Chapitre 2, des travaux ont amendé chaque partie mais des verrous de connaissance et certains points n’ont pas encore pu être abordés, comme l’exercice d’optimisation en tant que tel.

Dans la suite de mes travaux de recherche, je souhaite renforcer mes recherches autour de la filière méthanisation dans un contexte de transition agroécologique, avec une forte interaction avec les besoins « terrains » des acteurs de la filière. En effet, je souhaite renforcer les liens déjà établis avec les agriculteurs méthaniseurs, acteurs centraux de la thématique. La gestion des compromis décrite précédemment est un enjeu primordial lié à cette question de recherche et je souhaite approfondir l’identification des antagonismes et des synergies entre production d’énergie et production de fertilisants organiques recyclables en agriculture. J’ai abordé cette notion dans le Chapitre 2 en définissant la méthodologie à suivre pour y parvenir, notamment en identifiant les services rendus, en proposant des indicateurs pour les quantifier, en investiguant les leviers d’actions (procédés et intrants) impactant les services rendus et en formalisant les résultats par des modèles de prédiction. Toutefois, il est nécessaire d’approfondir l’évaluation des antagonismes et des synergies en me basant sur les résultats obtenus.

De plus, j'ai à cœur de rentrer dans le vif du sujet concernant l'exercice d'optimisation à proprement parlé. Tout au long de mes recherches, j'ai proposé les outils nécessaires (indicateurs et modèles) pour mener à bien cet exercice. La suite logique est donc désormais de les utiliser à ce but afin d'affiner les questions de recherche à creuser. Le schéma de ma problématique de recherche présenté dans le Chapitre 2 (Figure 5) a été mis à jour (Figure 41) et propose de se focaliser sur **l'Ingénierie Inverse au service de l'optimisation multi-objectif de la filière** en répondant aux trois questions de recherche suivantes :

- Comment utiliser la caractérisation de la matière pour gérer les compromis entre services rendus (définition approfondie des besoins des agroécosystèmes, identification des variables pertinentes à suivre à partir d'une analyse de sensibilité poussée des outils développés et approfondissement de la connaissance et description des dites variables) ?
- Quels sont les processus biologiques impliqués dans le devenir de la matière dans la filière méthanisation ayant un impact sur les agroécosystèmes (approche mécanistique, identification leviers d'actions) ?
- Comment la compréhension des processus et les indicateurs développés permettent de répondre à l'optimisation de la filière de valorisation des résidus organiques dans une approche multi-critère ?
- Comment l'optimisation multi-objectif va permettre d'affiner les questions de recherche relatives aux axes 1 et 2 et à son tour d'alimenter ces deux axes ?

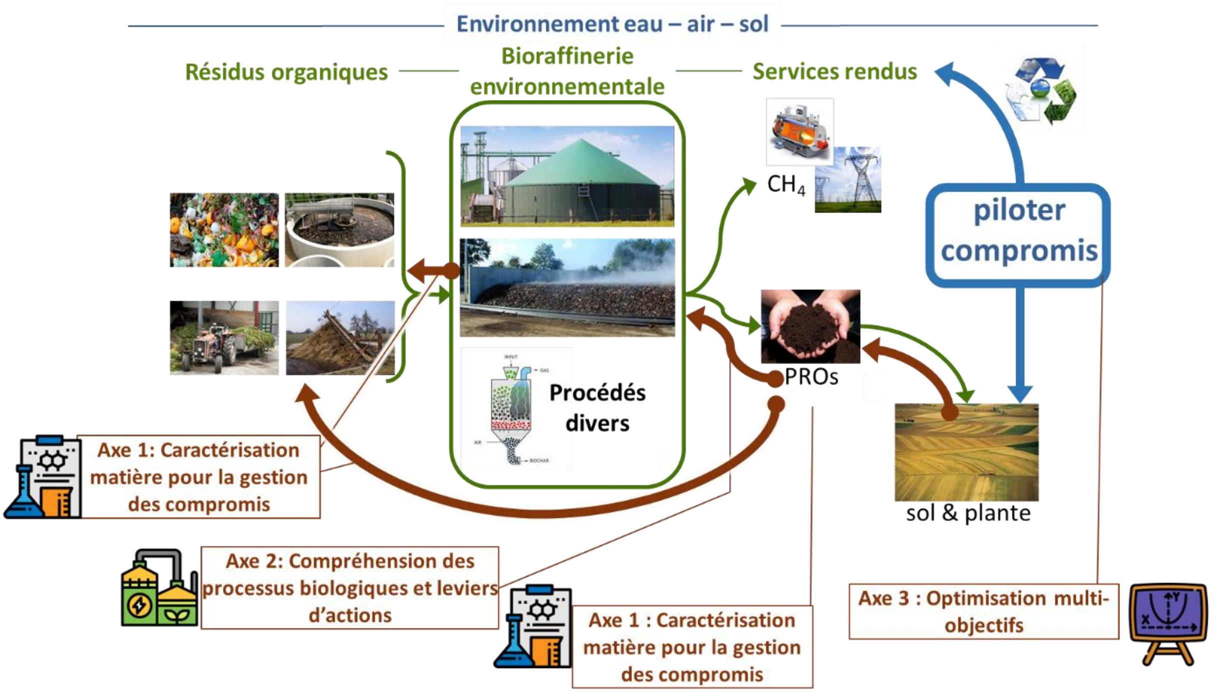


Figure 41 : Mise à jour de ma thématique de recherche déclinée en trois axes

Il est à noter que le système optimisé visé est la filière « procédés », du recettage optimum des intrants à la sortie de la filière. Cet exercice s'inscrit dans une volonté d'optimisation plus globale à l'échelle des exploitations, sous contraintes environnementales et sanitaires.

1. Axe 1 : Caractérisation et gestion des compromis

1.1. Variables de caractérisation pour affiner la description des services rendus (énergétiques et agroécosystémiques)

Dans un premier temps, je souhaite approfondir et affiner les liens entre produits issus des procédés de valorisation et besoins des agroécosystèmes afin de mieux identifier les synergies et antagonismes sur l'utilisation de la MO et des nutriments.

En effet, lors de mes travaux de recherche, j'ai commencé à définir les besoins des agroécosystèmes et des services écosystémiques rendus afin de proposer des indicateurs associés. Ces indicateurs ayant été validés par rapport aux réponses expérimentales biologiques, des questions restent en suspens concernant la qualité de la matière pour répondre aux besoins des agroécosystèmes, et plus précisémment quelles variables issues de ces indicateurs sont à cibler/investiguer. Si le raisonnement est poussé un peu plus loin et que ces besoins sont analysés, tous les ingrédients nécessaires à les satisfaire sont présents en théorie dans les digestats (Figure 42).

Si l'on synthétise les besoins des agroécosystèmes, on peut distinguer trois fonctionnalités du sol à alimenter à plus ou moins court-long terme, à savoir la fertilité biologique (promouvoir la biodiversité via la mesofaune et microfaune du sol), la fertilité chimique (apporter les nutriments nécessaires, sous la bonne forme disponible, aux plantes et sol) et la fertilité physique (apporter de la MO stable pour garantir une bonne structure du sol). Donc théoriquement, les ingrédients sont là mais sous quelle forme ? Sont-ils disponibles, bioaccessibles, complexes ? Quels impacts environnementaux associés ? Quels effets sur les agroécosystèmes ?

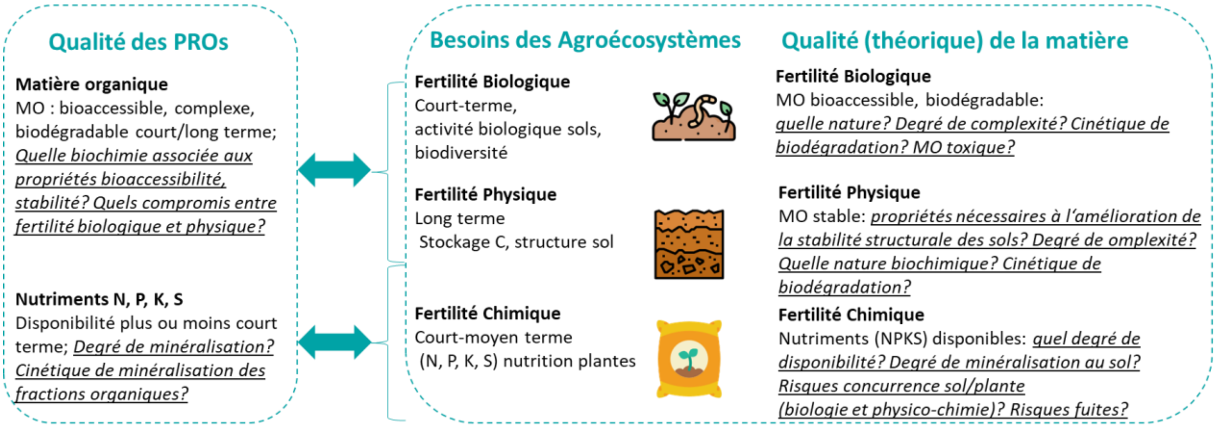


Figure 42 : Adéquations et besoins des agroécosystèmes : théorie et questions de recherche

Des indicateurs de caractérisation pour décrire la bioaccessibilité, la complexité et la biodégradabilité de la MO ainsi que la disponibilité des nutriments (N et P) ont été developpés et validés pour prédire certains des services rendus. Toutefois, la définition d'autres services rendus, comme l'utilisation de la MO par les microorganismes du sol, reste globale (carbone minéralisable au sol à court/moyen terme) et ne permet que partiellement d'apporter des éléments de réponse à la question sur l'impact de la méthanisation sur la MO du sol (à court, moyen et long terme).
En effet, il manque à définir un « cahier de charges » sur les propriétés spécifiques des besoins des agroécosystèmes pour réaliser une adéquation optimale entre qualités des PRO et les dits besoins.

Ainsi, je souhaite aller plus loin dans cette étude et répondre à plusieurs questions de recherche comme suit :

- Quelle qualité de MO serait plutôt souhaitable afin de mieux promouvoir la biodiversité ?
- Des molécules de type substances humiques-like seraient-elles à privilégier dans les digestats pour améliorer la stabilité structurale du sol ? La biodiversité ? Quel compromis à trouver entre MO labile et MO stable pour améliorer l'état d'un sol ? Quelle échelle de temps de biodégradation pour ces molécules ?
- Comment mesurer et quantifier les molécules conférant des propriétés de MO stable au digestat afin d'étudier leur provenance et devenir ? Est-ce que les indicateurs actuels sont suffisant pour cela ?
- Est-il est préférable de garder l'azote sous forme organique en contrôlant sa minéralisation au sol pour prévoir l'apport en azote aux plantes au moment désiré et ainsi prévenir les problèmes de volatilisation ?
- La méthanisation seule ou combinée avec d'autres procédés peut-elle répondre à ces besoins ? Si oui, comment ?

Ainsi je souhaite davantage me rapprocher de chercheurs (INRAE et hors INRAE) travaillant sur ces diverses fonctionnalités du sol afin de coupler nos approches et de mieux comprendre la biodégradation de la MO dans les sols et le devenir des nutriments dans les agroécosystèmes.

En parallèle, la gestion des compromis entre services rendus sur l'usage de la MO et nutriments déjà explicitée dans le Chapitre 2 n'a pas été approfondie. L'objectif est de mieux connaître la matière et son devenir dans les procédés et au-delà afin de contrôler sa production. La Figure 43 schématise ma vision de l'Axe 1 mis à jour avec les questions de recherche associées.

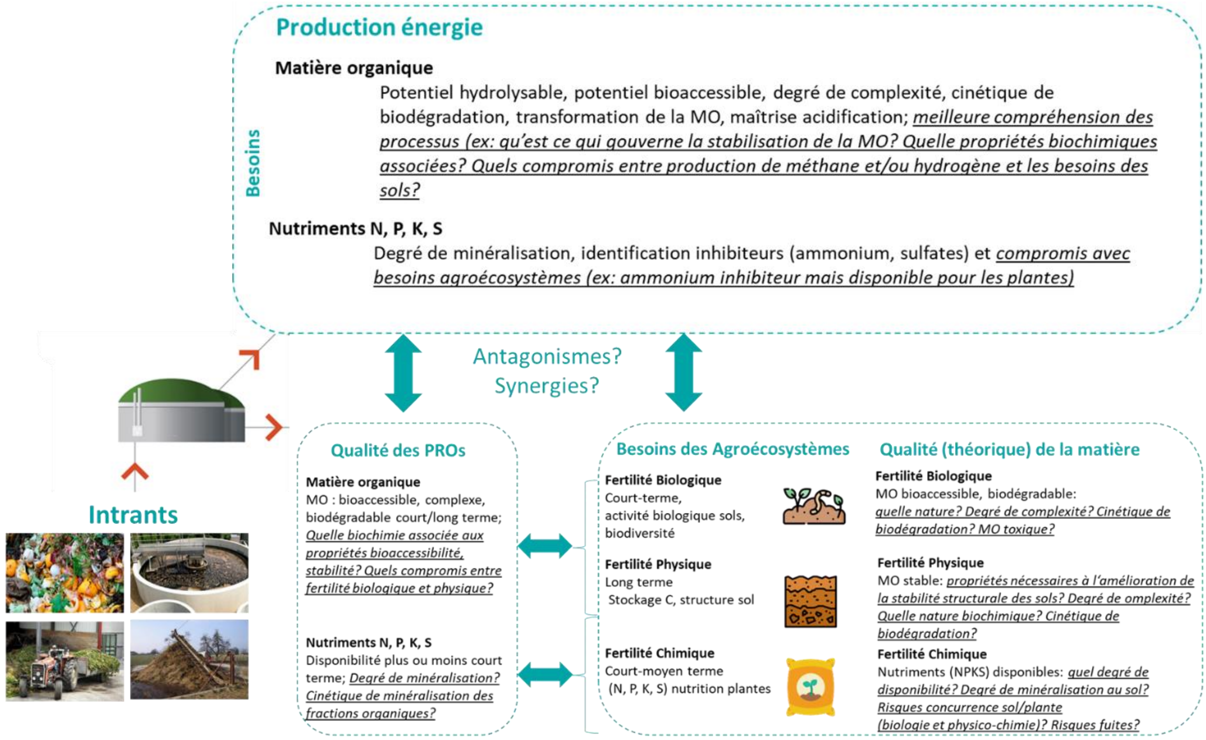


Figure 43 : Schématisation des questions de recherche associées à la gestion des compromis entre qualité des résidus organiques pour satisfaire les besoins pour la production d'énergie (méthanisation) et qualité des produits pour satisfaire les besoins des agroécosystèmes

Il est apparu très clairement, comme déjà évoqué dans le Chapitre 2, que des notions telles que la bioaccessibilité de la MO, sa complexité et la disponibilité des éléments sont des notions communes nécessaires à une meilleure compréhension des besoins des agroécosytèmes et de la production d'énergie. Toutefois, les microorganismes impliqués dans les processus biologiques, leur biodiversité et synergies, les conditions opératoires (procédés) et pédo-climatiques (pour le sol) et les temps de dégradation ne sont pas les mêmes dans les bioprocédés et dans les sols. C'est pourquoi une meilleure connaissance des processus dans ces deux systèmes est importante afin de les coupler. En outre, un travail doit être mené de concert avec des spécialistes de la biodégradation de la MO dans les sols afin d'identifier les méthodes pertinentes à tester pour aller plus loin. Par exemple, des mesures de carbone organique RockEval sur les digestats ont commencé à être réalisées. Cette méthode de dégradation thermique a historiquement été mise en place afin de quantifier la teneur en carbone organique et le potentiel pétrolier des roches mères génératrices d'hydrocarbures. Elle a permis plus récemment de quantifier la teneur en carbone organique des sols. Des approches interprétatives spécifiques ont été proposées pour analyser la part de la MO des sols la plus réactive ou pour quantifier la part de carbone persistant dans le sol à l'échelle centennale.

D'autres méthodes innovantes peuvent également être regardées en collaboration comme la caractérisation via l'étude des propriétés énergétiques de la MO liées à son utilisation par les microorganismes des sols (Bölscher et al., 2016).

1.2. Variables de caractérisation fine pour la compréhension des processus

Dans un premier temps, je souhaite utiliser les données générées par l'axe 1 et les indicateurs développés afin de réaliser une analyse de sensibilité de la filière globale pour avoir une première réponse sur le poids des variables issues de la caractérisation sur celui des variables prédites (potentiel méthane, C_bio, N_miné…). La démarche que je souhaite mener est schématisée par la Figure 44.

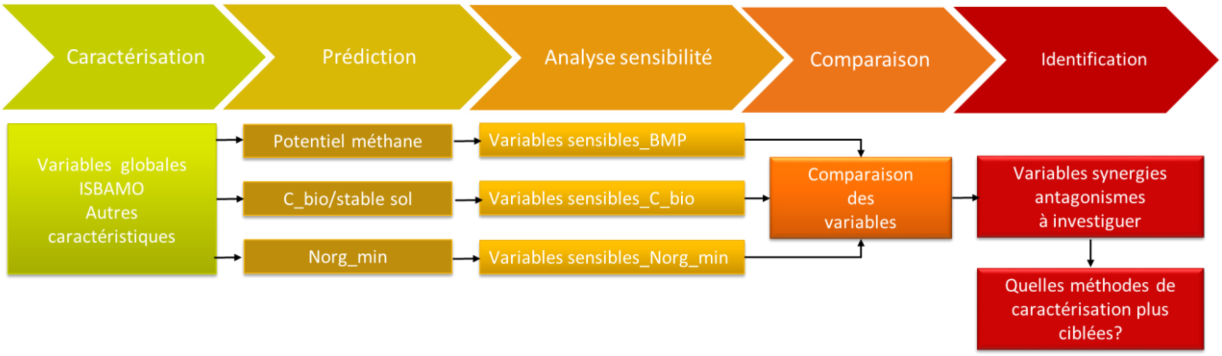


Figure 44 : Démarche à mettre en place pour déterminer les variables de caractérisation sensibles à la prédiction des services rendus

En effet, le modèle statistique PLS établi dans [JJ04] peut être couplé avec les modèles de prédiction du potentiel méthane (BMP) et du potentiel de Carbone minéralisable au sol (C_bio) publié dans [JJ18] puisque les variables explicatives sont les mêmes (i.e. IsBaMO®).

Comme évoqué précédemment, une analyse de sensibilité est à mettre en place sur les modèles de prédiction de la qualité des digestats et des services rendus afin d'évaluer l'impact de la méthanisation d'un substrat donné sur la qualité amendante de son digestat et sur son potentiel méthane. En comparant les variables ayant un poids significatif sur les différentes prédictions, les variables de caractérisation responsables des synergies et antagonismes sur lesquels aller investiguer peuvent être identifiées.

Dans un second temps et suite à cette analyse, les verrous de connaissance pourront être identifiés, les manques à combler en termes de caractérisation, voire le type de molécules à aller investiguer pour identifier des méthodes analytiques complémentaires pertinentes.

En outre, cet exercice viendra en parallèle alimenter l'axe 2 sur l'évaluation de leviers d'actions.

Par exemple, est-ce que certaines variables de caractérisation sont communes aux services rendus BMP et C_bio ? Si oui, comment gérer le compromis entre les deux et si non, comment faire en sorte d'augmenter cette synergie ?

Une revue de littérature réalisée dans le cadre de la thèse de David Fernandez-Dominguez [JJ01] a mis en évidence que des techniques spectrales complémentaires (comme Nuclear Magnetic Resonance et spectroscopie moyen infra-rouge) pourraient apporter un éclairage nouveau. Cette revue a également permis d'étudier les données existantes de la littérature en termes de devenir de la MO en méthanisation et d'ouvrir des perspectives d'études. Par exemple, l'identification de molécules complexes telles que les substances humiques-like pourraient permettre de mieux comprendre les processus de stabilisation de la MO pendant la méthanisation. En parallèle, la thèse de David Fernandez-Dominguez a mis en évidence que les méthodes statistiques d'interprétation des données spectrales avaient un impact signifcatif sur l'interprétation des résultats. Par exemple, en fluorimétrie 3D, l'utilisation de méthodes alternatives à celle développée et utilisée jusqu'à présent (régionalisation des spectres) pourrait permettre de considérer l'ensemble du spectre et de mieux appréhender la dynamique d'un changement dans un bioprocédé. Un travail en collaboration avec des statisticiens et des experts de la chimiométrie permettra de donner un nouvel éclairage aux données et d'aller plus loin dans les interprétations et conclusions, voire les prédictions.

Enfin, on pourrait s'inspirer des travaux de thèse de (Robin Beghin, 2020) sur l'utilisation de l'isotopie du C et réaliser un suivi de molécules (C et N) sur l'ensemble d'une filière jusqu'aux services rendus afin d'aller plus loin dans cette évaluation de synergie/antagonismes, en utilisant des intrants de qualité de MO diverse.

1.3. Elaboration d'une base de données pour l'utilisation de la plateforme de modélisation

L'axe 3 a abouti à des modèles mécanistiques et statistiques capables de prédire divers services rendus de la filière. Or, ces modèles, notamment les modèles mécanistiques, sont gourmands en données et en paramètres stoechiométriques (i.e. fraction biodégradable du C et degré de minéralisation du N) et cinétiques (i.e. constantes d'hydrolyse).

L'IsBaMO® est un indicateur relativement rapide à obtenir mais l'estimation de la biodégradabilité de chaque fraction associée à sa constante d'hydrolyse nécessite soit de faire un bilan matière au sein du réacteur, soit de réaliser des tests de biodégradation sur des digestats acclimatés, expérimentations coûteuses en temps. Il en est de même pour le compostage où le potentiel de compostabilité est, pour le moment, géré par le banc de compostage mis en place, très long à suivre. Un des objectifs de cet axe est de continuer d'amender une base de données intrants avec les caractérisations matière associées ainsi que l'estimation de leur cinétique et degré de biodégradation en réalisant des tests BMP avec digestats acclimatés, des bilans matières et des identifications de paramètres sur les courbes de production de méthane obtenus. Durant les études menées dans l'axe 1 ces dernières années, j'ai pu trouver des corrélations entre fractions biodégradables et indice de complexité issu de la fluorimétrie de chaque fraction. Cette base pourrait valider cette corrélation et permettre de s'affranchir, *in fine*, de tels tests coûteux en temps.

La Figure 45 présente la démarche envisagée. Les intrants caractérisés seront également (quand cela est possible) caractérisés en termes de biodégradabilité anaérobie de la MO (C et N). Les tests laboratoires où une caractérisation fine (IsBaMO®) a été réalisée seront également pris en compte et inventoriés, que ce soit en méthanisation et compostage.

A partir de bilans matières et d'identification de paramètres de biodégradation, voire de calage de modèles, la base de données sera alimentée par l'ensemble de ces paramètres.

De plus, lorsque cela sera également possible, les digestats et/ou composts (voire certains intrants) seront mis en incubation sol afin de récupérer des données en C et N minéralisable et de compléter les données actuelles des modèles statistiques.
Un premier stage prévu début 2023 dans le cadre de la la thèse de Margaud Pereme va commencer ce travail d'alimentation de la base, avec notamment un focus sur les tests BMP et une mise en place d'un protocole expérimental systématique à réaliser (inoculum acclimaté, suivi méthane, azote ammoniacal et bilan masse des fractions C et N).

Enfin, j'ai l'ambition d'aller jusqu'à la création d'un outil logiciel (base Rshiny) afin que les données soient facilement entrées, mises à jour et que l'on puisse visualiser, via des tests statistiques, les échantillons caractérisés et mettre à jour les modèles statistiques, voire en créer d'autres pour prédire des paramètres utiles à la modélisation à l'aide du machine learning. En effet, aujourd'hui, les bases de données ne sont pas systématiquement mises à jour et donc la validité des modèles créés non plus.

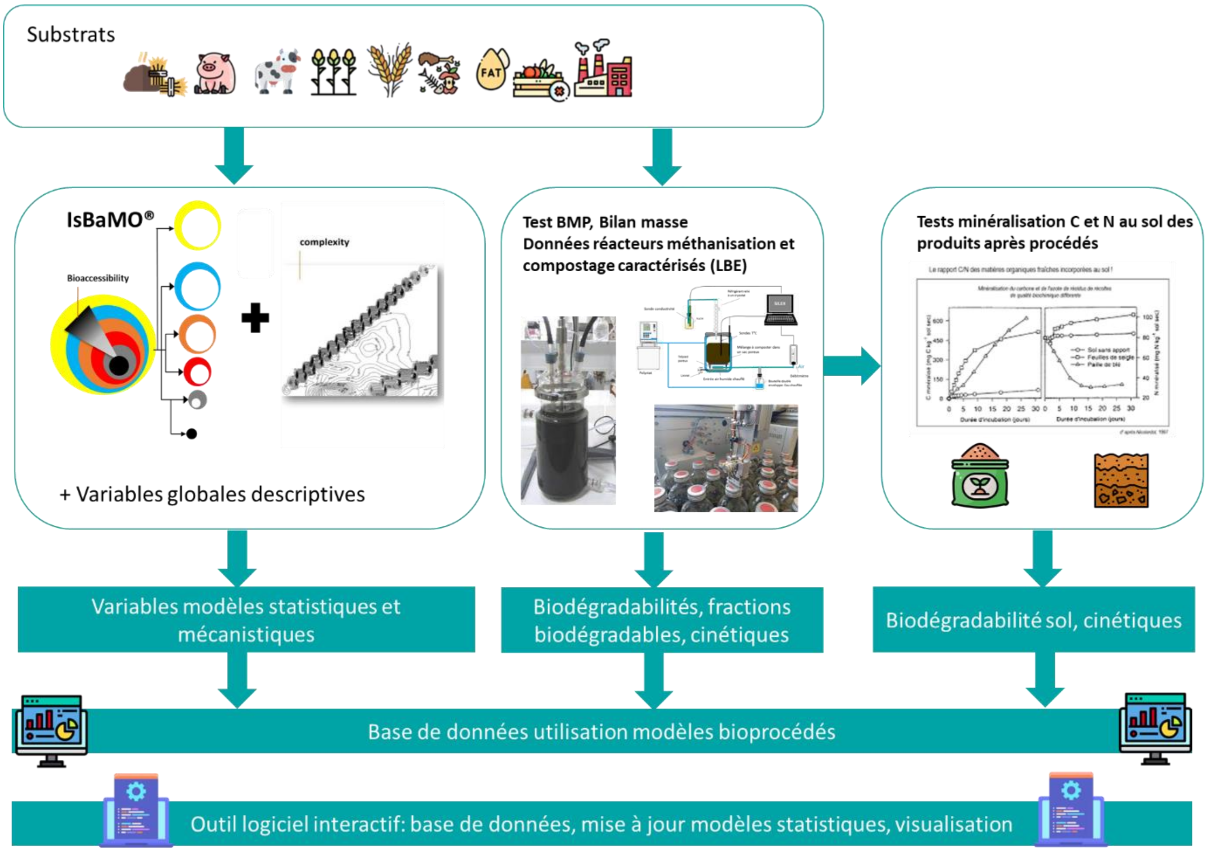


Figure 45 : Démarche envisagée pour mettre en place une base de données de caractérisation IsBaMO® durable et utilisable pour réaliser la modélisation de filière

2. Axe 2 : Compréhension des processus biologiques et leviers d’action

2.1. Etude des processus biologiques

Afin d’optimiser l’adéquation entre procédés et besoins issus des services rendus, les travaux commencés sur l’étude de la transformation de la MO pendant les procédés biologiques telles que la méthanisation sont à poursuivre. En effet, (Houot et al., 2022) a réalisé un premier bilan concernant l’impact de la méthanisation sur divers substrats (effluents d’élevage, CIVEs). Il apparaît que globalement la méthanisation a un effet de « stabilisation » de la MO observable expérimentalement via les cinétiques de minéralisations du C au sol. De plus, il a été observé que la fraction soluble d’un digestat a une couleur spécifique due aux acides humiques et acides fulviques. Des analyses spectrales ont corroboré cela [These_JJ] et des premiers tests de caractérisation des acides humiques par extraction alcaline également (Thèse Felipe Guilayn, 2019 ; projet Concept-Dig, 2019). Toutefois ces observations peuvent dépendre également des intrants de la méthanisation et des produits microbiens sécrétés selon les conditions opératoires des procédés.

Plusieurs hypothèses peuvent être émises afin d’expliquer les évolutions de la matière pendant la méthanisation mais restent à valider. Notamment l’augmentation de la complexité de la fraction DOM et des fractions facilement extractibles : est-ce dû seulement à la biodégradation de molécules simples ? A la génération de produits microbiens complexes ? A de l’humification ? Aux produits de l’hydrolyse des fractions moins accessibles vers les plus accessibles ? Comment découpler les processus pour les étudier ?

Toutes ces questions ont permis de proposer le sujet de thèse de David Fernandez-Dominguez en 2020 sur la caractérisation et modélisation du devenir de la MO pendant la méthanisation pour une meilleure utilisation des digestats en agriculture (Figure 46).

Afin de mieux comprendre ce phénomène de stabilisation et de mieux le piloter, la thèse s’intéresse à la part des molécules récalcitrantes à la biodégradation anaérobie provenant de sources exogènes (les intrants) et endogènes (les micro-organismes).

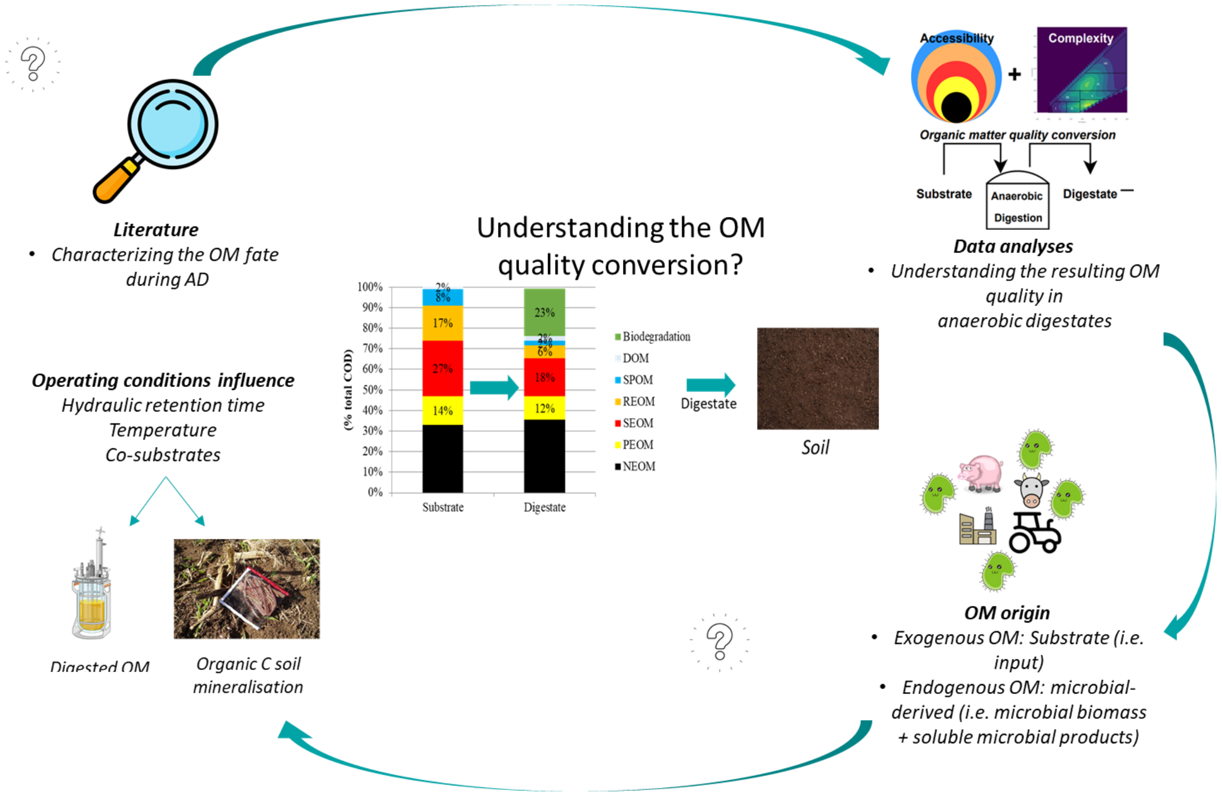


Figure 46 : Graphical abstract issu de la thèse de David Fernandez-Dominguez (2020-2023)

Les résultats sont en cours mais ont déjà commencé à montrer l'impact des produits endogènes sur la fraction soluble des digestats et que ces derniers peuvent avoir une signature de fluorescence différente selon le type d'inoculum [JJ36]. Une caractérisation via la Nuclear Magnetic Resonance (NMR) en collaboration avec l'université de Linköping (Suède) et une caractérisation des microorganismes ont été utilisées pour également caractériser la MO en sortie de digesteurs pilotes alimentés par des intrants réels versus intrants synthétiques. L'objectif était de découpler les sources de MO récalcitrante exogène (intrant réel) de la production de MO récalcitrante exogène (intrant synthétique biodégradable). Les résultats sont en cours d'interprétation. Certains sont prometteurs avec notamment une hypothèse sur l'impact des voies métaboliques sur la qualité de la MO comme déjà proposée par certains articles de la littérature [JJ01]. Comme proposé dans l'axe 1, l'utilisation d'un traçage isotopique pour découpler MO exogène/endogène pendant la méthanisation, avec divers intrants et conditions opératoires, pourrait permettre de quantifier les flux et la part de chacune dans la MO contenue dans le digestat.

De plus, afin de palier au manque de découplage intrants/conditions orpétaoires de la méthanisation, l'impact de ces dernières comme le temps de séjour, la température et la ration d'intrants sur la qualité de la MO et son devenir au sol seront également évalués dans le cadre de cette thèse.

En outre, comme décrit dans les perspectives de l'axe 1, l'analyse de sensibilité des modèles mis en place va permettre d'identifier des variables de caractérisation à investiguer et donc d'améliorer la connaissance des processus biologiques de la méthanisation pour mieux les modéliser et optimiser la filière dans son ensemble. Dans ce sens les axes 1 et 2 évolueront de concert et continueront d'apporter des éléments pour l'axe 3.

2.2. Leviers d'actions et hiérarchisation

Concernant l'identification des leviers d'actions, les travaux sur l'étude de la variabilité des digestats se poursuivent. Il a été montré précédemment par diverses études ([JJ12], [PO7], [JJ33]) que la variabilité des intrants et la variabilité des traitements ont un impact significatif sur la qualité agronomique des digestats et une typologie a été dans un premier temps proposée. Cette variabilité est une opportunité afin de mettre en adéquation digestat produit et besoins des agrosystèmes (Figure 47). Toutefois, cette variabilité que l'on souhaite rationnaliser doit être raisonnée avec la variabilité des pratiques agricoles afin de répondre aux verrous de connaissance et questions associées à la variabilité des effets observables sur les agroécosystèmes : y- a-t-il une typologie d'effets, d'impacts sur les besoins des agroécosystèmes ? Quelles relations avec les variabilités citées précédemment ? Et si oui quelle hiérarchie ?

Afin de répondre en partie à cette question, le projet Ferti-Dig que je coordonne (ADEME-GRDF, 2021-2024) est en cours. L'ambition de Ferti-Dig est d'approfondir la typologie des digestats proposée par Concept-Dig en inventoriant les données disponibles à la fois dans la bibliographie et en France. L'objectif principal étant de produire un guide d'utilisation agronomique des digestats basé sur leurs typologies, à destination des agriculteurs et des conseillers agricoles. Les questions de recherche autour de la corrélation entre typologies des digestats et typologies des effets vont être également regardées. Dans ce projet, les effets considérés sont l'activité biologique du sol via l'étude des vers de terre, la stabilité structurale du sol et le potentiel de fertilisation azotée via la mesure de coefficient équivalent azote issus d'essais aux champs.

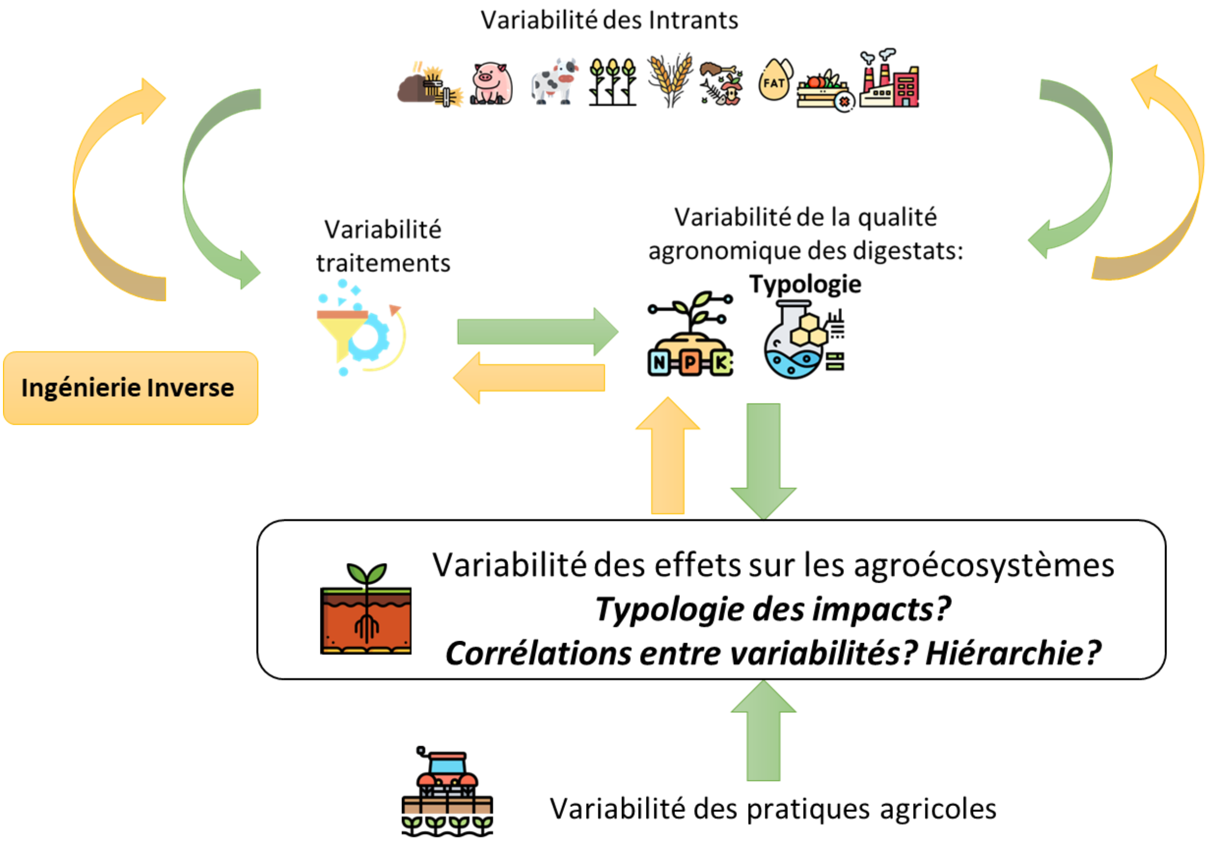


Figure 47 : Questions de recherche associées à la rationalisation et compréhension de la varibilité de la qualité agronomique des digestats et leurs impacts sur les agroécosystèmes

Les réponses apportées pourront permettre de mieux appréhender les effets des digestats sur les agroécosystèmes, de les classer selon une certaine typologie à affiner (intrants, procédés), ce qui donnera, *in fine*, l'opportunité d'utiliser la variabilité de ces produits comme levier d'action afin de mettre en adéquation les besoins des agroécosystèmes et la qualité des fertilisants organiques issus de la méthanisation via de l'ingénierie inverse.

2.3. Post-traitements

Je souhaite également poursuivre des travaux sur les traitements de digestats d'alimenter la « bibliothèques » de procédés possibles dans la filière méthanisation et plus largement de la Bioraffinerie Environnementale afin d'augmenter les possibilités de pilotage de la qualité des digestats et co-produits en sortie.

Les pertes en azote sont un enjeu pour le digestat. Certains digestats (d'origine urbaine) ont, en plus, d'autres enjeux comme la non-conformité aux normes en vigueur et le côut de transport des produits. Ainsi, je souhaite continuer à travailler sur le développement de solutions. Dans ce sens là, le projet en collaboration avec SUEZ, basé sur la Thèse de Felipe Guilayn (2019) sur les digestats d'origine urbaine va se poursuivre (cf. Fait Marquant Axe 2). Pour rappel, les deux voies de traitements considérées étaient une voie dite de « fractionnement » et la seconde de « conservation » de la matière. Dans la première, une extraction alcaline suivie d'une séparation de phases permet d'extraire des molécules d'intérêt type acides humiques ayant un fort potentiel de biostimulation pour les plantes tout en récupérant l'azote ammoniacal par stripping. L'étude et la caractérisation de ces extraits permettront également d'alimenter ma réflexion sur la compréhension des processus de stabilisation durant la méthanisation. Dans la seconde voie de traitement, un apport d'acide permet de conserver l'azote ammoniacal dans le digestat et un séchage doux en dépression permet de sécher le digestat, de concentrer sa matière et de l’hygiéniser afin de le rendre facilement transportable et utilisable en agronomie. De plus, les tests agronomiques sur l’effet biotimulant des extractions issues des digestats sont en cours ainsi que les analyses nécessaires à la normalisation des digestats acidifiés et séchés.

Enfin, étant donné que ma démarche est basée sur de l’ingénierie inverse, je souhaite également utiliser les besoins des agroécosystèmes comme cahier des charges pour concevoir des solutions innovantes en termes de procédés de traitement. En effet, la variabilité des intrants est un levier d’action significatif majeur. Toutefois, selon les installations en place et les contraintes des gisements des résidus disponibles dans le territoire, il est difficile de moduler la recette intrante. Dans ces cas-là, le levier d’action « post-traitements » est une solution à envisager.

3. Axe 3 : Modélisation et Optimisation : Ingénierie inverse

3.1. Modélisation des bio/procédés

Concernant les perspectives en terme de modélisation, des verrous restent à lever et génèrent des pistes de recherche à investiguer. Jusqu'à présent, mes travaux se sont focalisés sur la modélisation des procédés et du devenir de leur qualité, que ce soit via des modèles mécanistes ou statistiques. La thèse en cours de Margaud Pereme (2020-2023) a déjà permis de mettre en place une plateforme de simulation couplant modèles de méthanisation, post-traitements classiques et devenir des digestats au sol. Toutefois, ces modèles sont gourmands en données d'entrées et en calage de paramètres stoechiométriques (i.e. fraction biodégradable du C et degré de minéralisation du N) et cinétiques (i.e. constantes d'hydrolyse). C'est pourquoi, je souhaite continuer la compilation de données de susbtrats caractérisés avec, en parallèle, l'identification des paramètres associés comme évoqué dans les persceptives de l'axe 1.

Un verrou important a été identifié dans l'axe 1 du Chapitre 2 : la modélisation de la co-digestion comme illustrée par la Figure 48. En effet, que ce soit dans les modèles mécanistiques ou statistiques, les données d'entrée ont été caractérisées par intrant voire par mélange d'intrants déjà préparé. Or, malgré le fait que le fractionnement IsBaMO® soit additif, la fluorescence ne l'est pas et les questions sur les cinétiques d'hydrolyse, les synergies voire les interactions entre substrats n'ont pas été résolues. En effet, dans la littérature, les cinétiques de biodégradation entre co-substrats peuvent être additives selon le type de substrats et leur nombre. Par exemple, en considérant deux substrats, l'additivité existe (Kouas et al., 2018) mais au-delà et selon le type de substrats, cette additivité ne fait plus consensus et des phénomènes d'interactions apparaissent (Karki et al., 2022, 2021; Pagés Díaz et al., 2011; Xie et al., 2017). Ainsi, lorsque nous sommes face à un problème de simulation de la méthanisation avec plusieurs intrants (comme c'est le cas dans la majorité des méthaniseurs agricoles et territoriaux en France), comment modéliser leur biodégradation ?

Concernant la modélisation mécanistique, jusqu'à aujourd'hui, ma stratégie a été de faire une somme pondérée des fractions de chaque intrant en fonction de leur proportion en entrée, mais la détermination de leur biodégradabilité et leur constante d'hydrolyse est toujours source d'incertitude et bien souvent je cale les paramètres sur des données expérimentales. Ainsi, si l'on veut utiliser une base de données de paramètres et de paramètres associés, il faut aussi mettre en place une méthodologie d'utilisation de ces données pour la co-digestion. Une telle méthodologie n'existe pas, à ma connaissance. C'est pourquoi, je souhaite proposer un projet de thèse sur le sujet.

Actuellement, je suis la thèse de Lucie Perat (INRAE, LBE) supervisée par Eric Trably, sur la production d'hydrogène et la co-digestion. Lucie Perat utilise le fractionnement IsBaMO® et réalise des tests de co-fermentation avec divers constituants d'un biodéchet reconstitué. Les résultats préliminaires montrent qu'il n'y aurait pas additivité de la production d'hydrogène et que le fractionnement pourrait être un moyen de mieux comprendre la « règle » d'utilisation des substrats par les microorganismes, en lien avec les résultats publiés sur la modélisation simultanée ou séquentielle des fractions d'un substrat [JJ10]. Cet aspect est à creuser afin de proposer des expérimentations dédiées et de proposer une méthodologie pour la simulation de la co-digestion. En effet, on pourrait imaginer de mettre en place des expérimentations supplémentaires à celles de la thèse de Lucie Perat avec un suivi poussé afin de modéliser la co-digestion, que ce soit de manière mécanistique (afin de mieux comprendre les processus) ou à l'aide du machine learning afin de proposer une méthodologie/règle d'utilisation des données de co-digestion (Figure 48). J'ajouterais que l'utilisation du traçage isotopique selon les abondances naturelles du 13 C ou 15 N voire via un traçage de molécules marquées sur divers types d'intrants en co-digestion serait intéressant d'investiguer et pourrait participer à répondre à la question.

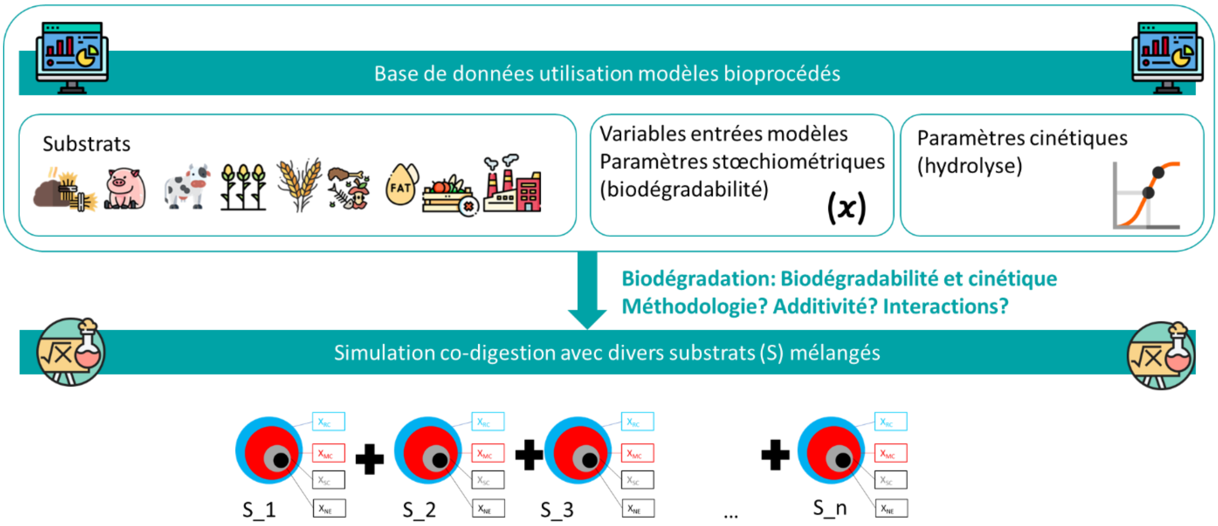


Figure 48 : Schématisation de la problématique de recherche autour de la modélisation de la co-digestion des intrants en méthanisation

Concernant la modélisation statistique, étant donné que la fluorescence n'est pas additive, l'utilisation de l'IsBaMO® ne peut être réalisée que si la caractérisation est faite sur le mix des intrants. L'autre solution serait de réaliser la modélisation par substrat et de pondérer les résultats avec la même incertitude que précédemment évoquée. Là aussi, des investigations sont à prévoir.

Enfin, l'axe 2 et la poursuite de travaux sur une meilleure compréhension des procédés permettra également de mettre à jour les modèles actuels.

3.2. Optimisation multi-objectif et couplage avec ACV

Enfin, un dernier enjeu à aborder est celui de l'optimisation multi-objectif de la filière. Jusqu'à présent, mes travaux se sont focalisés sur la modélisation des procédés et du devenir de leur qualité, que ce soit via des modèles mécanistiques ou statistiques. La thèse en cours de Margaud Pereme (2020-2023) a déjà permis de mettre en place une plateforme de simulation couplant modèles mécanistiques de méthanisation, post-traitements et devenir des digestats au sol. Les deux prochaines étapes sont de coupler cette modélisation au calcul des impacts environnementaux comme proposé par ACV et de réaliser une optimisation multi-objectif en utilisant des exemples d'étude de cas.

La première étape va nous permettre de mettre en exergue (i) les calculs non pris en compte dans la plateforme mais nécessaires pour l'ACV, (ii) la sensibilité de certains éléments de la filière sur les impacts environnementaux comme la recette des intrants, la gestion du stockage, les paramètres procédés et (iii) l'impact de la dynamique de la filière (perturbations systèmes, montée en charge organique, changement de recette saisonnière, …).

La seconde étape va ensuite consister en l'implémentation d'un algorithme d'optimisation multi-objectif (i.e. choix de l'algorithme génétique à valider d'après (Gésan-Guiziou, 2022)) appliqué à des études de cas/scénarios. Lors de cette étape, nous aurons à définir les contraintes environnementales et réglementaires, les objectifs à atteindre pour la production d'énergie et la production de fertilisants organiques ainsi que les paramètres sur lesquels jouer à partir de l'analyse de sensibilité précédente. Cet exercice ambitieux nous donnera un éclairage nouveau sur la gestion des compromis entre services rendus, l'impact de changements sur l'environnement et soulèvera des pistes à creuser pour aller plus loin dans l'analyse.

A ce sujet, je souhaiterais me rapprocher et m'inspirer des travaux proposés par les chercheurs du département TRANSFORM (Gésan-Guiziou et al., 2019) dans d'autres disciplines (agro-alimentaires,

alimentaires, ...) utilisant des outils d'optimisation et ayant développé un réseau sur le sujet au sein même de l'INRAE.
Le schéma de la Figure 49 s'inspire des livrables attendus de la thèse de Margaud Pereme (2023) qui s'inscrit dans une ambition d'optimisation plus générale avec des allers-retours entre optimisation et simulations, la définition du cahier des charges affiné (axe 1) selon le cas étudié (sol, conditions pédo-climatiques, cultures), l'utilisation des leviers d'actions identifiés (axe 2 : substrats, procédés) et d'une base de données substrats associée à la méthodologie développée de modélisation de la co-digestion si l'on réalise cette optimisation à partir de modèles mécanistiques.

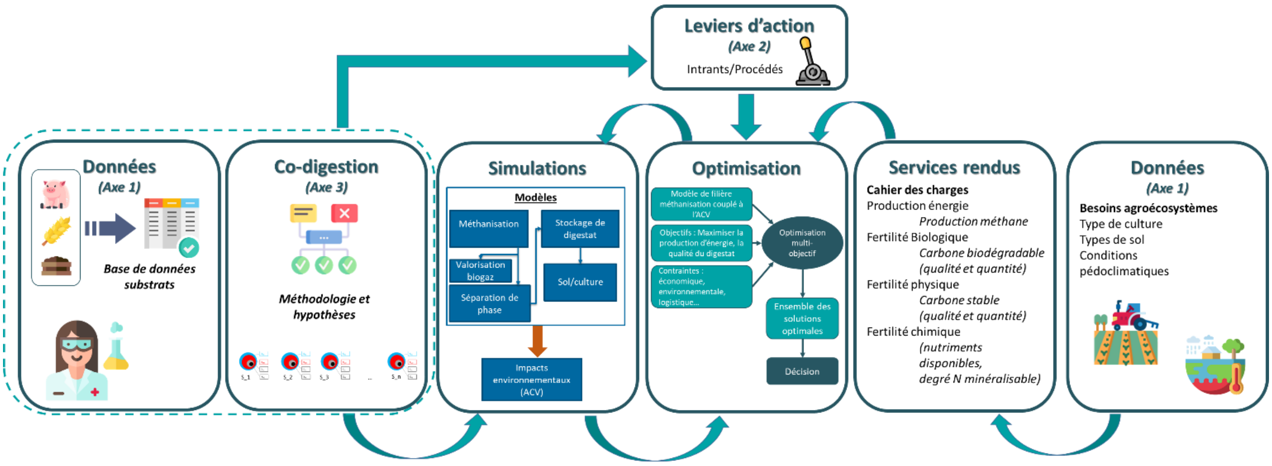


Figure 49 : Optimisation multi-objectif des services rendus par la filière méthanisation alimentée par les trois Axes de recherche

Les résultats des analyses effectuées et des optimisations permettront de prioriser les efforts à mettre en œuvre dans le degré de connaissance sur la filière méthanisation et d'alimenter les recherches des deux premiers axes et de générer des nouvelles questions de recherche. En effet, comme illustré par la Figure 50, l'optimisation multi-objectif pourra proposer à son tour des besoins et des pistes à creuser sur les trois axes de recherche.

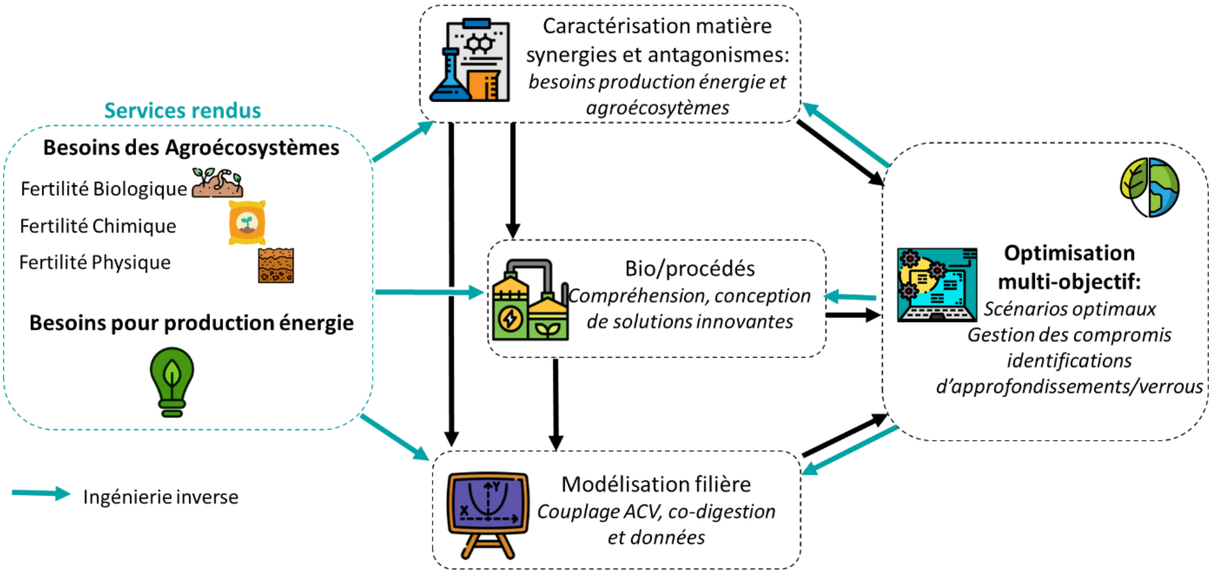


Figure 50 : L'ingénierie inverse, au-delà de l'optimisation multi-objectif de la filière de valorisation des résidus organiques

Ainsi l'ingénierie inverse à partir des services rendus via les besoins des agroécosystèmes est au cœur de mon projet de recherche, que ce soit pour mieux connaître la matière et son devenir et éco-concevoir des filières de valorisation optimales respectueuses de l'environnement, génératrices de gaz verts et en adéquation avec les principes de l'agroécologie (Figure 50).

3.3. Perspectives à long terme

Dans une vision à plus long terme de mes recherches, je souhaiterais élargir ma méthodologie à l'ensemble de la bioraffinerie environnementale, au-delà de la filière méthanisation, cas d'étude pour développer ladite méthodologie. En effet, la bioraffinerie environnementale comme définie au LBE vise à concevoir une filière de valorisation optimale d'un résidu donné dont la qualité matière est connue, tout en optimisant la combinaison des briques technologiques disponibles dans notre bibliothéque « d'outils bio/procédés » afin de proposer divers scénarios possibles de valorisation.

Les services rendus pouvant être divers, ils devront intégrer le devenir agronomique du co-produit final et donc de considérer son adéquation avec les besoins des agroécosystèmes. Ces propositions de scénari seront, comme décrit dans l'axe 3, obtenus via une méthodologie d'optimisation multi-objectif prenant en compte les objectifs souhaités par les services rendus aux divers bio/procédés possibles sous contraintes environnementales et sanitaires comme illustré par la Figure 51.

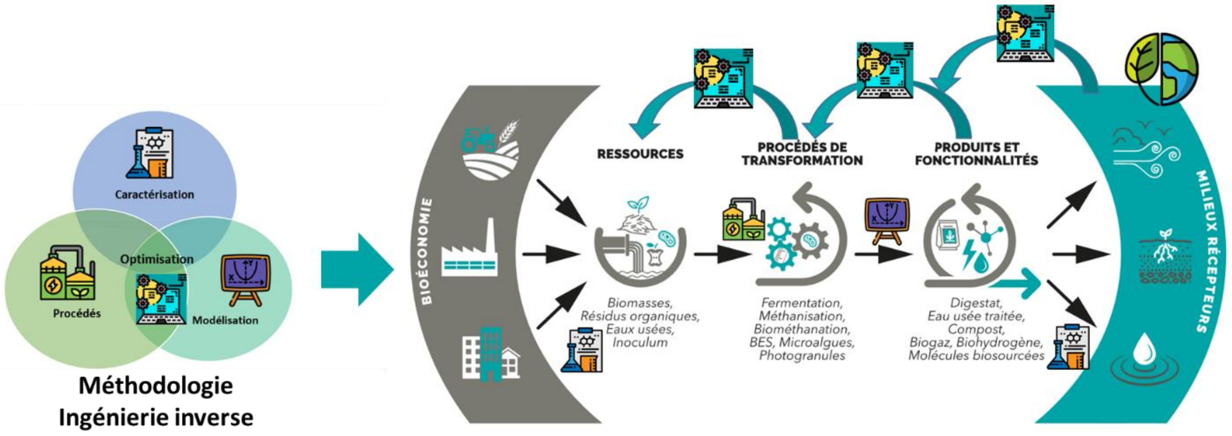


Figure 51 : Vision long-terme de ma thématique de recherche

Conclusion sur mes activités de recherche

Mes diverses expériences en tant qu'ingénieure de recherche dans le privé puis chargée de recherche dans l'académique m'ont permis de développer une activité de recherche autour de *l'intégration de bioprocédés de valorisation des résidus organiques pour une gestion durable des agroécosystèmes* dont les piliers font référence à mes compétences en caractérisation de la matière, génie des procédés et modélisation des procédés.

Dans un premier temps, j'ai utilisé ces compétences afin de construire ma thématique de recherche. Aujourd'hui, je souhaite consolider cette dernière, la diriger et la développer pour mieux la partager.

Durant mes expériences, j'ai eu l'opportunité de piloter des projets de recherche et d'encadrer des techniciens, stagiaires (DUT à master et ingénieurs), ingénieurs, post-doctorants et doctorants. Cela m'a permis d'avoir une expérience en direction de projet et en encadrement. La dynamique de mes activités montre que je suis prête à diriger des thèses et assumer la direction d'une thématique de recherche. J'apprécie de transmettre aux jeunes chercheurs la capacité à répondre à une thématique de recherche et à définir les questions de recherche autour d'une thèse qu'ils défendront.

D'un autre côté, l'expérience d'encadrer des doctorants me nourrit et me pousse à prendre du recul et de la hauteur vis-à-vis des résultats de recherche obtenus et des réflexions. C'est une expérience de partage mutuel où j'accompagne les jeunes chercheurs afin qu'ils s'approprient un sujet et eux m'apportent de nouvelles approches et réflexions nourrissant mes propres recherches.

Je souhaite donc poursuivre dans cette voie afin de pouvoir continuer ces synergies et alimenter ma thématique de recherche. Enfin, la possibilité de pouvoir expertiser des recherches (rapporter des thèses, HDR, projets …) me permettra d'ouvrir encore plus de perspectives.

Références Bibliographiques

Altieri, M.A., 2002. Agroecology: the science of natural resource management for poor farmers in marginal environments. Agric. Ecosyst. Environ. 93, 1–24. https://doi.org/10.1016/S0167-8809(02)00085-3

Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Bio/Technology 3, 117–129. https://doi.org/10.1007/s11157-004-2502-3

Aquino, S.F., Chernicharo, C.A.L., Soares, H., Takemoto, S.Y., Vazoller, R.F., 2008. METHODOLOGIES FOR DETERMINING THE BIOAVAILABILITY AND BIODEGRADABILITY OF SLUDGES. Environ. Technol. 29, 855–862. https://doi.org/10.1080/09593330802015508

Bareha, Y., Affes, R., Moinard, V., Buffet, J., Girault, R., 2021. A simple mass balance tool to predict carbon and nitrogen fluxes in anaerobic digestion systems. Waste Manag. 135, 47–59. https://doi.org/10.1016/j.wasman.2021.08.020

Bareha, Y., Girault, R., Guezel, S., Chaker, J., Trémier, A., 2019. Modeling the fate of organic nitrogen during anaerobic digestion: Development of a bioaccessibility based ADM1. Water Res. 154, 298–315. https://doi.org/10.1016/j.watres.2019.02.011

Barles, S., 2005. L'invention des déchets urbains, France, 1790-1970. Champ vallon.

Bayard, R., Benbelkacem, H., Gourdon, R., Buffière, P., 2018. Characterization of selected municipal solid waste components to estimate their biodegradability. J. Environ. Manage. 216, 4–12. https://doi.org/10.1016/j.jenvman.2017.04.087

Cavalli, D., Corti, M., Baronchelli, D., Bechini, L., Marino Gallina, P., 2017. CO 2 emissions and mineral nitrogen dynamics following application to soil of undigested liquid cattle manure and digestates. Geoderma 308, 26–35. https://doi.org/10.1016/j.geoderma.2017.08.027

Chauvin, M., 2004. Enjeux de la valorisation des déchets ou produits résiduaires organiques en Bretagne.

Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence Excitation−Emission Matrix Regional Integration to Quantify Spectra for Dissolved Organic Matter. Environ. Sci. Technol. 37, 5701–5710. https://doi.org/10.1021/es034354c

de la Fuente, C., Alburquerque, J.A., Clemente, R., Bernal, M.P., 2013. Soil C and N mineralisation and agricultural value of the products of an anaerobic digestion system. Biol. Fertil. Soils 49, 313–322. https://doi.org/10.1007/s00374-012-0719-9

Dou, Z., Toth, J.D., Galligan, D.T., Ramberg, C.F., Ferguson, J.D., 2000. Laboratory Procedures for Characterizing Manure Phosphorus. J. Environ. Qual. 29, 508–514. https://doi.org/10.2134/jeq2000.00472425002900020019x

Esparza-Soto, M., Westerhoff, P.K., 2001. Fluorescence spectroscopy and molecular weight distribution of extracellular polymers from full-scale activated sludge biomass. Water Sci. Technol. 43, 87–95.

Garcia-Bernet, D., Daboussi, F., 2016. Bioraffinerie environnementale et les usages en cascade de la biomasse. Innov. Agron. 54, 75–88.

Garnier, P., Néel, C., Aita, C., Recous, S., Lafolie, F., Mary, B., 2003. Modelling carbon and nitrogen dynamics in a bare soil with and without straw incorporation. Eur. J. Soil Sci. 54, 555–568. https://doi.org/10.1046/j.1365-2389.2003.00499.x

Gerke, J., 2015. Phytate (Inositol Hexakisphosphate) in Soil and Phosphate Acquisition from Inositol Phosphates by Higher Plants. A Review. Plants 4, 253–266. https://doi.org/10.3390/plants4020253

Gésan-Guiziou, G., 2022. Evaluation Multicritère, EMC et variabilité, in: Séminaire de Département Transform INRAE, Département Transform INRAE. Massy.

Gésan-Guiziou, G., Alaphilippe, A., Andro, M., Aubin, J., Bockstaller, C., Botreau, R., Buche, P., Collet, C., Darmon, N., Delabuis, M., Girard, A., Grateau, R., Kansou, K., Martinet, V., Membré, J.-M., Sabbadin, R., Soler, L.-G., Thiollet-Scholtus, M., Tonda, A., Van-Der-Werf, H., 2019. Annotation data about multi criteria assessment methods used in the agri-food research: The french national institute for agricultural research (INRA) experience. Data Br. 25, 104204. https://doi.org/10.1016/j.dib.2019.104204

Giovanela, M., Parlanti, E., Soriano-Sierra, E.J., Soldi, M.S., Sierra, M.M.D., 2004. Elemental compositions, FT-IR spectra and thermal behavior of sedimentary fulvic and humic acids from aquatic and terrestrial environments. Geochem. J. 38, 255–264. https://doi.org/10.2343/geochemj.38.255

Grau, P., Vanrolleghem, P., Ayesa, E., 2007. BSM2 Plant-Wide Model construction and comparative analysis with other methodologies for integrated modelling. Water Sci. Technol. 56, 57–65. https://doi.org/10.2166/wst.2007.602

GRDF, 2022. La dynamique du marché [WWW Document]. https://projet-methanisation.grdf.fr/la-methanisation/la-dynamique-du-marche.

Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C., 2015. Phosphorus in Digestate-Based Compost: Chemical Speciation and Plant-Availability. Waste and Biomass Valorization 6, 481–493. https://doi.org/10.1007/s12649-015-9383-2

Grigatti, M., Di Girolamo, G., Chincarini, R., Ciavatta, C., Barbanti, L., 2011. Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. Biomass and Bioenergy 35, 4619–4629. https://doi.org/10.1016/j.biombioe.2011.09.007

Gunaseelan, V.N., 2007. Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition. Bioresour. Technol. 98, 1270–1277. https://doi.org/10.1016/j.biortech.2006.05.014

Gunnarsson, A., Bengtsson, F., Caspersen, S., 2010. Use efficiency of nitrogen from biodigested plant material by ryegrass. J. Plant Nutr. Soil Sci. 173, 113–119. https://doi.org/10.1002/jpln.200800250

He, Z., Zhang, H., Toor, G.S., Dou, Z., Honeycutt, C.W., Haggard, B.E., Reiter, M.S., 2010. Phosphorus Distribution in Sequentially Extracted Fractions of Biosolids, Poultry Litter, and Granulated Products. Soil Sci. 175, 154–161. https://doi.org/10.1097/SS.0b013e3181dae29e

Henze, M., Gujer, W., Lino, T., van Loosdrecht, M.C.M., 2000. Activated sludge models ASM1, ASM2, ASM2d and ASM3. IWA Publishing.

Houot, S., Levavasseur, F., Launay, C., Moinard, V., Carton, S., Girault, R., 2022. Effet de la méthanisation sur les flux de matière organique au sein des exploitations, in: Workshop Agroécologie et Méthanisation Rennes (2022-10-19-2022-10-20).

Jeppsson, U., Pons, M.-N., Nopens, I., Alex, J., Copp, J.B., Gernaey, K.V., Rosen, C., Steyer, J.-P., Vanrolleghem, P.A., 2007. Benchmark simulation model no 2: general protocol and exploratory case studies. Water Sci. Technol. 56, 67–78. https://doi.org/10.2166/wst.2007.604

Jeuffroy, M.-H., 2022. Agroécologie: entre recherche et innovation, in: Workshop Agroécologie et Méthanisation Rennes (2022-10-19-2022-10-20). Rennes.

Journal Officiel de la République Française, 2020. Arrêté du 22 octobre 2020 approuvant un cahier des charges pour la mise sur le marché et l'utilisation de digestats de méthanisation d'intrants agricoles et/ou agro-alimentaires en tant que matières fertilisantes.

Karki, R., Chuenchart, W., Surendra, K.C., Shrestha, S., Raskin, L., Sung, S., Hashimoto, A., Kumar Khanal, S., 2021. Anaerobic co-digestion: Current status and perspectives. Bioresour. Technol. 330, 125001. https://doi.org/10.1016/j.biortech.2021.125001

Karki, R., Chuenchart, W., Surendra, K.C., Sung, S., Raskin, L., Khanal, S.K., 2022. Anaerobic co-digestion of various organic wastes: Kinetic modeling and synergistic impact evaluation. Bioresour. Technol. 343, 126063. https://doi.org/10.1016/j.biortech.2021.126063

Kaza, S., Yao, L.C., Bhada-Tata, P., Van Woerden, F., 2018. What a Waste 2.0 : A Global Snapshot of Solid Waste Management to 2050. Washington, DC: World Bank.

Kouas, M., Torrijos, M., Schmitz, S., Sousbie, P., Sayadi, S., Harmand, J., 2018. Co-digestion of solid waste: Towards a simple model to predict methane production. Bioresour. Technol. 254, 40–49. https://doi.org/10.1016/j.biortech.2018.01.055

LAMBERS, H., SHANE, M.W., CRAMER, M.D., PEARSE, S.J., VENEKLAAS, E.J., 2006. Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits. Ann. Bot. 98, 693–713. https://doi.org/10.1093/aob/mcl114

Lashermes, G., Nicolardot, B., Parnaudeau, V., Thuriès, L., Chaussod, R., Guillotin, M.L., Linères, M., Mary, B., Metzger, L., Morvan, T., 2010. Typology of exogenous organic matters based on chemical and biochemical composition to predict potential nitrogen mineralization. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2009.08.025

Lashermes, G., Nicolardot, B., Parnaudeau, V., Thuriès, L., Chaussod, R., Guillotin, M.L., Linères, M., Mary, B., Metzger, L., Morvan, T., Tricaud, A., Villette, C., Houot, S., 2009. Indicator of potential residual carbon in soils after exogenous organic matter application. Eur. J. Soil Sci. 60, 297–310. https://doi.org/10.1111/j.1365-2389.2008.01110.x

Lesteur, M., Latrille, E., Maurel, V.B., Roger, J.M., Gonzalez, C., Junqua, G., Steyer, J.P., 2011. First step towards a fast analytical method for the determination of Biochemical Methane Potential of solid wastes by near infrared spectroscopy. Bioresour. Technol. 102, 2280–2288. https://doi.org/10.1016/j.biortech.2010.10.044

Liu, X., Bayard, R., Benbelkacem, H., Buffière, P., Gourdon, R., 2015. Evaluation of the correlations between biodegradability of lignocellulosic feedstocks in anaerobic digestion process and their biochemical characteristics. Biomass and Bioenergy 81, 534–543. https://doi.org/10.1016/j.biombioe.2015.06.021

Malézieux, E., Lafontaine, H.O., 2013. Agro-écologie : quels principes dans les agro-écosystèmes tropicaux ?, in: http://www.inra.fr/Entreprises-Monde-agricole/Resultats-innovation-transfert/Tous-les-magazines/SIA-2013-Rencontre-AgroEcologie (Ed.), . Paris.

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59–86. https://doi.org/10.1016/j.geoderma.2017.01.002

Ministère de la Transition écologique et de la Cohésion des territoires, Ministère de la Transition Energétique, 2021. Traitement des déchets [WWW Document]. https://www.ecologie.gouv.fr/traitement-des-dechets.

Morvan, T., Nicolardot, B., Péan, L., 2006. Biochemical composition and kinetics of C and N mineralization of animal wastes: a typological approach. Biol. Fertil. Soils 42, 513–522. https://doi.org/10.1007/s00374-005-0045-6

Mottet, A., François, E., Latrille, E., Steyer, J.P., Déléris, S., Vedrenne, F., Carrère, H., 2010. Estimating anaerobic biodegradability indicators for waste activated sludge. Chem. Eng. J. 160, 488–496. https://doi.org/10.1016/j.cej.2010.03.059

Oudart, D., Robin, P., Paillat, J.M., Paul, E., 2015. Modelling nitrogen and carbon interactions in composting of animal manure in naturally aerated piles. Waste Manag. 46, 588–598. https://doi.org/10.1016/j.wasman.2015.07.044

Pagés Díaz, J., Pereda Reyes, I., Lundin, M., Sárvári Horváth, I., 2011. Co-digestion of different waste mixtures from agro-industrial activities: Kinetic evaluation and synergetic effects. Bioresour. Technol. 102, 10834–10840. https://doi.org/10.1016/j.biortech.2011.09.031

Pellerin, S., Bamière, L., Launay, C., Martin, R., Schiavo, M., Angers, D., Augusto, L., Balesdent, J., Basile-Doelsch, I., Bellassen, V., Cardinael, R., Cécillon, L., Ceschia, E., Chenu, C., Constantin, J., Darroussin, J., Delacote, P., Delame, N., Gastal, F., Gilbert, D., Graux, A.-I., Guenet, B., Houot, S., Klumpp, K., Letort, E., Litrico, I., Martin, M., Menasseri, S., Mézière, D., Morvan, T., Mosnier, C., Roger-Estrade, J., Saint-André, L., Sierra, J., Thérond, O., Viaud, V., Grateau, R., Le Perchec, S., Savini, I., Réchauchère, O., 2019. Stocker du carbone dans les sols français: quel potentiel au regard de l'objectif de 4 pour 1000 et à quel coût?

Peltre, C., Thuriès, L., Barthès, B., Brunet, D., Morvan, T., Nicolardot, B., Parnaudeau, V., Houot, S., 2011. Near infrared reflectance spectroscopy: A tool to characterize the composition of different types of exogenous organic matter and their behaviour in soil. Soil Biol. Biochem. 43, 197–205. https://doi.org/10.1016/j.soilbio.2010.09.036

Reibel, A., Leclerc, B., 2018. Valorisation agricole des digestats : Quels impacts sur les cultures, le sol et l'environnement ? .

Robin Beghin, 2020. Etude des conséquences de la digestion anaérobie du maïs sur ses caractéristiques amendantes: devenir des matières organiques, stockage du carbone, vie microbienne, capacité d'adsorption et stabilité structurale de l'horizon de surface de Luvisols cultivés. INPT, Toulouse.

Sole-Mauri, F., Illa, J., Magrí, A., Prenafeta-Boldú, F.X., Flotats, X., 2007. An integrated biochemical and physical model for the composting process. Bioresour. Technol. 98, 3278–3293. https://doi.org/10.1016/j.biortech.2006.07.012

Spérandio, M., 1998. Développement d'une procédure de compartimentation d'une eau résiduaire urbaine et application à la modélisation dynamique des procédés à boues activées. INSA Toulouse.

Swift, R.S., 2018. Organic Matter Characterization. pp. 1011–1069. https://doi.org/10.2136/sssabookser5.3.c35

Triolo, J.M., Sommer, S.G., Møller, H.B., Weisbjerg, M.R., Jiang, X.Y., 2011. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: Influence of lignin concentration on methane production potential. Bioresour. Technol. 102, 9395–9402. https://doi.org/10.1016/j.biortech.2011.07.026

Unifa, n.d. Le rôle des éléments nutritifs [WWW Document]. https://fertilisation-edu.fr/nutrition-des-plantes/le-role-des-elements-nutritifs/azote.html.

Van Soest, P.J., 1963. The Use of Detergents in the Analysis of Fibrous Feeds: II. A Rapid Method for the Determination of Fiber and Lignin. Off. Agric. Chem. 46, 829.

Vavilin, V.A., Fernandez, B., Palatsi, J., Flotats, X., 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. Waste Manag. 28, 939–951. https://doi.org/10.1016/j.wasman.2007.03.028

Vavilin, V.A., Rytov, S.V., Lokshina, L.Y., 1996. A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. Bioresour. Technol. 56, 229–237. https://doi.org/10.1016/0960-8524(96)00034-X

Xie, T., Xie, S., Sivakumar, M., Nghiem, L.D., 2017. Relationship between the synergistic/antagonistic effect of anaerobic co-digestion and organic loading. Int. Biodeterior. Biodegradation 124, 155–161. https://doi.org/10.1016/j.ibiod.2017.03.025

Yasui, H., Sugimoto, M., Komatsu, K., Goel, R., Li, Y.Y., Noike, T., 2006. An approach for substrate mapping between ASM and ADM1 for sludge digestion. Water Sci. Technol. 54, 83–92. https://doi.org/10.2166/wst.2006.529

Annexe : publications scientifiques majeures

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REVIEW PAPER

Characterising the stability of the organic matter during anaerobic digestion: a selective review on the major spectroscopic techniques

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Abstract Digestate landspreading is a key aspect of the circular economy. However, organic matter (OM) stability in digestates is usually either poorly assessed or done through laborious methods. Spectroscopic methods are useful and easy to deploy alternatives to assess several aspects in anaerobic digestion (AD) studies such as process performance, waste classification and both OM composition and transformation. In these studies, a lack of agreement on analytical techniques, indicators and reference values is evident. This unclear scenario brings to the forefront the need for a meta-analytical study providing benchmarking values and trends. This review aimed to fill up this gap through the identification and evaluation of: (i) the most frequently applied techniques, their principles, deployment methods and limitations, (ii) the quantitative spectroscopic indices to define OM stability, (iii) the common trends of these parameters due to AD effect on the OM and (iv) the relevance of each technique based on the frequency of statistically significant results reported. Ultraviolet–visible and fluorescence spectroscopy have been identified as the most relevant techniques for aqueous phase study whereas mid-infrared and ^{13}C cross-polarisation magic angle spinning nuclear magnetic resonance were the most appropriate for the solid phase. Their most applied indicators and their trends after AD have been summarised. Finally, the research studies that displayed statistically significant findings were described, the representativeness of the indices and the influence of sample preparation on their calculation were discussed and future research lines were suggested. Overall, this review demonstrates that spectroscopic methods provide relevant information for better digestate management.

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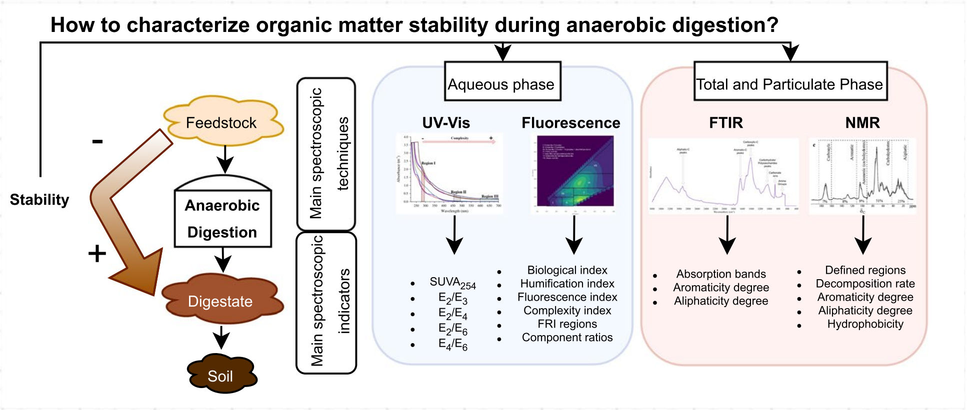
Graphical abstract



Keywords Biodegradability · Biogas effluent · Complexity · Digestate valorisation · Humic substances · Waste characterisation

Abbreviations

^{13}C CP-MAS NMR	^{13}Carbon cross-polarisation magic angle spinning nuclear magnetic resonance
A/O-A	Alkyl C to O-alkyl C ratio
AD	Anaerobic digestion
AcoD	Anaerobic co-digestion
BIX	Biological index
BMP	Biochemical methane potential
C/N	Carbon to nitrogen ratio
COD	Chemical oxygen demand
DOM	Dissolved organic matter
DOC	Dissolved organic carbon
EEM	Emission-Excitation matrix
EPS	Extracellular polymeric substances
ETC	Electron transfer capacity
FI	Fluorescence index
FCI	Fluorescence complexity index
FLA	Fulvic-like acid
F$_{max}$	Fluorescence intensity
FRI	Fluorescence regional integration
FTIR	Fourier transform infrared
HB	Hydrophobicity index
HIX	Humification index
HLA	Humic-like acid
HLS	Humic-like substances
OM	Organic matter
MIR	Mid-infrared spectroscopy
NDF	Neutral Detergent Fraction
NEOM	Non-Extractable Organic Matter
NMR	Nuclear magnetic resonance
PARAFAC	Parallel factor analysis
PCA	Principal component analysis
PEOM	Poorly Extractable Organic Matter
POM	Particulate organic matter
REOM	Readily Extractable Organic Matter
SEOM	Slowly Extractable Organic Matter
SMP	Soluble microbial product
SPOM	Extractable Soluble from Particulate Organic Matter
sCOD	Soluble chemical oxygen demand
T	Temperature
tCOD	Total chemical oxygen demand
TOC	Total organic carbon
TS	Total solids
UV–Vis	Ultraviolet–Visible
VFA	Volatile fatty acids
VS	Volatile solids

1 Introduction

Anaerobic digestion (AD) is a mature and effective biological process to stabilise organic matter (OM) and produce biogas (Astals et al. 2014). Digestate is the liquid to semi-solid by-product remaining after the biological process with both partially stabilised OM and nutrient-rich content (Monlau et al. 2016; Pigoli et al. 2021). The anaerobic digestates usually fulfil the OM minimal requirements for an organic soil amendment (Stutzenstein et al. 2018). Thus, returning digestates to soils is gaining attention to accomplish socio-economic and environmental concerns (Panuccio et al. 2019; Barłóg et al. 2020; Baştabak and Koçar 2020).

Physicochemical parameters (e.g. carbon to nitrogen ratio (C/N) and volatile fatty acids (VFA)) are the most common parameters to characterise digestates (Mottet et al. 2013; Bayard et al. 2015; Guilayn et al. 2019) and to describe stabilisation during AD (Mákadi et al. 2012; Möller and Müller 2012; Guilayn et al. 2020b; Fernández-Domínguez et al. 2020). Nonetheless, usual feedstock/digestate OM characterisation does not allow to properly assess OM transformation processes (e.g. polymerisation, molecular structure changes). Indeed, measuring carbon (C) concentration alone could be non-informative enough for an accurate OM characterisation (Domeizel et al. 2004; Teglia et al. 2011; Tambone et al. 2013). Besides, digestate stability tests require long experimentation times to solely assess C biodegradability in soils (Lashermes et al. 2009; Jimenez et al. 2017). Thus, a deeper analysis of the OM stability could provide significant information to further evaluate OM transformation processes during AD, the process performance as well as the fate of OM in soils to assure that digestates are safe to be spread on the land (Teglia et al. 2011; Papa et al. 2014; Tambone et al. 2019; Shakeri Yekta et al. 2019).

With that purpose, spectroscopic techniques are a non-destructive, relatively simple and fast alternative to characterise OM stability during AD (Marcato et al. 2009; Lesteur et al. 2010; Teglia et al. 2011; Li et al. 2018a, b). Moreover, they allow the detection of a wide range of functional groups to better understand the OM nature of an organic sample (Provenzano et al. 2014; Li et al. 2014b; Jimenez et al. 2015a; Kataki et al. 2017; Gómez et al. 2018). Spectroscopic techniques have been largely employed in AD studies to predict waste biodegradability (Schievano et al. 2009; Lesteur et al. 2010; Maynaud et al. 2017; Jimenez et al. 2017; Yang et al. 2021) and to evaluate the effect of OM application in the soil properties (Sarker et al. 2018; Iocoli et al. 2019; Tan et al. 2021). Concerning review articles, most of the published reviews were focused on the spectroscopic applicability for process monitoring (Madsen et al. 2011; Jimenez et al. 2015b; Mesquita et al. 2017; Wu et al. 2019; Awhangbo et al. 2020) although minor attention was paid to the characterisation of the OM. A limited number of reviews can be found related to the spectroscopic characterisation of the OM in freshwater ecosystems (Fellman et al. 2010), drinking water (Li et al. 2020), wastewater (Filho et al. 2015) or sludge (Xiao et al. 2020; Faixo et al. 2021). However, as far as the authors recognise, not a single review was focused on identifying the most used spectroscopic techniques to characterise OM transformation during AD even though spectroscopic indices were largely used to this end during the last decades.

It is accepted that AD enhances the organic soil amendment value compared to raw feedstocks due to the prevalence of more recalcitrant upon easily degradable compounds (Pognani et al. 2009; de la Fuente et al. 2013; Provenzano et al. 2014; Pigoli et al. 2021). Common spectroscopic indices trends with AD and their described values have shown significant results to characterise diverse factors in AD studies, such as waste classification, process performance and OM stability, including different types of feedstock. Therefore, spectroscopic parameters should be considered as a potential alternative to study AD. For instance, the complexity of the OM has been widely assessed using different spectroscopic methods based on the aromatic C of a sample. Nonetheless, the identification of further OM conversions and compounds will depend on each spectroscopic technique (Mei et al. 2020; Wang et al. 2021). Thus, the evaluation of the applicability of each spectroscopic technique in AD studies is a point of greater importance.

The goals of this review are: (i) identify the most used spectroscopic techniques and subsequent indices to assess OM stability during/after AD, (ii) show common trends on spectroscopic parameters revealed for each selected technique (impact of AD), and (iii) state the feasibility of the spectroscopic indices based on the significance of their related results. Additionally, the last section of this study was focused on discussing the most relevant points of the results as well as the influential points in the interpretation of the spectroscopic indices.

2 Spectroscopic techniques to characterise the OM stability during AD

To identify the most used spectroscopic techniques to characterise OM transformation during AD, a literature review was conducted by an advanced search in Web of Science®. The selected publications contained in the title, abstract, or author keywords, one or multiple variations of the words ‘‘spectroscopy’’ (e.g. spectroscopic, spectra), ‘‘organic matter’’ (e.g. digestate, biogas effluent, manure) and ‘‘anaerobic digestion” (e.g. methanization, anaerobic co-digestion). The exact query used to build the database was: “TS = (spectroscopy* OR spectroscopic* OR spectra* OR spectral*) AND TS = (digestate* OR organic matter* OR manure* OR slurry* OR biogas effluent* OR biogas slurry* OR anaerobically digested* OR biowaste*) AND TS = (anaerobic digestion* OR methanization OR anaerobic co-digestion). A total of 280 publications was obtained and manually verified. From these 280 studies, 88 papers properly aligned with the review topic were selected. From these 88 papers, the major spectroscopic techniques were identified based on the frequency of use, as shown in the Online Resource 1. The following topics were discarded for the selection of the major techniques: (i) main focus on pre-treatments effects or heavy metal impact on OM, (ii) lack of OM characterisation and consequent discussion. To complete the study, the selected literature (88 articles) was then extended to a total of 183 articles by checking meaningful citations.

Among the spectroscopic range, near-infrared spectra (NIR) (4000–10,000 cm^{-1}) has shown great potential for waste biodegradability prediction (Lesteur et al. 2011; Doublet et al. 2013; Ward 2016; Yang et al. 2021; Mallet et al. 2021), process monitoring and advanced control approaches (Krapf et al. 2012; Charnier et al. 2017; Stockl and Lichti 2018). NIR spectroscopy also allows rapid compositional analysis (Meder et al. 2007). However, spectroscopic analysis at longer wavelengths using mid-infrared spectroscopy (MIR) (400–4000 cm^{-1}) offers more specific chemical information since the vibrational resonances of most functional groups fall within the MIR range of excitation frequencies (Yang and Mouazen 2012; Hell et al. 2016). Besides, the extent of literature for the interpretation of the MIR spectra is remarkably higher than NIR (Bekiaris et al. 2015). Therefore, all these factors excluded the evaluation of NIR upon MIR.

Based on the designated literature, the following spectroscopic techniques were selected as the most used in AD studies to characterise dissolved, total and particulate OM stability (Fuentes et al. 2007; He et al. 2011; Guo et al. 2012; Liao et al. 2014; Hagos et al. 2017):

- Dissolved organic matter (DOM): ultraviolet–visible (UV–Vis) spectroscopy and fluorescence spectroscopy.
- Total and particulate organic matter (POM): MIR and ^{13}C cross-polarisation magic angle spinning nuclear magnetic resonance spectroscopy (^{13}C CP-MAS NMR).

It should be noted that fluorescence, MIR and ^{13}C CP-MAS NMR spectroscopic techniques can be performed on both the aqueous and the total or solid phases. However, only those suggested in the list above were discussed since they were the most used for the analysis of each phase.

2.1 Sample preparation before analysis

Several methods for sample preparation stated in literature are summarised in Table 1. Table 1 includes different extraction methods for DOM, dilutions and pH adjustments before analysis. Depending on the spectroscopic technique, the analyses were performed on the aqueous, the solid or the total phase of the sample, according to the previous list.

Aqueous sample preparation for fluorescence spectroscopy analysis is similar to UV–Vis (Brunetti et al. 2012; Zhang et al. 2013; Zheng et al. 2014; Qu et al. 2017). The aqueous phase is commonly extracted directly by centrifugation or after extraction with: basic solutions (i.e. NaOH, $NaHCO_3$), Mili-Q water or distilled water by shaking the sample between 2 and 24 h (Shao et al. 2009; Zhen et al. 2014; Zeng et al. 2017). Filtration through a 0.2–0.45 µm membrane filter is required before analysis (Zhang et al. 2013; Guo et al. 2018). Then, the pH is frequently adjusted (normally between 7 and 8.5) or the analysis is performed directly at the samples pH. Moreover, the Beer-Lambert Law should be respected (linearity with dilution). However, as shown in Table 1, most of

Table 1 Examples of sample preparation before spectroscopic analysis

Spectroscopic analysis	Sample preparation method	References
UV–Vis	0.1 g air-dried sample + 50 mL of 0.5 M NaOH shaken for 2 h Centrifugation at 1238 g. Dilution of 1:1 with distilled water	Fernandez-Bayo et al. (2018)
UV–Vis Fluorescence	3 mg of sample in 10 ml of 0.05 M $NaHCO_3$. Adjusted pH to 8.3 with NaOH Overnight equilibration at room temperature at a concentration of 100 mg L^{-1}. Adjusted pH to 8 with NaOH	Brunetti et al. (2012)
Fluorescence	20 g solid sample in 200 mL Milli-Q water (w/v) shaken for 24 h at room temperature. Centrifugation at 7000 rpm for 10 min at 4 °C. Filtration through a 0.45 µm filter membrane. TOC concentrations for all DOM samples were adjusted to 7 mg L^{-1}	Guo et al. (2012, 2018)
Fluorescence UV–Vis	TOC concentration in liquid phase was adjusted to 30 mg L^{-1} with deionised water TOC concentration in liquid phase was adjusted to 10 mg L^{-1} with deionised water	Gao et al. (2021)
UV–Vis	Samples added into 0.05 M $NaHCO_3$ solution at a final concentration of 200 mg L^{-1}	Liu et al. (2019)
Fluorescence	Centrifuged at 10,000 rpm for 10 min and filtered through a 0.45 µm filter membrane. Adjusted pH to 7.0 ± 0.05 by adding 0.1 M HNO_3 or NaOH solution The concentration was adjusted to a maximum of 10 mg C L^{-1}	Qu et al. (2017)
UV–Vis	Extraction at a solid to water ratio of 1:10 (w/v) and shaken for 24 h at room temperature. Centrifugation at 10,000 rpm for 10 min and filtered through a 0.45 µm filter membrane	Shao et al. (2009)
UV–Vis	3 mg in 10 mL of 0.05 M $NaHCO_3$. Adjusted pH to 8.3 using 0.02 M NaOH	Tang et al. (2018)
UV–Vis Fluo	Centrifuged at 8000 rpm for 10 min, supernatant filtration through a 0.45 µm filter membrane and diluted 50 times with Milli-Q water	Zhang et al. (2013)
UV–Vis	Extraction with 0.5 M NaOH by shaking for 2 h followed by centrifugation at 10,000 g for 10 min at 4 °C and filtered using a 0.45 µm filter membrane	Zhen et al. (2014)
Fluorescence	Soluble phases or extracted fractions from freeze-dried and milled samples from total or particulate phases (with $CaCl_2$ (0.01 M), NaOH + NaCl (0.01 M), NaOH (0.1 M), H_2SO_4 (72%)) were centrifuged at 18,600 g for 20 min at 4 °C and filtered using a 0.45 µm filter membrane. Dilutions were done according the Beer-Lambert law	Jimenez et al. (2017), Fernández-Domínguez et al. (2021)
Fluorescence	Diluted using pure water to TOC below 2 mg L^{-1}	Du and Li (2017), Wu et al. (2020)
Fluorescence	Diluted at a concentration of 100 mg L^{-1} after overnight agitation and adjustment to pH 8 with 0.05 N NaOH	Provenzano (2011), Provenzano et al. (2014)
Fluorescence	Centrifuged at 8000 rpm for 10 min and filtered through a 0.45 µm filter membrane Diluted with 0.1 M phosphate buffer to a DOC approximately of 30 mg L^{-1}	Wan et al. (2012), Zheng et al. (2014)

Table 1 (continued)

Spectroscopic analysis	Sample preparation method	References
MIR	Freeze-dried and milled samples from total or particulate phases (milling and/or freeze-drying could not be required). Thus, pellets are obtained by pressing under reduced pressure a mixture of 1 mg of sample and 100–400 mg of dried KBr	Brunetti et al. (2012), Guo et al. (2012), Provenzano et al. (2014), Guo et al. (2018), Tang et al. (2018), Liu et al. (2019)
^{13}C CP-MAS NMR	20–200 mg of freeze-dried and milled samples from total or particulate phases (milling and/or freeze-drying could not be required). Samples are packed in zirconia rotors Acid pre-treatment to remove paramagnetic trace elements. 600 µl of deuterated dimethyl sulfoxide (DMSO-d6) is added to the samples as solvent	Pichler et al. (2001), Fernandez-Bayo et al. (2018), Tambone et al. (2019), Fang et al. (2020a) Fierro et al. (2016), Laera et al. (2019), Shakeri Yekta et al. (2019)

the studies use large dilutions to fill this gap, where the final C concentration is adjusted to < 10 mg L^{-1} (Shao et al. 2009; Liu et al. 2019).

The total or the solid sample preparation (the solid fraction is usually obtained by a phase separation step such as centrifugation) is commonly freeze-dried before analysis. To homogenise the sample, it is milled into fine particles. For MIR analysis (typically measured using Fourier transform spectrometer (FTIR) (Griffiths and Airmet 2010)), the potassium bromide (KBr) pellet method is recurrently used to prepare the sample since alkali halides do not show any absorption spectrum due to a 100% transmission on the MIR spectra (4000–400 cm^{-1}). This method consists of mixing 1–2 mg of freeze-dried samples and 100–400 mg of KBr. Then, about 200 mg of the mixture is pressured under vacuum into pellets of 1 cm diameter and 1 mm thickness (Provenzano 2011; Brunetti et al. 2012; Provenzano et al. 2014; Tang et al. 2018; Liu et al. 2019). To obtain the ^{13}C CP-MAS NMR spectra of solid samples, powders after freeze-drying are tightly packed into cylindrical zirconia rotors (4 mm of outside diameter) (Chen et al. 2003; Fernandez-Bayo et al. 2018; Shakeri Yekta et al. 2019; Fang et al. 2020a). Pre-treatments with acid solutions to remove paramagnetic trace elements that could hinder the quality of the NMR spectra were suggested (Shakeri Yekta et al. 2018). Moreover, Deuterated DMSO, also known as dimethyl sulfoxide-d_6, is a common solvent used in NMR spectroscopy in which the hydrogen atoms are replaced with their isotope deuterium. DMSO shows no peaks in ^1H NMR spectroscopy depending on the purity degree, thus it is used to stabilise the field/frequency ratio due to its well-known chemical shift, low cost and stability (Tay et al. 2011; Shakeri Yekta et al. 2018; Laera et al. 2019). For total phase or POM samples (MIR or ^{13}C CP-MAS NMR), more homogeneity on sample preparation than for aqueous samples was reported (see Table 1).

3 Main spectroscopic techniques to characterise the aqueous phase in AD studies

3.1 Ultraviolet–visible spectroscopy (UV–Vis)

3.1.1 UV–Vis: spectroscopic indices to assess the OM stability in AD studies

Common absorption regions in the UV–Vis spectra related to various compounds have been frequently

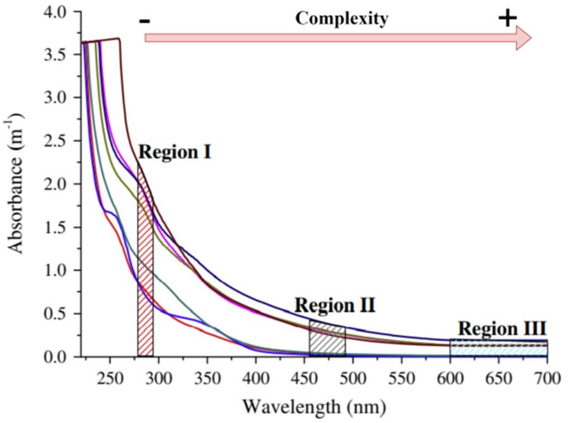


Fig. 1 Example of UV–Vis spectra and wavelength regions interpretation (adapted from Zhen et al. (2014))

reported to describe the DOM fraction during AD (Morán Vieyra et al. 2009; Albrecht et al. 2011; Zhen et al. 2014): Region I at 250–280 nm (lignin and quinone moieties), Region II at 400–500 nm (first stage humification compounds) and Region III at 600–670 nm (strongly humified compounds). These regions are displayed in Fig. 1 and further described in the Online Resource 1. Specific wavelength peaks of interest are usually identified by E_n. The "n" is the wavelength hundred where the peak is located. The origin of the nomenclature could not be traced. The most frequent peaks are E_2 (at 254 and 280 nm), E_3 (at 350 nm), E_4 (at 400, 436, 465, 472 nm) and E_6 (at 664 nm). These peaks have been used to build various spectroscopic indices to assess OM stability.

The $SUVA_{254}$ ratio and the absorption ratio E_4/E_6 (Eqs. 1 and 2) have been extensively described as biological stability indices in AD (Zheng et al. 2014; Zhen et al. 2014; Gao et al. 2021). The $SUVA_{254}$ increases together with the aromaticity degree while the E_4/E_6 ratio decreases as the aromaticity degree and the molecular weight increase. Similarly, the $SUVA_{280}$ ratio (calculated as $SUVA_{254}$ but with absorption at 280 nm) has been suitable to indicate humification due to the electron transition on the wavelength range 270–280 nm for some aromatic substances (Rodríguez et al. 2016). Nonetheless, the $SUVA_{280}$ has been poorly evaluated in AD compared with the $SUVA_{254}$ (Tang et al. 2018). The E_2/E_3 ratio (Eq. 3) has been used to assess changes in the relative size and aromaticity of DOM (Liu et al. 2019) although is frequently stated in composting processes (Helms et al. 2008; He et al. 2011). Whilst, the E_2/E_4 (Eq. 4) and the E_2/E_6 (Eq. 5) ratios have been used to assess the lignin content and the humification degree, respectively (Zhen et al. 2014; Fernandez-Bayo et al. 2018). Furthermore, the chromophoric DOM (a350), which is quantified at 350 nm, have been suggested as an index to define the concentration of the coloured fraction of DOM due to the presence of fulvic and humic-like acids (FLA and HLA). Whereas the E_2/E_3, the E_2/E_4, and the E_2/E_6 ratios have been punctually used in AD studies, the a350 was principally reported in soil or aquatic ecosystem studies (Helms et al. 2008; He et al. 2011; Zhang et al. 2011; Spencer et al. 2012; Tan et al. 2021).

$$SUVA_{254}\left(\frac{\mathrm{L}}{\mathrm{mg\,m}}\right)=\frac{\text{Absorption at 254 nm}(\mathrm{cm}^{-1})}{\mathrm{DOC}(\frac{\mathrm{mg}}{\mathrm{L}})}\times 100\left(\frac{\mathrm{cm}}{\mathrm{m}}\right) \tag{1}$$

$$\text{Decrease of aromatisation degree}:\ E_4/E_6=\text{Absorption at 465 nm}/\text{Absorption at 665 nm} \tag{2}$$

$$\text{Low molecular size and condensation}:\ E_2/E_3=\text{Absorption at 250 nm}/\text{Absorption at 365 nm} \tag{3}$$

$$\text{Relative amount of lignin}:\ E_2/E_4=\text{Absorption at 280 nm}/\text{Absorption at 472 nm} \tag{4}$$

$$\text{Non-humified upon highly humified material}:\ E_2/E_6=\text{Absorption at 280 nm}/\text{Absorption at 665 nm} \tag{5}$$

The values stated from numerous source type in AD studies using the UV–Vis indices are displayed in Table 2.

Regarding the $SUVA_{254}$ ratio, Akhiar et al. (2017) characterised 11 liquid digestate fractions and the respective values ranged from 1.6 to 3.0 L mg^{-1} m^{-1}. Those values were in the range with FLA compounds (0.6–3.9 L mg^{-1} m^{-1}) whereas values up to 5.0 L mg^{-1} m^{-1} were stated for HLA reference compounds. Zheng et al. (2014) observed values between 0.9 and 3.1 L mg^{-1} m^{-1} for liquid digestates. These authors suggested the $SUVA_{254}$ ratio as a suitable stability index to evaluate the humidification degree, which increased by 0.16–10.93 times during the methane production phase. More recently, Gao et al.

Table 2 Most relevant UV–Vis indices ranges and evolution trends in different AD studies (data from 11 papers and over 8 different samples)

Index	Description	Source type	Values before AD (L mg^{-1} m^{-1})[a]	Values after AD (L mg^{-1} m^{-1})[a]	Effect of AD[b]	References
SUVA$_{254}$	Humification degree	Liquid digestate	–	1.6–3	–	Akhiar et al. (2017)
		Liquid digestate	0.3–1	0.9–3.1	↑	Zheng et al. (2014)
		Sludge	0.13–0.37	1.17–2.03	↑	Gao et al. (2021)
		Sludge	–	<2	–	Du and Li (2017)
		Municipal Solid Waste	0.04	0.90–3.10	↑	Shao et al. (2009)
		Humic substances from excess activated sludge	0.98–2.47	2.79–5.20	↑	Liu et al. (2019)
		Digestate from maize silage, chicken manure and beef waste	–	5.92	–	Mukherjee et al. (2016)
SUVA$_{254}$ and SUVA$_{280}$	Humification degree	Extracted humic acids from sewage sludge	2–2.5	3–4.5	↑	Tang et al. (2018)
E$_2$/E$_3$	Low molecular size and condensation	Humic substances from excess activated sludge	4.59–6.41	3.63–3.10	↓	Liu et al. (2019)
E$_2$/E$_4$	Relative amount of lignin at the beginning of humification	Green waste and food waste mixture	>8.0	7.5–8.5	↓	Fernandez-Bayo et al. (2018)
		Activated sludge	–	6.8	–	Zhen et al. (2014)
E$_2$/E$_6$	Non-humified upon highly humified material	Green waste and food waste mixture	70–150	90–170	↑	Fernandez-Bayo et al. (2018)
		Activated sludge	–	15.2	–	Zhen et al. (2014)
E$_4$/E$_6$	Decrease of humification degree	HLA from waste activated sludge	11.50–13.48	7.11–8.01	↓	Liu et al. (2019)
		Waste activated sludge	–	2.0–4.6	↓	Zhen et al. (2014)
		Green waste and food waste mixture	8–17	10–20	↑	Fernandez-Bayo et al. (2018)
		Liquid digestate	–	2.44–5.12	–	Zheng et al. (2014)
		Sludge	5.21–9.51	4.22–2.53	↓	Gao et al. (2021)
		HLA from Grapemarc	9.3	6.1	↓	Brunetti et al. (2012)
		Activated sludge	–	2.2	–	Zhen et al. (2014)

[a] Unit for SUVA indices. Peak ratios are dimensionless. [b] ↑, increase in the index described; ↓, decrease increase in the index described

(2021) reported an increase of the SUVA$_{254}$ after sludge AD from 0.13–0.37 L mg^{-1} m^{-1} to 1.17–2.03 L mg^{-1} m^{-1}. These authors considered that sludge OM with the SUVA$_{254} > 1.10$ L mg^{-1} m^{-1} acquired greater anaerobic stability than incoming materials. Exceptionally, higher SUVA$_{254}$ values than HLA references were observed for a digestate composed of maize silage, chicken manure and beef waste (5.92 L mg^{-1} m^{-1}). These values were notably higher than low and high temperature (T) biochar from woodchips (1.26 and 1.06 L mg^{-1} m^{-1}, respectively), indicating that the digestates' aromaticity degree was related to the process and the feedstock type (Mukherjee et al. 2016). The trend of the SUVA$_{254}$ ratio during AD was evaluated by Tang et al. (2018) in HLA extracts from sewage sludge. A decrease of the humification degree followed by an increase for the periods 8–24 and 24–48 days was stated, respectively. The re-polymerisation of new aromatic structures was suggested, where final values around 4.00 and 3.25 L mg^{-1} m^{-1}

were reached for the $SUVA_{254}$ and the $SUVA_{280}$ ratios, respectively. Additionally, both indices shared a similar pattern. Liu et al. (2019) also presented that the extracted FLA and HLA compositions during AD of excess activated sludge lost aliphatic moieties and gained aromatic moieties. As a result, the $SUVA_{254}$ ratio increased after AD from 0.98–2.47 L mg^{-1} m^{-1} to 2.79–5.20 L mg^{-1} m^{-1}.

The impact of both the T and the incubation time on the $SUVA_{254}$ ratio varied among studies (Du and Li 2017; Rosadi et al. 2020). The $SUVA_{254}$ ratio from water treatment sludge was remarkably higher under anaerobic incubation at 20 °C (maximum value of 8 L mg^{-1} m^{-1} on day 14) compared with other aerobic and anaerobic incubation times (7, 14, 21 days) and T (5, 40 °C) (Rosadi et al. 2020). Interestingly, Rosadi et al. (2020) showed a final decrease of the $SUVA_{254}$ ratio below 1 L mg^{-1} m^{-1} after 21 days of incubation associated with the enrichment production of non-aromatic compounds (possible aliphatic structures) from microbial metabolism. Nonetheless, thermophilic AD led to higher $SUVA_{254}$ ratio values (around 3 L mg^{-1} m^{-1}) after 21 days of incubation. Du and Li (2017) reported a $SUVA_{254}$ below 2 L mg^{-1} m^{-1} for both 61 days aerobic and anaerobic treatments besides not a clear trend of this index was related to TS concentration or T. These authors also observed that aerobic treatments displayed higher $SUVA_{254}$ ratio values than anaerobic. In that line, Shao et al. (2009) have shown an increase of the $SUVA_{254}$ ratio from 0.04 to 3.13 L mg^{-1} m^{-1} for the DOM from MSW in a column reactor with aeration besides a slight variation during 100 days of stabilisation in open piles. Indeed, an increase of the $SUVA_{254}$ ratio is widely stated after aerobic treatments such as composting (He et al. 2011; Guo et al. 2018).

Liu et al. (2019) described a decrease for the E_2/E_3 and the E_4/E_6 ratios for excess activated sludge after AD from 6.41–4.59 to 3.63–3.10 and from 11.50–13.48 to 7.11–8.01, respectively. This is accordant with Brunetti et al. (2012), who displayed an E_4/E_6 decrease from 9.3 to 6.1 in the extracted humic-like substances (HLS) after the AD of grapemarc distillery effluents. After sludge AD, the E_4/E_6 ratio decreased from 5.21–9.51 to 4.22–2.53 due to an increase in both the humification and the polymerisation degree of the benzene ring skeleton (Gao et al., 2021). In addition, an E_4/E_6 ratio <4 was suggested as stabilisation criteria. Additionally, Zhen et al. (2014) observed E_4/E_6 values ranging 2.00–4.60 for different digested sludge regardless of the electro-alkali pretreatment. The E_4/E_6 stated values were below 5.0, which is a core characteristic value of matured HLS (Albrecht et al. 2011). Interestingly, Fernandez-Bayo et al. (2018) showed higher E_2/E_6 and E_4/E_6 ratios after 14 days of thermophilic AD compared with aerobic processes. The increase of the ratios was associated with incomplete stabilisation. Nonetheless, not a regular trend was found for the E_4/E_6 ratio by Zheng et al. (2014) during AD likely due to the influence of different factors (e.g. pH, free radicals, total acidity, protein and polysaccharide). An increase of the E_4/E_6 ratio was also associated with a possible result of low aromatic condensation, higher aliphaticity and higher content of particular functional groups as carboxylic groups (Fialho et al. 2010). Indeed, the ratio E_4/E_6 was poorly correlated to aromaticity compared with $SUVA_{254}$ since the absorption at 665 nm is usually weak/not measurable in DOM (Fuentes et al. 2007; Helms et al. 2008).

3.1.2 UV–Vis spectroscopy: statistically significant results

$SUVA_{254}$ ratio was positively correlated with the hydraulic retention time (HRT) ($R^2=0.52$, $p<0.01$). Moreover, the higher the $SUVA_{254}$ ratio, the lower biogas yields were found due to higher aromatic C content and stabilisation of the OM (Akhiar et al. 2021). Indeed, the $SUVA_{254}$ ratio was suitable to evaluate the humification degree by Zheng et al. (2014), who noticed a higher aromaticity degree after the conversion of the biodegradable fraction (i.e. end of methane production). In this line, the $SUVA_{254}$, the $SUVA_{280}$ and the E_2/E_3 ratios showed the highest correlations ($R^2>0.95$) with the aromatic C region previously defined using the ^{13}C CP-MAS NMR spectra of extracted HLS (Rodríguez et al. 2016). Indeed, Chen et al. (2003) reported that the $SUVA_{254}$ ratio was correlated with the aromatic C region from the ^{13}C CP-MAS NMR spectra ($R^2=0.62$) although not significantly. The $SUVA_{254}$ and the $SUVA_{280}$ were also correlated to the electron transfer capacity (ETC) of HLS during chicken manure AD (Wang et al. 2021). Furthermore, the E_2/E_6 and E_4/E_6 ratios displayed a linear correlation with the C/N ratio ($p<0.05$) (Fernandez-Bayo et al. 2018). The increase of the OM stability degree with increasing the C/N ratio was related to the addition of more lignified compounds. Guo et al. (2010) also found correlations between the a350 (defined in Sect. 3.1.1) and humic-like components (C4, C5) of $r = 0.96$ ($n = 9$) and $r = 0.83$ ($n = 9$), respectively. Besides, a correlation between protein-like (C1) and DOC was revealed ($r = 0.99$, $n = 9$). To confirm the feasibility of the $SUVA_{254}$ ratio to assess stability, a negative correlation between $SUVA_{254}$ and aerobic respirometric activity was noticed by Shao et al. (2009) ($r = -0.898$, $p < 0.001$). Interestingly, Tang et al. (2018) observed that seed germination indices were positively correlated with $SUVA_{254}$ and $SUVA_{280}$.

3.2 Fluorescence spectroscopy: the excitation-emission matrix

3.2.1 Fluorescence regions to assess the OM stability in AD studies

Scan models to study the fluorescence properties of the DOM in AD studies are commonly performed using the fluorescence excitation-emission (Ex/Em) matrix (EEM). The EEM matrix can be treated and visualised as an emission map, which provides all the fluorescence information of the DOM composition (Chen et al. 2003). The EEM subsequent data is frequently combined with fluorescence regional integration (FRI) analysis or parallel factor analysis (PARAFAC) for a quantitative data approach rather than peak picking and quantification (Sanchez et al. 2013; He et al. 2013; Zheng et al. 2014; Martin-Mata et al. 2015). Therefore, the articles that used the EEM matrix combined with FRI or PARAFAC were selected to discuss this section. Specific fluorescent Ex/Em wavelengths (nm) regions have been associated to compound-like groups: tyrosine region (200–280/280–350), tryptophan region (200–250/330–400), FLA (200–250/390–550) and HLA ($320-360/420-460$) (Wan et al. 2012; Zhang et al. 2017; Wu et al. 2020). Nonetheless, further compounds identification among publications was collected in the Online Resource 1.

After AD, an increase of fluorescent compounds-like such as tryptophan-like, tyrosine-like (i.e. protein-like) and HLA compounds as the main components of the DOM is widely stated in the literature (Wan et al. 2012; Zhang et al. 2013, 2017; Li et al. 2014b; Provenzano et al. 2016). However, several trends during AD were identified depending on the study: (i) tryptophan-like and soluble microbial product (SMP) decrease along with the dissolved organic C concentration (Shao et al. 2009), (ii) stability of HLA and FLA compounds (Brunetti et al. 2012; Dai et al. 2013), (iii) tyrosine-like substances conversion into tryptophan-like substances (Dai et al. 2013), (iv) conversion of protein-like substances into more stable HLS (Ni et al. 2017). During the AD of 29 types of biodegradable solid waste, Zheng et al. (2014) assessed the evolution of the percentage of fluorescence response (Pi,n) for a specific fluorescent region (I to V): tyrosine-like protein (I), tryptophan-like protein (II), FLA compounds (III), SMP-like materials (IV) and HLA compounds (V) (Chen et al. 2003, 2014; Ma et al. 2018; Wang et al. 2020). These authors displayed an increase by 0.01–0.54 and 0.03–0.64 times of FLA percentage (Ex/Em of 230/436 nm) and tyrosine-like compounds (Ex/Em of 220(270)/304 nm), respectively. These Ex/Em pairs were consistent as humification and protein indicators, respectively. Nonetheless, interferences were observed during AD between dissolved lignin-derived compounds and nicotinamide adenine dinucleotide (NADH) with HLA compounds, suggesting that HLA compounds (Ex/Em of 260(350)/436 nm) were unsuitable as a humification degree index. Furthermore, tryptophan peaks were inconsistent with the dissolved protein content due to interferences of FLA compounds. The protein zone emission wavelength at < 300 nm was used as an indicator of incomplete anaerobic degradation, where the Ex/Em pairs at 250–300 nm and 280/320–340 nm were associated with SMP-like materials (Fellman et al. 2010; Liao et al. 2014; Jia et al. 2017a). In that line, Maqbool et al. (2016) showed during the operation of a membrane bioreactor that tyrosine-like component was related to the sludge stability whereas tryptophan-like peaks with extra polymeric substances (EPS) production. As a result, EPS could be transformed into microbial HLS. Interestingly, the Ex/Em at 275/430 nm was associated with the fluorescent coenzyme of NADH substances and was used as an index to describe the microbial activity due to microbial metabolism decomposition (Jia et al. 2017b). From the above-mentioned fluorescence regions, the respective fluorescence indices used to assess the composition and stability of the DOM in AD studies are described below.

3.2.2 *Fluorescence indices to assess the OM stability in AD studies*

Wan et al. (2012) proposed the tyrosine-like/FLA ratio to indicate the conversion of organic nitrogen. This ratio increased the first 15 days of co-digestion (AcoD) of cattle dung and duck manure together with the total nitrogen concentration, indicating that tyrosine was one of the main forms of nitrogen. However, the same ratio was decreased after 40 days of AD both for co-digestion and mono-digestion, suggesting the conversion of protein into non-fluorescent compounds. Moreover, the protein-like/(HLA + FLA) ratio presented a complex evolution with similar values for mono and AcoD (about 2.8). The accumulation of humified material during AD was noticed since protein-like intensities decreased whereas the intensity of fulvic-like and humic-like compounds was relatively stable. In the same study, the variation of the microbial activity was associated with the tryptophan-like/FLA ratio. After 15 days, the tryptophan-like/FLA ratio was higher and more stable for AcoD (3.0–3.6) than for mono-digestion (0.8–1.6). Consequently, higher VS removal besides an improved microbial activity during AcoD were reported. Provenzano et al. (2014) displayed an increase of tryptophan-like/FLA ratio from 0.6 to 1.1 after AD of pig slurry while the composted feedstock presented the lowest ratio (0.5). Thus, higher humification processes in aerobic systems were suggested. Indeed, AD showed a higher content of aromatic proteins such as tryptophan and tyrosine aromatic compounds compared with aerobic digestion (Ramdani et al. 2012; Du and Li 2017). The same ratio was used by Baker (2002) to evaluate OM stability with a controlled incubation T during 50 days. Different trends and values were attained depending on the feedstock: silage liquor (> 20), pig and cattle slurry (~2–5), and sheep barn waste (~0.5–4.0). Stable tryptophan/FLA ratios and tryptophan intensities were indicators of more stable samples due to higher ages and decomposition states.

Using EEM-PARAFAC, the ratio FLA/tyrosine-like increased with the methane production phase, ranging 0.97–4.06 (Zheng et al. 2014). That increase was related to the degree of stabilisation and simple protein degradation. This was also noticed during dry-AD, where higher OM stabilisation was found with a ratio evolution from 0.40 to 4.69 from 28 to 84 days (Ni et al. 2017). PARAFAC-EEM is a chemometric method which allows to process the EEM data to assess DOM stability. PARAFAC method reduces the number of variables and provides further information of the DOM composition using semi-quantitative analysis compared with peak picking and FRI (Guo et al. 2012; Shao et al. 2013; Zheng et al. 2014; Qu et al. 2017; Zhang et al. 2017; Penghe et al. 2020). Despite PARAFAC is one of the most popular tools for the multivariate analysis of the EEM spectra, fluorescence overlapping is a major problem besides the use of chemometric tools could be more complex than FRI. Additionally, the definition and use of the region boundaries in FRI is widely supported in the literature and considerably consistent, justifying the frequent use of FRI for EEM data interpretation (Li et al. 2020).

Other indices such as the humification index (HIX) (i.e. proportions of substances with high molecular weights and complex molecular structures) and the biological index (BIX) (i.e. autochthonous biological activity) have been commonly calculated as described in Eq. 6 and Eq. 7 (Qu et al. 2017; Wang et al. 2020, 2021). Complementary to the biological index and described in Eq. 8, the fluorescence index (FI) has been used as an index to differentiate the sources of DOM derived from microbial (i.e. extracellular release and leachate of algae and bacteria) versus terrestrial substances (i.e. originated from decomposition and leaching of plant and soil organic matter) (McKnight et al. 2001; Qu et al. 2017; Wang et al. 2020). Samples presenting BIX values higher than 0.6 are considered to contain high biological material, as well as FI values higher than 1.9. Besides, both indices seem to follow the same trend (Qu et al. 2017).

$$\mathrm{HIX} = \frac{\text{Emission intensity between 435 nm and 480 nm}}{\text{Emission intensity between 300 nm and 345 nm}}\ \text{(with excitation at 254 nm)} \tag{6}$$

$$\mathrm{BIX} = \frac{\text{Emission intensity at 380 nm}}{\text{Emission intensity at 430 nm}}\ \text{(with excitation at 310 nm)} \tag{7}$$

$$FI = \frac{\text{Emission intensity at 450 nm}}{\text{Emission intensity at 500 nm}} \text{ (with excitation at 370 nm)} \quad (8)$$

Zhang et al. (2017) showed a decrease of the HIX on raw sludge from 4.2 to 2 after hydrolysis whereas it increased from 1.7 to 2.1 during methane production. Moreover, the FI decreased from 2.1 to 1.1 after hydrolysis and until a minimum of 0.5 at the end of methane production. This FI variation indicated that the DOM origin changed from microbial (> 1.9) in raw sludge to terrestrial (0.5) at the end of the methane production. Therefore, the bioavailability of the DOM decreased. Higher FI values (2.21–2.47) have been reported in dry anaerobic fermentation (Qu et al. 2017) compared with wet anaerobic fermentation (1.68–1.72), which is likely influenced by the incoming exogenous DOM from wastewater (Guo et al. 2012). Qu et al. (2017) also identified a noticeable increase of BIX (up to 6) after 12 days of dry AD. Thus, an increase of the microbial-derived DOM was noticed. Indeed, Wang et al. (2020) showed values of the BIX > 0.77 and the HIX < 4 for digestate samples. Contrarily, Wang et al. (2021) stated a declining trend of the BIX value after the AD of chicken manure (from 1.01–1.06 to 0.65–0.69) and corn stover (from 0.95–1.03 to 0.81–0.82). However, the HIX increased from 0.58 to 1.18 and from 1.49–2.77 to 4.19–4.52 for chicken manure and corn stover, respectively. Shao et al. (2013) also reported a gradual increase of HIX towards 18 days until the stabilisation after 40 days for low-solid AD, suggesting that refractory SMP was increased in the DOM. Furthermore, Su et al. (2021) displayed a HIX decrease during the hydrolysis of oil crop straw followed by a gradual increase up to 1.53–1.65 probably due to the methanogenic activity, which is in accordance with Zhang et al. (2017). These authors also confirmed humification after AD by the increase of the ratio HLA/tryptophan fluorescence, in that study called A:T ratio. Surprisingly, Rosadi et al. (2020) showed higher HIX at 21 days of incubation for anaerobic incubation than

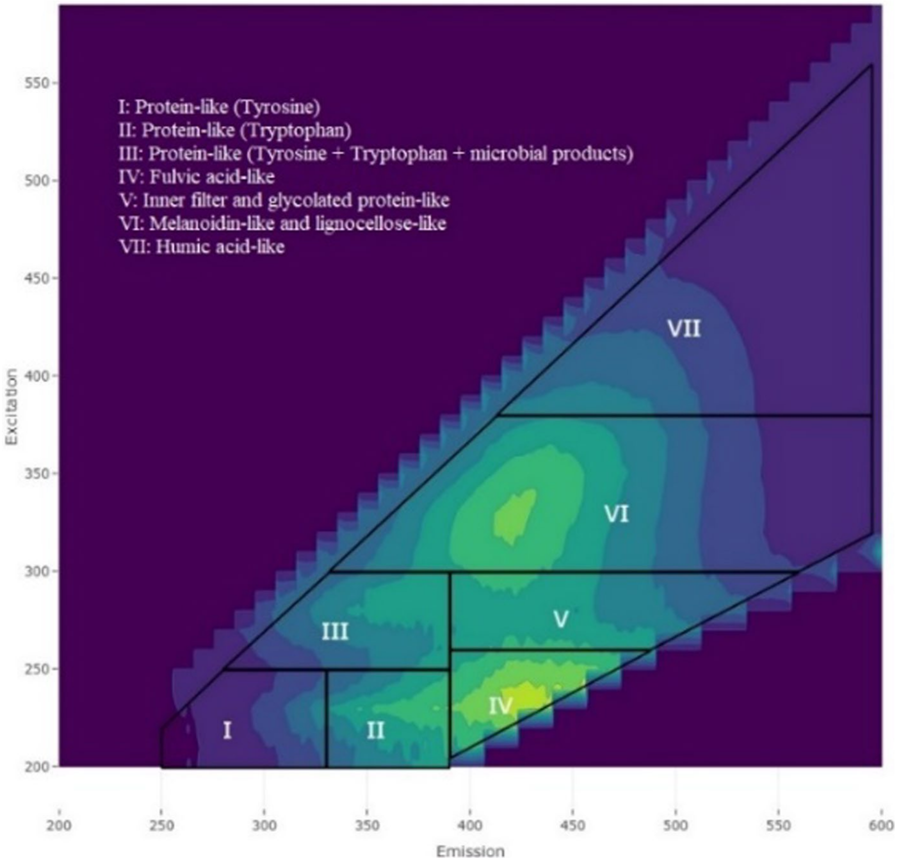


Fig. 2 Example of 3D-EEM fluorescence map coupled with FRI. Polygons indicate the volumes zones integration (map obtained from the publicly available package (Guilayn 2018))

for aerobic incubation compared to other studies (Du and Li 2017). It is important to state that the interpretation of the evolution of the specific fluorescence indices can be challenging since many publications do not clearly specify the evolution of each component, which is indispensable.

He et al. (2013) compared different humification indices and a new ratio was proposed, namely humification parameter, as the more suitable humification index. This index was calculated by the sums of the fluorescence zone volumes of FLA and HLA (III,V) between the sum of the volume values of protein-like (I, II, IV), as described in Eq. 9. This humification parameter ranged from 3.4 to 6.2 for landfill leachate wastes. More recently, Muller et al. (2014) proposed a fluorescence complexity index (FCI) built from seven fluorescence volume zones (Eq. 10): I, protein-like (Tyrosine); II, protein-like (Tryptophan); III, protein-like (Tyrosine, Tryptophan and microbial products); IV, FLA; V, inner filter, glycolated protein-like; VI, melanoidin-like and lignocellulose-like; VII, HLA (see Fig. 2). The FCI was also applied to evaluate the DOM stability of liquid extracted OM fractions from biochemical fractionation methods (Jimenez et al. 2015a). The extracted fractions, which are mentioned below, are defined as: (1) Extractable Soluble from Particulate Organic Matter (SPOM), (2) Readily Extractable Organic Matter (REOM), (3) Slowly Extractable Organic Matter (SEOM), (4) Poorly Extractable Organic Matter (PEOM), (5) Non-Extractable Organic Matter (NEOM). Moreover, the FCI was included in studies to predict waste biodegradability during AD or carbon mineralisation in soil (Jimenez et al. 2015a, 2017; Zhang et al. 2019; Somers et al. 2021; Fernández-Domínguez et al. 2021). From the more to the last accessible fractions, an increase of the FCI from <0.05 to 0.1–0.3 was stated (Muller et al. 2014). After AD, an increase of the FCI for the most accessible fractions on sewage sludge ranging values between 0.48–1.38 was observed (Aemig et al. 2016, 2019). More recently, Mei et al. (2020) stated an increase of the FCI of the DOM of raw sludge after AD from 0.6–2.0 to a maximum of 7.45. Interestingly, mechanical dehydration reduced the FCI due to the loss of soluble HLS. Besides, Mei et al. (2020) suggested an FCI >5.0 to evaluate the stabilisation level of anaerobically digested sludge since the FCI was strictly related to the protein and HLS contents. Gao et al. (2021) also described an increase from 0.99–1.51 to 1.54–1.87 for the FCI in DOM during AD of sludge. This is in accordance with Fernández-Domínguez et al. (2021) and Somers et al. (2021), who showed an increase of the FCI after AD on the most accessible fractions of 34–94% and 34%, respectively. Guilayn et al. (2020a) also used the FCI to compare the composition of extracted HLS from digestates with a commercial reference. The leonardite commercial reference presented a considerably higher FCI (7) than manure (4) or sludge (0.5) digestate HLS extracts.

$$\text{Humification parameter} = \sum \mathrm{V_f(III, V)} \,/ \sum \mathrm{V_f(I, II, IV)} \tag{9}$$

$$\mathrm{FCI} = \frac{\sum_{i=4}^{7} \mathrm{V_f(i)}}{\sum_{j=1}^{3} \mathrm{V_f(i)}} \tag{10}$$

where $\mathrm{V_f}$ (i) is the normalised volume of a fluorescence zone i (U.A./mg $\mathrm{O_2 \cdot L^{-1}}$).

The fluorescence indices values reported in the literature using the EEM matrix and their trends during AD were summarised in Table 3 and further described below.

3.2.3 *Fluorescence spectroscopy: statistically significant results*

The fluorescence intensities ($\mathrm{F_{max}}$) of tyrosine-like, tryptophan and protein-like components, were correlated with the raw protein concentration ($\mathrm{R^2_{C1}} = 0.851$, $p < 0.05$; $\mathrm{R^2_{C3}} = -0.86$, $p < 0.05$) whereas were negatively correlated with the removal of total chemical oxygen demand (tCOD) (Li et al. 2014a). EEM-PARAFAC components were significantly correlated ($p < 0.05$) with several parameters from AD performance (e.g. accumulated biogas production, TS, VS, pH, electrical conductivity) (Li et al., 2014b). Ni et al. (2017) also showed that the $\mathrm{F_{max}}$ of five components (C1: humic-like, C2: protein-like, C3: fulvic acid-like, C4: similar to fulvic acid-like and C5: humic-like organic substances) besides the C4/C2 ratio (FLA/protein-like) were correlated with several leachate bulk parameters (pH, soluble chemical oxygen demand (sCOD), and VFA). These fluorescent components were suggested as possible indicators to monitor AD. Indeed, Du and Li (2017) successfully

Table 3 Most relevant fluorescence indices values and their evolution trends in AD studies (data from 18 papers and over 19 different samples)

Index	Description	Source type	Value before AD	Values after AD	Effect of AD[a]	References
Tyrosine-like/ fulvic-like acid	Conversion of organic nitrogen	Cattle Dung, Duck manure	4.5, 2.5	2.5, 1.8	↓	Wan et al. (2012)
		Cattle Dung + Duck manure	2.9	1.5	↓	Wan et al. (2012)
Tryptophan-like/ fulvic-like acid	Microbial activity	Cattle Dung, Duck manure	3.5, 2.8	1.6, 2.3	↓	Wan et al. (2012)
		Cattle Dung + Duck manure	3.1	3.5	↑	Wan et al. (2012)
		Pig Slurry	0.6	1.1	↑	Provenzano et al. (2014)
		Silage liquor	21.5	8.8	↓	Baker (2002)
		Pig Slurry	4.2	3.4–4.1	↓	Baker (2002)
		Cattle slurry	3.3	3.7	↑	Baker (2002)
		Sheep barn waste	1.8	1.5	↓	Baker (2002)
Fulvic-like acid/ Humic-like acid	Accumulation of humified material	Cattle Dung, Duck manure	2.3, 1.2	1.6, 1.2	↓, ↔	Wan et al. (2012)
		Cattle Dung + Duck manure	1.4	1.3	↓	Wan et al. (2012)
Protein-like/ humic and fulvic-like	Protein versus humified compounds	Cattle Dung, Duck manure	5.5, 3.1	2.8, 2.8	↓	Wan et al. (2012)
		Cattle Dung + Duck manure	2.9	2.8	Complex trend	Wan et al. (2012)
Humification index (HIX)	Humification degree	Biogas slurry	–	2.29–2.66	–	Wang et al. (2020)
		Waste activated sludge	0.3	0.8–0.9	↑	Shao et al. (2013)
		Water from DWTS	0.6	<0.8	↑	Rosadi et al. (2020)
		Chicken manure	0.58	1.18	↑	Wang et al. (2021)
		Corn stover	1.49–2.77	4.19–4.52	↑	Wang et al. (2021)
		Sludge	4.2	2.1	↓	Zhang et al. (2017)
		Oil crop straw	0.5–1.2	1.5–1.7	↑	Su et al. (2021)
Biological index (BIX)	Autochthonous biological activity	Biogas slurry	–	0.83–0.90	–	Wang et al. (2020)
		Chicken manure	1.01–1.06	0.65–0.69	↓	Wang et al. (2021)
		Corn stover	0.95–1.03	0.81–0.82	↓	Wang et al. (2021)
		Rice straw and cattle Manure	0.7–0.8	0.7–0.8	Complex trend	Qu et al. (2017)
Fluorescence index (FI)	Microbial versus terrestrial substances	Rice straw and cattle manure	2.4	2.5	Complex trend	Qu et al. (2017)
		Dewatered sludge	2–2.5	2–2.5	↔	Rosadi et al. (2020)
		Sludge	2.1	0.5	↓	Zhang et al. (2017)
		Swine manure slurry	1.72	1.68–1.69	↔	Guo et al. (2012)
Humification parameter	Humification degree	Landfill and landfill Leachates samples		3.4–6.2	–	He et al. (2013)

Table 3 (continued)

Index	Description	Source type	Value before AD	Values after AD	Effect of AD[a]	References
Fluorescence complexity index (FCI): 7 zones	Organic matter complexity	Sludge	0.3–2	1–7.45	↑	Mei et al. (2020)
		Sludge	0.99–1.51	1.54–1.87	↑	Gao et al. (2021)
		SPOM and REOM of pig slurry mixture	0.38–1.86	0.81–3.20	↑	Fernández-Domínguez et al. (2021)
		SPOM and REOM of sludge	0.34–0.42	0.45–1.34	↑	Fernández-Domínguez et al. (2021)
		SPOM and REOM of manure	1.1–3.3	1.6–4.5		Somers et al. (2021)
		REOM and NDF[b] of sludge	–	0.23–0.83	–	Jimenez et al. (2015a)
		RE-EPS[c] and HLS of sludge	–	0.1–0.3	–	Muller et al. (2014)
		Extracted fractions sludge	0.44–0.35	0.59–1.38	↑	Aemig et al. (2016)
		Commercial Leonardite	–	~7	–	Guilayn et al. (2020a)
		Sludge (HLS)	–	~0.5	–	Guilayn et al. (2020a)
		Manure (HLS)	–	~4.0	–	Guilayn et al. (2020a)

[a] ↑, increase in the index described; ↓, decrease increase in the index described.; ↔, stability in the index described

[b] NDF, Neutral Detergent Fraction

[c] RE-EPS, readily extractable extracellular polymeric substances

used principal component analysis (PCA) to relate TOC, sCOD, VFA, UV_{260} and fluorescence peaks. Evaluating single peaks, He et al. (2013) noticed that the F_{max} of the HLS component was positively correlated with the emission spectra area with an excitation wavelength at 465 nm. Zheng et al. (2014) found that the tyrosine fluorescence peaks were appropriate to evaluate the protein content during AD besides the Ex/Em at 230/436 nm was suitable to assess the humification degree. Robust correlations were found by Baddi et al. (2013) between the wavelength pairs Ex/Em at 280/360 and 360/390 nm with CH_4 production. Similarly, Qu et al. (2017) reported a significant correlation between the CH_4 content and microbial HLS, suggesting that the increase of stable complex materials from microorganisms improved the methane content ratio. Moreover, the COD content was negatively correlated ($p<0.05$) with the BIX and FI. Thus, higher BIX and FI ratios likely indicated higher degradation rates. Furthermore, BIX and FI ratios were correlated with the five fluorescent components. That could be explained by an improved production of fluorescent components due to higher microbial activity. In that line, Su et al. (2021) displayed that the HLA/tryptophan fluorescence ratio (called as A/T ratio by the authors) and HIX presented similar trends with a strong correlation ($r=0.92$, $p<0.001$). However, the newly DOM that was produced during AD was considered to have low aromatic C content (i.e. lower A/T ratio or HIX values). During corn stover AD, only the HIX was correlated with the ETC of HLS (Wang et al. 2021).

Nonetheless, the ETC of HLS was correlated to the HIX whereas negatively correlated with the BIX and C/N ratio for chicken manure AD.

Significant findings were also found using EEM-FRI coupled with the biochemical fractionation method. The obtained variables from the biochemical fractionation coupled with 3D fluorescence were used to predict both biochemical methane potential tests (BMP) and C mineralisation in soil using Partial Least Square (PLS) regression (Jimenez et al. 2015a, 2017; Guilayn, 2019). Jimenez et al. (2017) displayed that BMP values were negatively correlated to the SEOM fraction and the complex fluorescence zones (IV-VII) from all the fractions. Regarding waste classification, the same variables were used in PCA and hierarchical clustering analysis (HCA) to successfully classify a wide variety of organic wastes (Jimenez et al. 2015a; Fernández-Domínguez et al. 2021). Biodegradability of the OM based on the maximum respirometry rate of post-treated digestates was correlated ($R^2 = 0.5–0.7$, p-values < 0.05) with fluorescence zones I-III of the most accessible extracted fractions (SPOM + REOM + SEOM) (Maynaud et al. 2017). Similarly, Aemig et al. (2016) reported that the COD of the DOM was correlated with the fluorescence zones I-III and anti-correlated to the fluorescence zones IV-VII for sewage and digested sludge. Indeed, the FCIs of the last extracted fractions (SEOM and PEOM) were correlated with the digestion time (Somers et al. 2021) and negatively correlated with BMP values (Zhang et al. 2019). Interestingly, the HLA content was linearly correlated to the sum of the fluorescence zones from IV to VII as well as to the FCI (Guilayn et al. 2020a, b). However, the HLA area of digestates and $SUVA_{254}$ were not correlated (Akhiar et al. 2021). This is in accord with Shao et al. (2009), who noticed no correlation between the biodegradation kinetics of fluorescent DOM and UV-absorbing molecules. Furthermore, the highest cumulative EEM volume (ΦT,n) and the fluorescence percentage in a specific region (*P*i) lacked significant correlation ($R^2 < 0.5$) with the aromatic C region from ^{13}C CP-MAS NMR spectra (Chen et al. 2003).

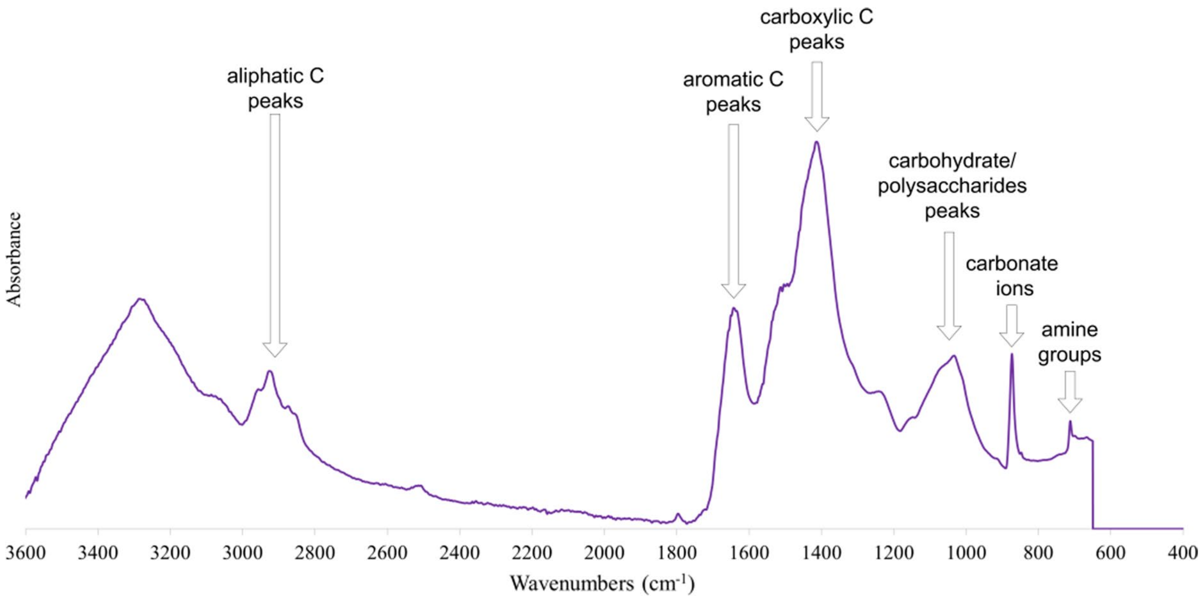


Fig. 3 Example of MIR spectra from a digested sludge. Arrows indicate the usual peaks location to calculate MIR ratios and evaluate OM stability (data from the authors)

4 Main spectroscopic techniques to characterise total and POM in AD studies

4.1 Mid-infrared spectroscopy (MIR)

4.1.1 MIR indices to assess the OM transformation in AD studies

According to the absorption band, different compounds have been assigned to analyse the MIR spectra, as specified in the Online Resource 1. In AD studies, the aromatic C (1590–1689 cm^{-1}, aromatic C=C, C=O in amide I, ketones, and quinones), the aliphatic C (2800–3000 cm^{-1}, related to aliphatic C–H stretching), carbohydrates (1034–1040 cm^{-1}, C-O stretching of polysaccharides) and carboxylic C (~ 1430 cm^{-1}, carboxylic acids and/or C=N stretching of amide III) are the most discussed regions to evaluate OM transformation (Cuetos et al. 2010; Provenzano 2011; Roccotelli et al. 2020). The different regions are identified in Fig. 3. After AD, Provenzano et al. (2018) characterised digestates from two primary digesters that were regularly fed with maize silage. The authors showed a higher relative increase of the peak at 1640 cm^{-1} (C=O stretching conjugated to aromatic rings of lignin) than at 2924 cm^{-1} (attributed to methylene stretching in aliphatic molecules). Besides, a relative decrease of polysaccharides peaks at 1160 cm^{-1} (C–O–C asymmetrical stretching of cellulose and hemicellulose) was also found. Nonetheless, the aromatic and polysaccharides content for the two digesters differed even that were operated under the same conditions (i.e. feedstock, temperature, organic loading rate (OLR), HRT). This fact was associated with a possible difference in the microbial community led by stochastic factors. Furthermore, anaerobic post-digestion increased the relative intensity of the peaks at 1510 and 1460 cm^{-1} (ascribed to aromatic skeletal vibrations and CH_2 deformation stretching of lignin and xylan, respectively) whereas storage induced the appearance of a peak around 1600 cm^{-1} related to aromatic ring vibration of lignin. Iocoli et al. (2019) analysed the structure of cattle manure, poultry litter and pig slurry during AD. Uniformity in the functional groups' distribution among digestates regardless of the feedstock was found with a concentration of aliphatic C in digestates upon aromatic C. AD effect in OM transformation was reported by a reduction of: (i) the content of structural polysaccharide-type compounds (C-H stretching at 2920, 2850 and C-O stretching at 1030 cm^{-1}), (ii) aromatic compounds (C=O stretching at 1650 cm^{-1}) and (iii) high molecular weight olefinic compounds (1515–1505 cm^{-1}, lignin type). However, an increase of: (i) free ammonium (1384–1400 cm^{-1}, C-H in plane), (ii) short-chain organic acids (1640 and 1397 cm^{-1}, C=O from carboxylates and C-H in plane, respectively) and (iii) amines and low molecular weight amides (1570–1600 cm^{-1} and 863 cm^{-1}, C=O amide/N–H in plane and NH_2 out in plane, respectively) was described. Li et al. (2018b) showed that both anaerobic or aerobic digested sewage sludge contained lower proportions of amide I and II groups (protein-like groups) and higher percentages of phenolic, COO- (carboxylic acids) and/or C-H (cellulose-like) groups compared with raw sewage sludge. These changes were explained by the lower intensity bands at 1610–1700 cm^{-1} (aromatic C=C, C=O stretching of amide groups, quinonic C=O and/or C=O of H-bonded conjugated ketones) and 1530–1590 cm^{-1} (N–H deformation and C=N stretching of amides) regions besides the higher intensity bands at 1370–1420 cm^{-1} (O–H deformation and C-O stretching of phenolic OH, antisymmetric stretching of COO^- groups, and/or C-H bending) and 1000–1170 cm^{-1} (C-O stretching of polysaccharide-like substances) regions. Nonetheless, Martínez et al. (2016) identified an intense region at around 1185–900 cm^{-1} (related the functional groups C–O–C, C–O, C–C, C–H in cellulose and hemicellulose (Naumann et al. 2010)) after AcoD of sludge with fats. Besides, two high-intensity bands at around 1560 and 1500–1400 cm^{-1} were also reported, ascribed to proteins (primary amines) in the samples and the adsorption of lipids onto the biomass surface, respectively. Fernández et al. (2015) mainly showed an increase in the protein region at 1640 cm^{-1} (attributed to C=O stretching of amide groups) after cheese whey AD. Additionally, a decrease in the absorption of the peaks at 2920 and 2850 cm^{-1} (aliphatic C–H stretching) was related to aliphatic C conversion into biogas (Wu et al. 2011; Fierro et al. 2016). Indeed, Cuetos et al. (2013) indicated digestion failure by the accumulation of VFA and consequently, the prevalence of carbohydrates (at 1070–1020 cm^{-1}) and organic material (C=O and C=C bonds at 1650–1620 cm^{-1}) was detected. Thus, an increase of the aliphatic C region could be expected for failed

AD processes due to incomplete degradation (Cuetos et al. 2010).

Further transformations and compounds identification after AcoD of fleshings with primary and secondary sludge were described by Sri Bala Kameswari et al. (2014). The main peaks evolution related to carboxylic acids, hydrocarbons, amines, alkynes, and nitro compounds showed that different compounds were transformed into secondary metabolites (e.g. primary, secondary, and tertiary alcohols, amino acids, methane, and ammonia). The MIR spectra extracted from microalgal biomass also showed that carbohydrate and proteins were the major components of microalgal cell wall (Lee and Kim 2018). Peaks at 1657–1546 cm^{-1} (N–H bending band groups) and 1047 cm^{-1} (P–O–C stretching groups) were associated with amino acids and phospholipids derived from the microbial cell walls, respectively (Peng et al. 2019). Those peaks decreased when effluents were treated with $NaNO_3$ and microbial electrolysis during AD of secondary sludge. In this line, Smidt and Parravicini (2009) described the characteristic bands of microbial biomass comparing anaerobically digested with primary sludge: (i) aliphatic methylene bands at 2920 and 2850 cm^{-1}, (ii) amide bands I (C=O vibration) at 1640 cm^{-1}, amide II (N–H vibration) at 1540 cm^{-1} and amide III (C-N vibration) at 1240 cm^{-1}. Li et al. (2014a, b) reported an increase of the polysaccharide-like group, aryl ethers, phenols and/or sulfonates besides a decrease of carboxylic acids, primary and secondary amines and aliphatic C after AD of dewatered sewage sludge. A signal at 1400 cm^{-1} was related to the biodegradation of O–H and C-O functional groups during fermentation whereas organic acids production was identified at 1369 cm^{-1} (C-H deformation of CH_2 and CH_3 groups and/or antisymmetric stretching of COO- groups) (Guo et al. 2012). Tang et al. (2018) used the MIR spectra to identify the functional groups of HLS, where moderate and strong peaks at 1600–1680 cm^{-1} (aromatic C=C vibrations of alkenes, stretching C=O of amide I, O-C=O of ester and carboxyl, and C=O of ketones and quinone groups) were detected. Interestingly, aromatic peaks were strengthened after 30 days AD likely due to the formation of aromatic structures in HLA from derived aromatic compounds released from lignin. This re-polymerisation of aromatic structures was supported by other authors during AD and composting processes (Amir et al. 2010; Rodríguez et al. 2016; Tallou et al. 2020). The MIR spectra were also used to identify inhibition issues during AD performance, such as recalcitrant nitrogen compounds from Maillard reactions on thermal pretreated poultry wastes (Rodríguez-Abalde et al. 2013). The increase of aromatic compounds upon easily biodegradable aliphatic substances was commonly stated during composting processes as well (Castaldi et al. 2005; Huang et al. 2006; Meissl and Smidt 2007; Spaccini and Piccolo 2008; He et al. 2011; Ge et al. 2015; Martín-Ramos and Martín-Gil 2020). However, a partial decrease of aromatic compounds at 1730 cm^{-1} and 1607 cm^{-1} (C=O groups of hemicellulose and lignin) treating lignified compounds led to secondary products at 1560 and 1410 cm^{-1} (characteristic of carboxylate groups of acetic or formic acids) was noticed by other authors (Xia et al. 2018; de Diego-Díaz et al. 2019).

Usual changes in the MIR spectra upon AD have been evaluated to mainly assess the OM aromaticity and aliphaticity through different ratios (cm^{-1} unity omitted), as shown in Eqs. 11, 12, 13, 14, 15, 16 and 17) (Cuetos et al. 2010; Provenzano et al. 2016; Kataki et al. 2017). Table 4 shows the MIR ratios ranges and their trends during AD for the different revised studies.

$$\text{Aromatic C/aliphatic C} = \text{Absorption at } 1630\,\text{cm}^{-1}/\text{Absorption at } 2930\,\text{cm}^{-1} \tag{11}$$

$$\text{Aromatic C/aliphatic C} = \text{Absorption at } 1630\,\text{cm}^{-1}/\text{Absorption at } 2851\,\text{cm}^{-1} \tag{12}$$

Table 4 Most relevant MIR ratios values and trends in AD studies (data from 3 papers and over 5 different samples)

Index	Description	Source type[a]	Values before AD	Values after AD	Effect of AD[b]	References
1630/2930	Aromatic C/aliphatic C	SHW-50	–	1.02	Decreased as HRT decreased	Cuetos et al. (2010)
		SHW-36	–	1.03		
		SHW-25	–	0.72		
		SHW + OFMSW-50	–	1.19		
		SHW + OFMSW-36	–	1.08		
		SHW + OFMSW-25	–	0.88		
		Biogas by-products	–	0.42–1.03	–	Kataki et al. (2017)
		LD *Ipomoea carnea*	–	3.12	–	Kataki et al. (2017)
1630/2851	Aromatic C/aliphatic C	SHW-50	–	1.7	Decreased as HRT decreased	Cuetos et al. (2010)
		SHW-36	–	1.7		
		SHW-25	–	1.06		
		SHW + OFMSW-50	–	3.0		
		SHW + OFMSW-36	–	2.0		
		SHW + OFMSW-25	–	1.5		
		Biogas by-products	–	0.38–1.23	–	Kataki et al. (2017)
		LD *Ipomoea carnea*	–	3.56	–	Kataki et al. (2017)
1640/1030	Aromatic C/carbohydrate	Fruit and vegetable wastes	0.95–1	1.05	Proportional to biogas production	Provenzano et al. (2016)
1640/1430	Aromatic C/carboxylic C	Fruit and vegetable wastes	0.95–1	1.05–1.10	Proportional to biogas production	Provenzano et al. (2016)
1530/2930	Aromatic C/aliphatic C	SHW-50	–	0.7	Decreased as HRT decreased	Cuetos et al. (2010)
		SHW-36	–	0.71		
		SHW-25	–	0.44		
		SHW + OFMSW-50	–	0.72		
		SHW + OFMSW-36	–	0.77		
		SHW + OFMSW-25	–	0.63		
2930/1034	Aliphatic C/carbohydrates	SHW-50	–	0.77	Increased as HRT decreased	Cuetos et al. (2010)
		SHW-36	–	0.79		
		SHW-25	–	1.57		
		SHW + OFMSW-50	–	0.51		
		SHW + OFMSW-36	–	0.85		
		SHW + OFMSW-25	–	1.62		
		Biogas by-products	–	0.9–1.8	–	Kataki et al. (2017)

Table 4 (continued)

Index	Description	Source type[a]	Values before AD	Values after AD	Effect of AD[b]	References
		LD Rice straw	–	2.7	–	Kataki et al. (2017)
2925/1640	Aliphatic C/aromatic C	Fruit and vegetable waste	0.95–1	1–1.05	Inversely proportional to biogas production	Provenzano et al. (2016)

[a] SHW, slaughterhouse waste; OFMSW, Organic fraction of Municipal Solid Waste; LD, liquid digestate

[b] ↑, increase in the index described; ↓, decrease increase in the index described

$$\text{Aromatic C/aliphatic C} = \text{Absorption at } 1530\,\text{cm}^{-1}/\text{Absorption at } 2930\,\text{cm}^{-1} \tag{13}$$

$$\text{Aliphatic C/carbohydrates} = \text{Absorption at } 2930\,\text{cm}^{-1}/\text{Absorption at } 1034\,\text{cm}^{-1} \tag{14}$$

$$\text{Aliphatic C/aromatic C} = \text{Absorption at } 2925\,\text{cm}^{-1}/\text{Absorption at } 1640\,\text{cm}^{-1} \tag{15}$$

$$\text{Aromatic C/carbohydrates} = \text{Absorption at } 1640\,\text{cm}^{-1}/\text{Absorption at } 1030\,\text{cm}^{-1} \tag{16}$$

$$\text{Aromatic C/carboxylic C} = \text{Absorption at } 1640\,\text{cm}^{-1}/\text{Absorption at } 1430\,\text{cm}^{-1} \tag{17}$$

Cuetos et al. (2010) displayed that higher 1630/2930 (1.02–1.19), 1630/2851 (1.70–3.05) and 1530/2930 (0.70–0.72) ratios were found at higher HRT (50 and 36 days) due to OM stabilisation whereas the 2930/1034 ratio (0.51–0.77) was decreased due to incomplete digestion. Moreover, the ratios 1634/2852, 1634/2930 and 2930/1028 were used by Kataki et al. (2017), where the reported values ranged 0.38–3.56, 0.83–3.12 and 0.97–2.68, respectively, for different digestates from bioenergy by-products similar to organic manure. Interestingly, not a significant ratio variation between solid (solid fraction after phase separation) and ash digestate was demonstrated regardless of the nature of the feedstock. However, the values varied for liquid digestates (liquid fraction after phase separation). These ratios are expected to increase in digestates compared with their raw feedstocks due to a higher aromaticity degree. Indeed, Fierro et al. (2016) showed that all the characterised digestates in their study shared absorption bands at ~ 3449 cm^{-1} (O–H stretching vibration of carboxylic, phenolic and alcoholic groups), at 2921 and 2852 cm^{-1} (associated with methylene in long-chain fatty acids) and 1634 cm^{-1} (aromatic C region). Thus, the increase of the aromaticity and aliphaticity degree could occur after AD. Provenzano et al. (2016) also evaluated the ratios at 2925/1640, 1640/1030, and 1640/1430 during AcoD of fruit and vegetable waste in sludge digesters. The 2925/1640 ratio decreased while the biogas production was increasing (from 0.95–1.00 to 0.80–0.85) followed by a ratio increase once the biogas production dropped (from 0.80–0.85 to 1–1.05). Contrarily, the 1640/1030 and 1640/1430 ratios increased with the biogas production (from 0.95–1.00 to 1.15–1.20 and from 0.95–1.00 to 1.20–1.25, respectively) while decreased when the biogas production ceased (from 1.15–1.20 to 1.05 and from 1.20–1.25 to 1.05–1.10). However, overlapping issues in the region 1500–1700 cm^{-1} produced by the complexity of the organic matrix impeded the use of the absorption relations 1650/2920 and 1650/2850 to estimate the degree of aromaticity in Iocoli et al. (2019).

4.1.2 MIR: statistically significant results

Main bands of MIR spectra were significantly correlated with other chemical parameters (e.g. biogas production and dissolved organic C content) (Li et al. 2014b). A high correlation between the ratio

2920/1640 and the C/N ratio was also reported (r = 0.95). Thus, the 2920/1640 ratio was suggested as a biological stability indicator of MSW during landfill stabilisation (Wu et al. 2011). MIR specific absorption of aromatic C at 1627 cm^{-1} and 1230 cm^{-1} (aromatic rings and aromatic esters or amide III) were positively correlated ($p < 0.05$) with seed germination index (Tang et al. 2018). Based on PLS regressions, a correlation coefficient of $R^2 = 0.91$ was found between the whole wavenumber range from 4000 to 400 cm^{-1} and the measured COD, suggesting that a prediction model for COD determination using MIR could be developed (Smidt and Parravicini 2009). Furthermore, the ETC of HLA was correlated to the carbonyl and carboxyl groups (1020–1260 cm^{-1}) and ketone groups (1650–1800 cm^{-1}) during chicken manure AD. Nonetheless, for corn stover AD, ETC was only correlated with carbonyl and carboxyl groups (Wang et al. 2021). The MIR spectra was also used for waste classification in PCA analysis to differentiate between solid and post-treated digestates (Kataki et al. 2017). In that last study, the wavelength at 3460 cm^{-1} (which corresponds to carboxyl, phenol and alcohol groups) besides the wavelength at 1089 cm^{-1} (assigned to Si–O stretch) were the main explicative variables for the Component 1. Similarly, the MIR spectra was successfully used to compare the composition of stearic and palmitic acid with sewage sludge co-digested with Butcher's Fat Waste (Martínez et al. 2016).

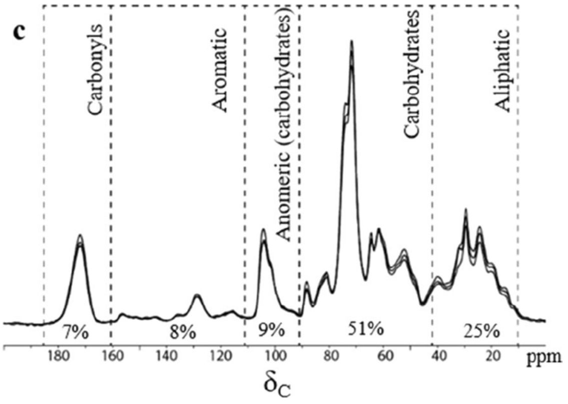


Fig. 4 Example of ^{13}C CP-MAS NMR spectra and C type regions (extracted from Laera et al. (2019))

4.2 Nuclear magnetic resonance spectroscopy: ^{13}C CP-MAS NMR

4.2.1 Feedstock characterisation using ^{13}C CP-MAS NMR regions proportions

Spectra regions have been associated with different structures from molecular groups, as described in the Online Resource 1. In NMR, the variations in the absorption frequency, which take place due to the different shielding of the nuclei, receive the name of chemical shifts (δ or ppm units). The chemical shift scale of ^{13}C CP-MAS NMR spectra is commonly referred to tetramethylsilane (TMS) (δ = 0 ppm). Most protons absorb at fields smaller than TMS, so the δ scale increases towards the smaller fields. Most proton signals (^1H) vary between 0 and 12 δ, while the ^{13}C signals vary from 0 to 250 δ. Based on the ^{13}C signals, the 13C CP-MAS NMR spectra are currently divided into different C type regions according to shared molecular structures: (i) alkyl C (e.g. terminal methyl and methylene groups assigned to proteins, lipids and aliphatic C (δC 0–47 ppm), (ii) O-alkyl C, that is the sum of O-methoxyl C, O-alkyl and N-alkyl C signals (e.g. polysaccharides as cellulose, hemicellulose and proteins) (δC 47–113 ppm), (iii) aromatic C, that is the sum of aryl C, including O-aryl C (e.g. lignin, phenols, aromatic esters or amine moieties) (δC 110–160 ppm), (iv) carboxyl C, that is the sum of carboxyl, carbonyl and amide C (e.g. ketones, aldehydes, organic acids) (δC 160–220 ppm) (Kögel-Knabner 2002; Smernik et al. 2004; Piterina et al. 2009; Tambone et al. 2019). Figure 4 displays the main regions evaluated through ^{13}C CP-MAS NMR spectra in AD studies. The proportions of the C regions bring a semi-quantitative idea of the distribution of the molecular groups of a feedstock. To assess the AD influence on the molecular structure conversion, the ^{13}C CP-MAS NMR spectra reported for feedstocks and digestates in different publications was collected and processed in Table 5. The spectra were divided according to the four above-mentioned regions to expose general trends.

As shown in Table 5, main considerations can be underlined concerning the ^{13}C CP-MAS NMR spectra of feedstocks and their corresponding digestates. Alkyl C (resonances of aliphatic CH_2 and terminal CH_3 units of lipids and proteins), and O-alkyl C (potential overlap of signals from CH and CH_2 in glycerol, N-alkyl C in proteins, methoxyl C in lignin and high field aromatic C) regions displayed the highest proportions in digestates (12–51% and 19–68%,

Table 5 Common ^{13}C CP-MAS NMR defined regions and reported values in AD studies (data from 12 papers and over 18 different samples)

References	Source type[a]	Alkyl C $\delta = 0$–47 ppm		O-alkyl C $\delta = 47$–115 ppm		Aromatic C $\delta = 115$–160 ppm		Carboxyl C $\delta = 160$–210 ppm	
		Feedstock value	Digestate value	Feedstock value	Digestate value	Feedstock value	Digestate value	Feedstock value	Digestate value
Shakeri Yekta et al. (2019)	Agrowaste	8.5–26	13–20	56–81	58–67	6–13	15–16	2–8	6–8
	Food waste	17–39	13–38	44–67	39–53	8–11	11–18	7–10	8–12
Fernandez-Bayo et al. (2018)	Green waste + Food waste	12.4–21.2	12.2–16.7	63.6–69.1	63.1–67.8	7.5–14.1	14.3–16	4.5–7.7	5.5–6.6
Pichler et al. (2001)	Municipal Solid Waste	50	51	34	26	7	13	9	10
Pigoli et al. (2021)	Sewage Sludge	–	29.2	–	54.8	–	7.6	–	8.4
Laera et al. (2019)	OFHW, SHW, IW	25	33	60	42	8	13	7	9
Fang et al. (2020b)	Sewage Sludge	44.4	38.7	28.9	19.0	0.5	4.5	26.1	26.1
	Manure	33.1	41.1	45.4	29.9	4.3	2.4	17.0	26.6
Tambone et al. (2009)	58% OFMSW, 21% CS, 18% AIW	21.8	39.1	65.5	42.1	4.81	9.1	7.9	9.8
Tambone et al. (2010)	80% OFMSW	25.9	44.5	59.1	35.4	5.13	7.9	9.8	12.2
	48%PS, 24% MS	22.5	26.9	63.4	57.4	5.3	8	9	7.8
	65% PS	19.8	35.5	65.8	51.2	8.0	8.1	6.3	8.1
Tambone et al. (2013)	70% Sewage sludge	16.2	21.2	68.5	57.3	9.1	12.7	6.2	8.8
	70% Sewage sludge	18.4	21.1	65.6	60.4	8.2	10.9	7.8	7.4
	PS	38.5	29.5	44.5	38.5	7.0	6.9	10.1	25.1
Tambone et al. (2019)	Liquid digestate	–	29.3–43.5	–	37.5–51.5	–	7.4–14.2	–	8.2–11.9
Corno et al. (2015)	Corn mixture	12.4	22.3	75.6	58.5	6.9	10.3	5.1	8.9
	Giant cane mixture	9.7	11.7	78.4	74.6	7.9	9.6	4.0	4.1
Gómez et al. (2007)	Cattle manure	20.3	24.7	61.1	52.6	5.6	7.2	11.1	14.4
	Poultry manure	21.9	28.2	62.0	45.8	4.8	9.6	8.6	14.1

[a] OFHW, organic fraction household waste; SHW, slaughterhouse waste; IW, industrial waste; OFMSW, Organic fraction of Municipal Solid Waste; CS, cow slurry; AIW, agroindustrial waste; PS, Pig slurry; MS, milk serum

respectively). Nonetheless, aromatic C (e.g. lignin, phenols, aromatic esters or amine moieties) and carboxyl C (mainly related to the presence of proteins) regions accounted for 2.4–17.5% and 4.1–25.1%, respectively. Similarly, the feedstocks globally presented low values for alkyl C, aromatic C and carboxyl C whereas high O-alkyl C proportionalities. Indeed, it has been widely reported that the alkyl C, aromatic C and carboxyl C regions relatively increase after AD whereas O-alkyl C decreases likely due to: (i) a preferable consumption of labile compounds upon aromatic C and phenols, (ii) re-polymerisation of aromatic compounds and (iii) prevalence of alkyl C recalcitrant compounds (Tambone et al. 2013; Tang et al. 2018; Laera et al. 2019). Therefore, as a general rule, feedstocks will be characterised for easier or partially easier degradable compounds (i.e. carbohydrates, N-alkyl proteins, hemicellulose) than their subsequent digestates. Moreover, the aromatic compounds such as lignin, cutin, HLA, steroids, and complex proteins will possibly remain after the digestion due to their recalcitrant nature (Schievano et al. 2009; Tambone et al. 2013). Based on the collected data, aromatic C and alkyl C usually showed the highest relative increase after AD (data not shown). However, alkyl C and O-alkyl C kept the highest proportions after AD compared with aromatic C and carbonyl C regions. Alkyl C concentration in digestates was associated with O-alkyl C degradation even though short-chain aliphatic C at 0–28 ppm (e.g. VFA and steroid-like molecules) are easily transformed into CO_2 and CH_4 during AD (Schievano et al. 2009; Tambone et al. 2009; Pognani et al. 2010). This results in a lower O-alkyl C/alkyl C ratio than their corresponding feedstocks (Tambone et al. 2019). Nonetheless, a large proportion of O-alkyl C in digestates could also indicate incomplete digestion processes or the presence of non-easily degradable compounds. An aliphatic C increase has been also related to methyl groups in polypropylene and to methylene groups in polyamide (22–26 ppm) and polystyrene (128 and 147 ppm) that are present in plastics (Pichler et al. 2001; Fonoll et al. 2016). Thus, impurities present in OM could be reflected in the spectroscopic results.

Table 5 also displays a link between the ^{13}C CP-MAS NMR spectra and the feedstock type. Values between 60 and 70% for O-alkyl C in digestates were reported for fibrous feedstocks, such as green waste mixtures (Fernandez-Bayo et al. 2018), corn giant cane mixture (Corno et al. 2015) or agricultural digestates (Shakeri Yekta et al. 2019). Nonetheless, higher aliphatic C content (30–45%) was found for sewage sludge (Fang et al. 2020a; Pigoli et al. 2021), manure (Gómez et al. 2007; Fang et al. 2020a) or food wastes (Tambone et al. 2009; Laera et al. 2019; Shakeri Yekta et al. 2019). The aromatic C ranged between 15 and 20% for digestates from agricultural wastes, green waste or food waste mixtures although values lower than 10% were mostly reported for sludge, manure and slurry samples. The highest aromatic C values can be compared with extracted HLS from composts extracts (14.1–19.8%) (Spaccini and Piccolo 2008; Albrecht et al. 2015). However, higher aromatic C proportions were found on extracted HLS from different samples, such as compost (48.4–45.6%) (Amir et al. 2010), reference HLS compounds (48.7–79.5%) (Fuentes et al. 2007) or bulk soils samples (27–36%) (Kögel-Knabner 1997).

4.2.2 *^{13}CP-MAS NMR indices to assess the OM transformation in AD studies*

According to the four reported ^{13}CP-MAS NMR regions, different ratios have been proposed in the literature to assess the OM transformation. These ratios are described by Eqs. 18, 19, 20 and 21 (Jackson and Line 1997; Hsu and Lo 1999; Veeken et al. 2001; Piterina et al. 2009; Tambone et al. 2013):

$$\text{Aromaticity index} = \frac{\text{Aromatic C (110−160 ppm)}}{\text{Alkyl C (0−45 ppm)} + \text{O-Alkyl C (60−110 ppm)}} \tag{18}$$

$$\frac{\text{A}}{\text{O-A}}\text{ratio} = \frac{\text{Alkyl C (0−45 ppm)}}{\text{O-Alkyl C (60−110 ppm)}} \tag{19}$$

$$\text{Hydrophobicity index} = \frac{\text{Alkyl C (0−45 ppm)} + \text{Aromatic C (110−160 ppm)}}{\text{O-Alkyl C (60−110 ppm)} + \text{Carboxyl C (160−210 ppm)}} \tag{20}$$

Table 6 Most relevant ^{13}C CP-MAS NMR ratios values and trends in AD studies (data from 9 papers and over 11 different samples)

Index[a]	Source type	Before treatment[b]	After treatment	Effect of AD[c]	References
A/O-A ratio	Agricultural waste	0.1–0.5	0.3–0.5	↑	Shakeri Yekta et al. (2019)
	Food waste	0.25–0.88	0.9–1.1	↑	Shakeri Yekta et al. (2019)
	Municipal waste	0.42	0.79	↑	Laera et al. (2019)
	Pig feces*	–	0.57	–	Hsu and Lo (1999)
	Cattle manure*	0.27	0.56	↑	Veeken et al. (2001)
	Wheat Straw + Feces*	–	0.23	–	Veeken et al. (2001)
	Wheat Straw*	–	0.15	–	Veeken et al. (2001)
Aromaticity	Sludge	14	10.1	↓	Piterina et al. (2009)
	Cattle manure	6.2	9.5	↑	Gómez et al. (2007)
	Poultry manure	6.3	13.8	↑	Gómez et al. (2007)
	Sludge	20.5	30.4	↑	Bartoszek et al. (2008)
	Sludge	11.9	14.3	↑	Polak et al. (2007)
	Sludge*	–	20.5–28.7	–	Hsu and Lo (1999)
	Manure*	–	10	–	Hsu and Lo (1999)
	Feces + straw*	–	15	–	Veeken et al. (2001)
	Wheat Straw*	–	6	–	Veeken et al. (2001)
Aliphaticity	Organic wastes and pig slurry	0.6–0.9	0.6–0.8	↓	Tambone et al. (2013)
Hydrophobicity	Sludge	1.12	0.62	↓	Piterina et al. (2009)
	Feces*	–	0.64	–	Hsu and Lo (1999)
	Wheat Straw + Feces*	–	0.39	–	Veeken et al. (2001)
	Wheat Straw*	–	0.29	–	Veeken et al. (2001)

[a] Definitions in Sect. 4.3 (Eqs. 1 to 4)

[b] Treatments include AD and composting

[c] ↑, increase in the index described; ↓, decrease increase in the index described. *Composting treatment

$$\text{Aliphaticity index} = \frac{\text{Alkyl C (0–45 ppm)}}{\text{Alkyl C (0–45 ppm)} + \text{Aromatic C (110–160 ppm)}} \tag{21}$$

For most of the revised publications, these ratios were used to estimate both the OM degradation and potential maturation whereas the whole ^{13}C CP-MAS NMR spectra were used to identify specific compounds and describe waste composition (Pichler et al. 2001; Piterina et al. 2009). Interestingly, the A/O-A ratio was used to reflect OM stability since it specifies the relative content of protein to carbohydrate (Shakeri Yekta et al. 2019) and recalcitrant to labile compounds (Piterina et al. 2009; Chen et al. 2018). Relevant information was extracted from the literature associated with ^{13}C CP-MAS NMR ratios in AD studies. For that reason, the main ^{13}C CP-MAS NMR ratios values and trends in AD studies are displayed in Table 6.

The ^{13}C CP-MAS NMR ratios were not widely used to assess OM transformation, as previously observed for MIR ratios. Most of the discussions in the literature are qualitative or performed on specific spectra peaks/zones. Nonetheless, the increase of the A/O-A and the aromaticity ratios after AD could be expected since aliphatic C and aromatic C relatively increased during AD whereas O-alkyl C decreased (see Table 5). This is in accordance with Shakeri Yekta et al. (2019), who found as a general trend that the O-alkyl C fraction decreased upon alkyl C during AD. Consequently, a higher A/O-A ratio in digestates than feedstocks was stated, which ranged between 0.1 and 1.2. Moreover, higher values were displayed for food waste digestates (0.9–1.1) than agricultural waste digestates (0.3–0.5). However, it should be noted that all the C regions are expected to be degraded on an absolute basis and at different extensions, depending on the feedstock type and the process performance (Corno et al. 2015). An increase of the aromaticity index was also described by Gómez et al. (2007) for digested cattle manure and poultry manure from 6.2 to 9.5 and 6.3 to 13.8, respectively. During sludge AD, Bartoszek et al. (2008) and Polak et al. (2007) showed an increase of the aromaticity index for extracted HLA from the primary settling tank to the digestion chambers from 20.5% to 30.4% and 11.9% to 14.3%, respectively. Thus, humification was proved during sewage sludge treatment. However, Piterina et al. (2009) reported a decrease of the aromaticity index from 14 to 10% after a mesophilic digestion phase. Interestingly, a storage period increased the aromaticity index from 0.43 to 8.4% probably due to the neo-synthesis of protein-rich EPS whereas the hydrophobicity index (HB) decreased from feed (1.12%) to storage (0.68%). Higher values of the aromaticity index were presented for sludge and manure composted samples (10–28.7) (Hsu and Lo 1999) although similar values were found for pig feces (0.57) and pulp industry sludge (0.44) (Jackson and Line 1997). Nonetheless, lower A/O-A values were reported for raw cattle manure (0.27) or straw (0.15) (Veeken et al. 2001), suggesting an influence of the feedstock type on the C regions extents. Moreover, HB indices were higher for pig feces composting (0.64) than straw and straw co-digestion with feces (0.29 and 0.39, respectively).

Tambone et al. (2013) proposed an aliphaticity index (Eq. 21) expressed as the ratio of alkyl C divided by alkyl C and aromatic C. After AD, a concentration of aliphatic molecules due to an average increase of alkyl C (68.4%) from O-alkyl C degradation (−27.6%) was stated with aliphaticity indices between 0.6 and 0.8. Nonetheless, the aliphaticity index slightly decreased from feedstocks to digestates probably due to aromatic C accumulation. Composting processes showed higher aromaticity indices related to more stable and humified compounds whereas AD concentrated recalcitrant alkyl C and aromatic C (Tambone et al. 2019). However, O-alkyl C content will vary depending on the lignocellulosic material added in composts as a bulking agent (Schievano et al. 2009; Gómez et al. 2010; Tambone et al. 2015; Albrecht et al. 2015). Indeed, the A/O-A ratio was relatively constant or decreased during 12 months of soil incubation under aerobic conditions whereas it was increased under anaerobic conditions. Thus, anaerobic environments appear to enrich more the OM in aliphatic components than aerobic conditions (Chen et al. 2018).

Table 7 Most used spectroscopic techniques and relative significant results reported

Spectroscopic technique	No. of publications[a]	Statistically significant findings[b,c]
UV–Vis	20	50%
Fluorescence	42	45%
MIR	31	23%
^{13}C CP-MAS NMR	17	53%

[a] Total number of publications using each technique from the selected ones reviewed to discuss Sects. 3 and 4 ($n = 82$)

[b] Expressed as: significant finding (statistically proved) associated with spectroscopic parameters/total of publications revised using each technique

[c] Statistical significance determination may vary from study to study and not every study performed statistical analysis

4.2.3 ^{13}C CP-MAS NMR: statistically significant results

^{13}C CP-MAS NMR spectra displayed a strong positive relationship between the respiration rate and the O-alkyl C content ($r = 0.72$) for pretreated MSW using PCA analysis (Pichler et al. 2001). Indeed, an increase of the biological stability of digestates was suggested since the cumulative oxygen uptake (OD_{20}) was correlated with both O-alkyl C ($r = 0.98$; $p < 0.05$) and alkyl C ($r = -0.99$; $p < 0.05$) regions. The O-alkyl C region was inversely correlated to alkyl C and carboxyl C contents in different studies both for feedstocks and digestates (Tambone et al. 2009, 2013). In Tambone et al. (2013), the O-alkyl C content in digestates was significantly ($p < 0.05$) and positively correlated with those in feedstocks ($r = 0.88$, for alkyl C and $r = 0.68$, for O-alkyl C). Whereas, the alkyl C and O-alkyl C regions were significantly ($p < 0.05$) and inversely correlated ($r = 0.93$, $p < 0.05$ for feedstocks and $r = 0.88$, $p < 0.05$ for digestates). Similarly, Shakeri Yekta et al. (2019)

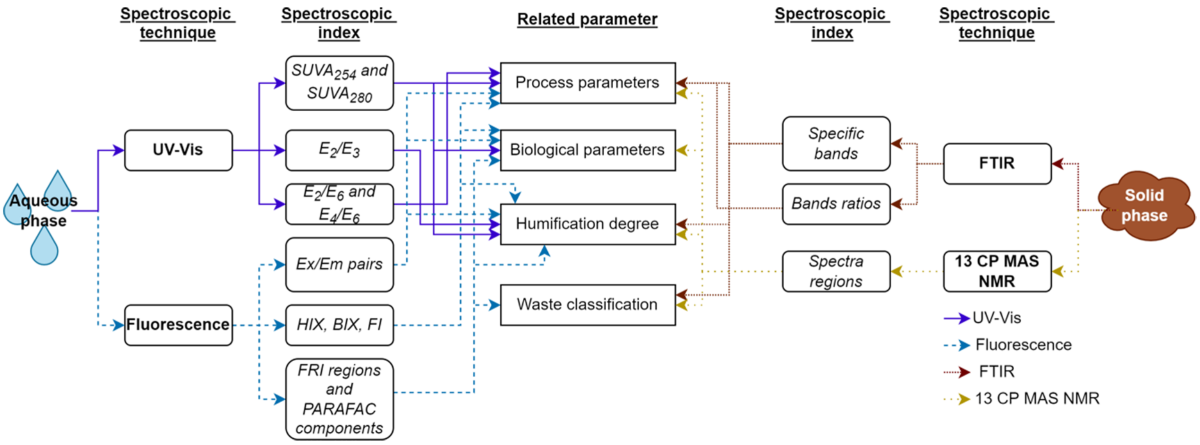


Fig. 5 Scheme of significant correlations between spectroscopic indices from the evaluated spectroscopic techniques and different parameters reported in the literature in AD studies. Arrows indicate significant correlations reported from the literature reviewed

observed that the integrals of alkyl C and carbonyl C spectra of digested POM were correlated to each other and to the N contents of the POM ($r=0.97$, $p<0.05$). Besides, O-alkyl C and anomeric C regions were significantly correlated with xylose, glucose and arabinose content ($r=0.87$–0.97, $p<0.05$) (carbohydrate content) while alkyl C region was positively correlated with lipid content in digestates. Furthermore, the aromatic C region was negatively correlated with VS and TOC content (Tambone et al. 2010). Schievano et al. (2009) evaluated the ^{13}C CP-MAS NMR spectra to classify 12 feedstocks and their subsequent digestates using PCA. The PC1 was defined by the biological stability gradient. Besides, the PC1 was correlated ($r>0.6$) with O-alkyl C and anti-correlated with alkyl C, aromatic C, and carboxyl C whereas the PC2 was defined by the type of C links. PC2 was correlated ($r>0.6$) with Aliphatic-C and anti-correlated with O-alkyl C and aromatic C fractions. Waste classification approaches were successfully evaluated using the ^{13}C CP-MAS NMR spectra based on mean values differences, which showed great reproducibility and robustness (Tambone et al. 2010, 2013, 2019; Pigoli et al. 2021).

5 Relevant points and knowledge gaps

5.1 Summary of the relevance of each spectroscopic technique

To assess the relevance of each spectroscopic technique, the frequency of use of each technique and their statistically significant results were reported from a total of 82 publications included in Sects. 3 and 4. All the selected publications used at least one of the reviewed spectroscopic techniques (UV–Vis, Fluorescence, MIR or ^{13}C CP-MAS NMR) to characterise OM during AD. Table 7 displays the number of publications that used each spectroscopic technique. Moreover, the number of publications that showed significant results (statistically proved) between spectroscopic parameters and other parameters (e.g. AD performance, biological parameters, waste classification) were calculated over the total of publications reviewed for each technique. Thus, this section provides an idea of which techniques and their relative data have been frequently used in statistical analysis to find statistical significance between spectroscopic parameters with other parameters. The statistical method to determine significance varied depending on the study and not every study performed statistical analyses (see Online Resource 1).

Based on the statistically significant findings for the selected studies, Fig. 5 summarises the spectroscopic parameters and indices that have shown significant correlations with other parameters in AD studies.

UV–Vis indices ($SUVA_{254}$, $SUVA_{280}$ and E_2/E_3 ratio) were described as good indicators of process parameters and biological stability by means of the aromaticity degree. However, the E_2/E_6 and the E_4/E_6 ratios were only correlated with the C/N ratio. Fluorescence components from EEM-PARAFAC and single Ex/Em pairs were used to monitor AD process and define the biological stability. The use of the HIX, BIX and FI ratios provided further information about biological responses in the digester. The EEM coupled with FRI was successfully applied for: (i) waste classification, (ii) characterising the HLA content and (iii) predicting BMP or soil incubation values. Nonetheless, waste classification and waste composition (e.g. HLA, carbohydrates, lipid content) determination was mainly assessed using MIR and ^{13}C CP-MAS NMR spectroscopy. Furthermore, both spectroscopic techniques performed on solid samples were correlated to the humification degree of a sample, process parameters and biological stabilisation.

As shown in Table 7 and Fig. 5, every technique allowed to find significant results either concerning OM structure or concerning OM stability. Most of the studies that displayed significant results between spectroscopic parameters and other AD parameters were based on statistical correlations (e.g. pearson correlations, PCA, HCA). However, significant differences in average values were also presented, principally using the ^{13}C CP-MAS NMR spectra to evaluate waste classification since this type of spectra is calculated on a semi-quantitative way. It should be noted that even though numerous spectroscopic parameters provided significant results: (i) a large part of the studies did not include the spectroscopic parameters in the statistical analysis (ii) not all studies that performed spectroscopic characterisation of OM stability also used statistical analyses, which kept the discussion of the obtained results by means of qualitative assessment based on visual spectra interpretation.

5.2 Representativeness of aqueous versus solid phase indices

Spectroscopic analyses on the aqueous phase were frequently focused on specific compound identification or bacterial metabolism monitoring (e.g. biological activity, microbial DOM) whereas spectroscopy of solid samples was mainly used to describe the composition of the OM. This could be explained by the fact that spectroscopic analysis on solid samples can provide information from the whole molecular groups in the molecular structure of the sample (expected to be more representative of the samples). Nonetheless, aqueous indices are just analysing the presence of specific compounds in the soluble fraction (after filtering at a maximum of 0.45 µm), which likely provide a partial view of the sample. Interestingly, the use of fluorescence spectroscopy on liquid extractions obtained from the biochemical fractionation on freeze-dried samples (same sample that would be used for MIR or ^{13}C CP-MAS NMR analysis) is increasing in literature to assess waste classification as well as to characterise OM after AD or specific pre-treatments (Jimenez et al. 2015a; Aemig et al. 2016; Zhang et al. 2019; Laera et al. 2019; Somers et al. 2021; Fernández-Domínguez et al. 2021). Therefore, this methodology has shown that fluorescence spectroscopy on the aqueous phase could also be used to improve waste classification approaches.

Organic compounds need to be solubilised so that they can be consumed, which makes aqueous indices of great interest to further understand AD performance. That being stated, aqueous phase analysis is a fraction of a whole, but at the opposite, possibly solid state spectroscopy can provide irrelevant information for particular issues of biological processes. Despite different studies used spectroscopic techniques on both the solid and the aqueous phase, the lack of correlations between indicators of these two different phases could suggest that they describe different aspects of the sample. Consequently, new studies on the possible correlations between spectroscopic indices from solid and aqueous phases require further research.

5.3 Influence of the sample preparation on the spectroscopic indices

Diverse sample preparation methods before spectroscopic analyses were described in the literature as can be observed in Table 1 (Sect. 2.1). Liquid samples were frequently filtered before analysis since DOM is defined as the part of the OM after filtration from

0.22 to 0.7 µm. Nimptsch et al. (2014) evaluated the influence of the pore size of filter membranes (0.7, 0.45 or 0.22 µm) on several spectroscopic indices calculations (including $SUVA_{254}$, E_2/E_3, FI, humification index) and fluorescence components from different water sources (e.g. river water, wetland, sewage effluent). Based on that study, the meta-analysis of DOM composition using different filter sizes can be performed since filter types had significant but minor effects on the chromophoric DOM composition. Furthermore, the effect of the filter membrane materials was evaluated for specific UV–Vis absorption determinations (Karanfil et al. 2003). These authors noticed that hydrophilic polyethersulfone and hydrophilic polypropylene filters were the best options to perform spectroscopic analyses. However, different measures were suggested before the analysis: (i) rise the filter surface area with 30 mL of distilled and deionised water per cm^2 of the filter membrane, (ii) the first 25 mL of the filtrate on a 47 mm filter must be wasted to minimise DOM losses from filter membranes, (iii) do a filtered blank experiment. Common filters are composed of organic material. Thus, the filter material (subjected to a specific pressure) could be expected to release or adsorb DOM (Zsolnay 2003). An increase of the pH value also showed a linear relationship with the fluorescence intensities of specific fluorophores (Patel-Sorrentino et al. 2002). However, weaker effects of the pH variation (natural pH ± 2) on the tryptophan-like and humic-like fluorescence intensities from freshwater were reported compared with the filter size (between 1.2 and 0.2 µm) (Baker et al. 2007). The biochemical fractionation performed on freeze-dried samples could be considered as a sample preparation method. Indeed, the chemical extractions possibly impact on the original OM composition.

For solid sample preparation, the milling procedure, the particle size, and the surface quality of the samples were identified as influencing factors on the spectroscopic analyses. Hein et al. (2010) showed that the effect of milling the sample was higher than the particle size to enhance the estimation of chemical properties using NIR. More recently, Kamnev et al. (2021) presented the importance of sample preparation in MIR analysis of microbiological materials. These authors reported that preliminary sample preparation steps (e.g. grinding, washing and drying) could alter the composition and properties of the samples. As a general rule, if the sample preparation could alter the properties of the sample, a suggestion would be to analyse the sample before and after the pre-treatment. Durak and Depciuch (2020) tested the influence of different sample preparation (control, dried, frozen, frozen and dried and frozen and lyophilised) on the MIR spectra of plant samples. The spectra from lyophilised samples were the most distinctive although the evaluation of the chemical composition of plants was suggested using fresh plants. Nonetheless, the differences in the results using different methods of measurement were higher than the differences from sample preparation. For ^{13}C CP-MAS NMR analysis, freeze-drying of the samples, paramagnetic compounds removal with acid solutions, and the use of deuterated DMSO as solvent were commonly conducted (Shakeri Yekta et al. 2018). To the author's knowledge, further specific studies that evaluate the effect of sample preparation on ^{13}C CP-MAS NMR analysis of organic wastes are missing. Despite the preparation methods for MIR and ^{13}C CP-MAS NMR analyses are reasonably homogeneous, the broad diversity of preparative and analytical methods (either for aqueous or solid samples) brings potential discrepancies in the results' conclusions and comparability. To compare the spectroscopic indices in a suitable way, it is highly recommended to reveal their calculation inconsistencies depending if there is an extraction step or not (for aqueous phases) or if the particulate phase is different (total phase, solid phase, freeze-dried sample).

6 Conclusions

Over 183 articles were systematically reviewed where at least one of the major spectroscopic techniques (UV–Vis, fluorescence, MIR and ^{13}C CP-MAS NMR) was used to characterise OM. The wide applicability of spectroscopy to assess DOM, total and POM composition during AD was displayed along with this publication. For each spectroscopic technique, the most relevant indicators and the trends with AD could be found and comprehensively summarised. This review is a unique piece of work by showing (i) how spectroscopy is employed in AD studies, (ii) what the most used spectroscopic indices are, (iii) how AD affects these indices while summarising their value ranges regarding different types of waste (before/after AD).

Every technique allowed obtaining statistically significant findings (in 23–53% of studies) concerning OM structure, AD process parameters and OM stability. Therefore, spectroscopic techniques can be considered as an effective and robust tool for AD studies. For instance, by applying spectroscopic techniques and reference indicators from this review, studies aiming to optimise AD (e.g. increase organic load) could more easily consider digestate OM stability, which is essential given all the stakes around AD beyond a renewable energy-producing process. Nonetheless, not all the revised articles included the spectroscopic parameters in the statistical analysis. Indeed, a major part of the studies discussed the spectroscopic results qualitatively. Spectroscopic indices on the solid phase were mainly used to describe waste composition and OM structure while spectroscopic indices on the aqueous phase were principally used to assess biological parameters as well as to identify specific soluble compounds. Moreover, the broad methods for sample preparation (e.g. membrane filter pore size, pH, milling) and even the measurement methods can hinder the quality and comparability of the results. Novel studies on the correlation between aqueous and solid spectroscopic indices as well as the evaluation of the impact of the sample phase on the spectroscopic indices calculation remain as important gaps to be filled.

Each spectroscopic technique is based on different principles. However, the information that can be obtained from them is possibly complementary to have the best idea of the OM characterisation of a sample. Therefore, combining spectroscopic methods and including spectroscopic parameters in statistical analyses instead of using spectra only for qualitative discussions are a highly recommended option to improve the global performance of AD.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Aemig Q, Chéron C, Delgenès N et al (2016) Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in sludge organic matter pools as a driving force of their fate during anaerobic digestion. Waste Manag 48:389–396. https://doi.org/10.1016/j.wasman.2015.11.045

Aemig Q, Doussiet N, Danel A et al (2019) Organic micropollutants' distribution within sludge organic matter fractions explains their dynamic during sewage sludge anaerobic digestion followed by composting. Environ Sci Pollut Res 26:5820–5830. https://doi.org/10.1007/s11356-018-4014-7

Akhiar A, Battimelli A, Torrijos M, Carrere H (2017) Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. Waste Manag 59:118–128. https://doi.org/10.1016/j.wasman.2016.11.005

Akhiar A, Guilayn F, Torrijos M et al (2021) Correlations between the Composition of Liquid Fraction of Full-Scale Digestates and Process Conditions. Energies 14:971. https://doi.org/10.3390/en14040971

Albrecht R, Le Petit J, Terrom G, Périssol C (2011) Comparison between UV spectroscopy and nirs to assess humification process during sewage sludge and green wastes co-composting. Bioresour Technol 102:4495–4500. https://doi.org/10.1016/j.biortech.2010.12.053

Albrecht R, Sebag D, Verrecchia E (2015) Organic matter decomposition: bridging the gap between Rock-Eval pyrolysis and chemical characterization (CPMAS 13C NMR). Biogeochemistry 122:101–111. https://doi.org/10.1007/s10533-014-0033-8

Amir S, Jouraiphy A, Meddich A et al (2010) Structural study of humic acids during composting of activated sludge-green waste: Elemental analysis, FTIR and 13C NMR. J Hazard Mater 177:524–529. https://doi.org/10.1016/j.jhazmat.2009.12.064

Astals S, Batstone DJ, Mata-Alvarez J, Jensen PD (2014) Identification of synergistic impacts during anaerobic co-digestion of organic wastes. Bioresour Technol 169:421–427. https://doi.org/10.1016/j.biortech.2014.07.024

Awhangbo L, Bendoula R, Roger JM, Béline F (2020) Multi-block SO-PLS approach based on infrared spectroscopy for anaerobic digestion process monitoring. Chemometr

Intell Lab Syst 196:103905. https://doi.org/10.1016/j.chemolab.2019.103905

Baddi GA, Antizar-Ladislao B, Alcuta A et al (2013) Municipal Solid Waste Stabilization Efficiency Using Fluorescence Excitation-Emission Spectroscopy. Environ Eng Sci 30:232–240. https://doi.org/10.1089/ees.2012.0041

Baker A (2002) Fluorescence properties of some farm wastes: implications for water quality monitoring. Water Res 36:189–195. https://doi.org/10.1016/S0043-1354(01)00210-X

Baker A, Elliott S, Lead JR (2007) Effects of filtration and pH perturbation on freshwater organic matter fluorescence. Chemosphere 67:2035–2043. https://doi.org/10.1016/j.chemosphere.2006.11.024

Barłóg P, Hlisnikovsky L, Kunzová E (2020) Effect of Digestate on Soil Organic Carbon and Plant-Available Nutrient Content Compared to Cattle Slurry and Mineral Fertilization. Agronomy 10:379. https://doi.org/10.3390/agronomy10030379

Bartoszek M, Polak J, Sułkowski WW (2008) NMR study of the humification process during sewage sludge treatment. Chemosphere 73:1465–1470. https://doi.org/10.1016/j.chemosphere.2008.07.051

Baştabak B, Koçar G (2020) A review of the biogas digestate in agricultural framework. J Mater Cycles Waste Manag 22:1318–1327. https://doi.org/10.1007/s10163-020-01056-9

Bayard R, Gonzalez-Ramirez L, Guendouz J et al (2015) Statistical Analysis to Correlate Bio-physical and Chemical Characteristics of Organic Wastes and Digestates to Their Anaerobic Biodegradability. Waste Biomass Valor 6:759–769. https://doi.org/10.1007/s12649-015-9411-2

Bekiaris G, Bruun S, Peltre C et al (2015) FTIR–PAS: A powerful tool for characterising the chemical composition and predicting the labile C fraction of various organic waste products. Waste Manag 39:45–56. https://doi.org/10.1016/j.wasman.2015.02.029

Brunetti G, Farrag K, Plaza C, Senesi N (2012) Advanced techniques for characterization of organic matter from anaerobically digested grapemarc distillery effluents and amended soils. Environ Monit Assess 184:2079–2089. https://doi.org/10.1007/s10661-011-2101-z

Castaldi P, Alberti G, Merella R, Melis P (2005) Study of the organic matter evolution during municipal solid waste composting aimed at identifying suitable parameters for the evaluation of compost maturity. Waste Manag 25:209–213. https://doi.org/10.1016/j.wasman.2004.12.011

Charnier C, Latrille E, Jimenez J et al (2017) Fast characterization of solid organic waste content with near infrared spectroscopy in anaerobic digestion. Waste Manag 59:140–148. https://doi.org/10.1016/j.wasman.2016.10.029

Chen W, Westerhoff P, Leenheer JA, Booksh K (2003) Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. Environ Sci Technol 37:5701–5710. https://doi.org/10.1021/es034354c

Chen X, Zhang Y, Gu Y et al (2014) Enhancing methane production from rice straw by extrusion pretreatment. Appl Energy 122:34–41. https://doi.org/10.1016/j.apenergy.2014.01.076

Chen X, Xu Y, Gao H et al (2018) Biochemical stabilization of soil organic matter in straw-amended, anaerobic and aerobic soils. Sci Total Environ 625:1065–1073. https://doi.org/10.1016/j.scitotenv.2017.12.293

Corno L, Pilu R, Tambone F et al (2015) New energy crop giant cane (*Arundo donax L.*) can substitute traditional energy crops increasing biogas yield and reducing costs. Bioresour Technol 191:197–204. https://doi.org/10.1016/j.biortech.2015.05.015

Cuetos MJ, Gómez X, Otero M, Morán A (2010) Anaerobic digestion of solid slaughterhouse waste: study of biological stabilization by Fourier Transform infrared spectroscopy and thermogravimetry combined with mass spectrometry. Biodegradation 21:543–556. https://doi.org/10.1007/s10532-009-9322-7

Cuetos MJ, Gómez X, Martínez EJ et al (2013) Feasibility of anaerobic co-digestion of poultry blood with maize residues. Bioresour Technol 144:513–520. https://doi.org/10.1016/j.biortech.2013.06.129

Dai X, Luo F, Dai L, Dong B (2013) Degradation of Extracellular Polymeric Substances (EPS) in Anaerobic Digestion of Dewatered Sludge. Procedia Environ Sci 18:515–521. https://doi.org/10.1016/j.proenv.2013.04.069

de Diego-Díaz B, Duran A, Álvarez-García MR, Fernández-Rodríguez J (2019) New trends in physicochemical characterization of solid lignocellulosic waste in anaerobic digestion. Fuel 245:240–246. https://doi.org/10.1016/j.fuel.2019.02.051

de la Fuente C, Alburquerque JA, Clemente R, Bernal MP (2013) Soil C and N mineralisation and agricultural value of the products of an anaerobic digestion system. Biol Fertil Soils 49:313–322. https://doi.org/10.1007/s00374-012-0719-9

Domeizel M, Khalil A, Prudent P (2004) UV spectroscopy: a tool for monitoring humification and for proposing an index of the maturity of compost. Bioresour Technol 94:177–184. https://doi.org/10.1016/j.biortech.2003.11.026

Doublet J, Boulanger A, Ponthieux A et al (2013) Predicting the biochemical methane potential of wide range of organic substrates by near infrared spectroscopy. Bioresour Technol 128:252–258. https://doi.org/10.1016/j.biortech.2012.10.044

Du H, Li F (2017) Characteristics of dissolved organic matter formed in aerobic and anaerobic digestion of excess activated sludge. Chemosphere 168:1022–1031. https://doi.org/10.1016/j.chemosphere.2016.10.108

Durak T, Depciuch J (2020) Effect of plant sample preparation and measuring methods on ATR-FTIR spectra results. Environ Exp Bot 169:103915. https://doi.org/10.1016/j.envexpbot.2019.103915

Faixo S, Gehin N, Balayssac S et al (2021) Current trends and advances in analytical techniques for the characterization and quantification of biologically recalcitrant organic species in sludge and wastewater: A review. Anal Chim Acta 1152:338284. https://doi.org/10.1016/j.aca.2021.338284

Fang C, Huang R, Dykstra CM et al (2020a) Energy and Nutrient Recovery from Sewage Sludge and Manure via Anaerobic Digestion with Hydrothermal Pretreatment. Environ Sci Technol 54:1147–1156. https://doi.org/10.1021/acs.est.9b03269

Fang F, Wang S-N, Li K-Y et al (2020b) Formation of microbial products by activated sludge in the presence of a metabolic uncoupler o-chlorophenol in long-term operated sequencing batch reactors. J Hazard Mater 384:121311. https://doi.org/10.1016/j.jhazmat.2019.121311

Fellman JB, Hood E, Spencer RGM (2010) Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. Limnol Oceanogr 55:2452–2462. https://doi.org/10.4319/lo.2010.55.6.2452

Fernández C, Cuetos MJ, Martínez EJ, Gómez X (2015) Thermophilic anaerobic digestion of cheese whey: Coupling H_2 and CH_4 production. Biomass Bioenergy 81:55–62. https://doi.org/10.1016/j.biombioe.2015.05.024

Fernandez-Bayo JD, Yazdani R, Simmons CW, VanderGheynst JS (2018) Comparison of thermophilic anaerobic and aerobic treatment processes for stabilization of green and food wastes and production of soil amendments. Waste Manag 77:555–564. https://doi.org/10.1016/j.wasman.2018.05.006

Fernández-Domínguez D, Astals S, Peces M et al (2020) Volatile fatty acids production from biowaste at mechanical-biological treatment plants: Focusing on fermentation temperature. Bioresour Technol 314:123729. https://doi.org/10.1016/j.biortech.2020.123729

Fernández-Domínguez D, Patureau D, Houot S et al (2021) Prediction of organic matter accessibility and complexity in anaerobic digestates. Waste Manag 136:132–142. https://doi.org/10.1016/j.wasman.2021.10.004

Fialho LL, Lopes da Silva WT, Milori DMBP et al (2010) Characterization of organic matter from composting of different residues by physicochemical and spectroscopic methods. Bioresour Technol 101:1927–1934. https://doi.org/10.1016/j.biortech.2009.10.039

Fierro J, Martinez EJ, Rosas JG et al (2016) Co-Digestion of Swine Manure and Crude Glycerine: Increasing Glycerine Ratio Results in Preferential Degradation of Labile Compounds. Water Air Soil Pollut 227:78. https://doi.org/10.1007/s11270-016-2773-7

Filho EGA, Silva LMA, e, Ferreira AG, (2015) Advancements in waste water characterization through NMR spectroscopy: review. Magn Reson Chem 53:648–657. https://doi.org/10.1002/mrc.4158

Fonoll X, Astals S, Dosta J, Mata-Alvarez J (2016) Impact of paper and cardboard suppression on OFMSW anaerobic digestion. Waste Manag 56:100–105. https://doi.org/10.1016/j.wasman.2016.05.023

Fuentes M, Baigorri R, González-Gaitano G, García-Mina JM (2007) The complementary use of 1H NMR, 13C NMR, FTIR and size exclusion chromatography to investigate the principal structural changes associated with composting of organic materials with diverse origin. Org Geochem 38:2012–2023. https://doi.org/10.1016/j.orggeochem.2007.08.007

Gao J, Zhao X, Chen S et al (2021) New insights into the evaluation of anaerobic properties of sludge: Biodegradability and stabilization. J Environ Sci 100:158–166. https://doi.org/10.1016/j.jes.2020.07.021

Ge J, Huang G, Huang J et al (2015) Mechanism and kinetics of organic matter degradation based on particle structure variation during pig manure aerobic composting. J Hazard Mater 292:19–26. https://doi.org/10.1016/j.jhazmat.2015.03.010

Gómez X, Diaz MC, Cooper M et al (2007) Study of biological stabilization processes of cattle and poultry manure by thermogravimetric analysis and 13C NMR. Chemosphere 68:1889–1897. https://doi.org/10.1016/j.chemosphere.2007.02.065

Gómez X, Cuetos MJ, Tartakovsky B et al (2010) A comparison of analytical techniques for evaluating food waste degradation by anaerobic digestion. Bioprocess Biosyst Eng 33:427–438. https://doi.org/10.1007/s00449-009-0343-8

Gómez X, Meredith W, Fernández C et al (2018) Evaluating the effect of biochar addition on the anaerobic digestion of swine manure: application of Py-GC/MS. Environ Sci Pollut Res 25:25600–25611. https://doi.org/10.1007/s11356-018-2644-4

Griffiths PR, Airmet R (2010) FTIR vs. FT-IR Vs mid-IR Appl Spectrosc 64:40A-40A. https://doi.org/10.1366/000370210790619582

Guilayn F, Jimenez J, Martel J-L et al (2019) First fertilizing-value typology of digestates: A decision-making tool for regulation. Waste Manag 86:67–79. https://doi.org/10.1016/j.wasman.2019.01.032

Guilayn F, Benbrahim M, Rouez M et al (2020a) Humic-like substances extracted from different digestates: First trials of lettuce biostimulation in hydroponic culture. Waste Manag 104:239–245. https://doi.org/10.1016/j.wasman.2020.01.025

Guilayn F, Rouez M, Crest M et al (2020b) Valorization of digestates from urban or centralized biogas plants: a critical review. Rev Environ Sci Biotechnol 19:419–462. https://doi.org/10.1007/s11157-020-09531-3

Guilayn F (2018) A R package for processing, plotting and integrating fluorescence excitation-emission matrices

Guilayn F (2019b) Digestats de méthanisation : caractérisation et nouvelles voies de valorisation. PhD Thesis, Univ. Montpellier

Guo W, Xu J, Wang J et al (2010) Characterization of dissolved organic matter in urban sewage using excitation emission matrix fluorescence spectroscopy and parallel factor analysis. J Environ Sci 22:1728–1734. https://doi.org/10.1016/S1001-0742(09)60312-0

Guo X, He X, Zhang H et al (2012) Characterization of dissolved organic matter extracted from fermentation effluent of swine manure slurry using spectroscopic techniques and parallel factor analysis (PARAFAC). Microchem J 102:115–122. https://doi.org/10.1016/j.microc.2011.12.006

Guo X, Li C, Zhu Q et al (2018) Characterization of dissolved organic matter from biogas residue composting using spectroscopic techniques. Waste Manag 78:301–309. https://doi.org/10.1016/j.wasman.2018.06.001

Hagos K, Zong J, Li D et al (2017) Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. Renew Sust Energ Rev 76:1485–1496. https://doi.org/10.1016/j.rser.2016.11.184

He X, Xi B, Wei Z et al (2011) Spectroscopic characterization of water extractable organic matter during composting of municipal solid waste. Chemosphere 82:541–548. https://doi.org/10.1016/j.chemosphere.2010.10.057

He X-S, Xi B-D, Li X et al (2013) Fluorescence excitation-emission matrix spectra coupled with parallel factor and regional integration analysis to characterize organic matter humification. Chemosphere 93:2208–2215. https://doi.org/10.1016/j.chemosphere.2013.04.039

Hein PRG, Lima JT, Chaix G (2010) Effects of sample preparation on NIR spectroscopic estimation of chemical properties of *Eucalyptus urophylla* S.T. Blake Wood Wood Res Technol 64:45–54. https://doi.org/10.1515/hf.2010.011

Hell J, Prückler M, Danner L et al (2016) A comparison between near-infrared (NIR) and mid-infrared (ATR-FTIR) spectroscopy for the multivariate determination of compositional properties in wheat bran samples. Food Control 60:365–369. https://doi.org/10.1016/j.foodcont.2015.08.003

Helms JR, Stubbins A, Ritchie JD et al (2008) Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol and Oceanogr 53:955–969. https://doi.org/10.4319/lo.2008.53.3.0955

Hsu J-H, Lo S-L (1999) Chemical and spectroscopic analysis of organic matter transformations during composting of pig manure. Environmen Pollut 104:189–196. https://doi.org/10.1016/S0269-7491(98)00193-6

Huang GF, Wu QT, Wong JWC, Nagar BB (2006) Transformation of organic matter during co-composting of pig manure with sawdust. Bioresour Technol 97:1834–1842. https://doi.org/10.1016/j.biortech.2005.08.024

Iocoli GA, Zabaloy MC, Pasdevicelli G, Gómez MA (2019) Use of biogas digestates obtained by anaerobic digestion and co-digestion as fertilizers: Characterization, soil biological activity and growth dynamic of *Lactuca sativa* L. Sci Total Environ 647:11–19. https://doi.org/10.1016/j.scitotenv.2018.07.444

Jackson MJ, Line MA (1997) Organic Composition of a Pulp and Paper Mill Sludge Determined by FTIR, 13C CP MAS NMR, and Chemical Extraction Techniques. J Agric Food Chem 45:2354–2358. https://doi.org/10.1021/jf960946l

Jia X, Xi B, Li M et al (2017a) Evaluation of biogasification and energy consumption from food waste using short-term hydrothermal pretreatment coupled with different anaerobic digestion processes. J Clean Prod 152:364–368. https://doi.org/10.1016/j.jclepro.2017.03.029

Jia X, Xi B, Li M et al (2017b) Biodegradation of Potamogeton pectinatus biomass by hydrogen and methane coproduction: Effect of different pretreatments and parallel factor analysis. Int J Hydrog Energy 42:18315–18324. https://doi.org/10.1016/j.ijhydene.2017.04.178

Jimenez J, Aemig Q, Doussiet N et al (2015a) A new organic matter fractionation methodology for organic wastes: Bioaccessibility and complexity characterization for treatment optimization. Bioresour Technol 194:344–353. https://doi.org/10.1016/j.biortech.2015.07.037

Jimenez J, Latrille E, Harmand J et al (2015b) Instrumentation and control of anaerobic digestion processes: a review and some research challenges. Rev Environ Sci Biotechnol 14:615–648. https://doi.org/10.1007/s11157-015-9382-6

Jimenez J, Lei H, Steyer J-P et al (2017) Methane production and fertilizing value of organic waste: Organic matter characterization for a better prediction of valorization pathways. Bioresour Technol 241:1012–1021. https://doi.org/10.1016/j.biortech.2017.05.176

Kamnev AA, Dyatlova YA, Kenzhegulov OA et al (2021) Fourier Transform Infrared (FTIR) Spectroscopic Analyses of Microbiological Samples and Biogenic Selenium Nanoparticles of Microbial Origin: Sample Preparation Effects. Molecules 26:1146. https://doi.org/10.3390/molecules26041146

Karanfil T, Erdogan I, Schlautman MA (2003) Selecting Filter Membranes for measuring DOC and UV254. Journal AWWA 95:86–100. https://doi.org/10.1002/j.1551-8833.2003.tb10317.x

Kataki S, Hazarika S, Baruah DC (2017) Investigation on by-products of bioenergy systems (anaerobic digestion and gasification) as potential crop nutrient using FTIR, XRD, SEM analysis and phyto-toxicity test. J Environ Manage 196:201–216. https://doi.org/10.1016/j.jenvman.2017.02.058

Kögel-Knabner I (1997) 13C and 15N NMR spectroscopy as a tool in soil organic matter studies. Geoderma 80:243–270. https://doi.org/10.1016/S0016-7061(97)00055-4

Kögel-Knabner I (2002) The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol Biochem 34:139–162. https://doi.org/10.1016/S0038-0717(01)00158-4

Krapf LC, Gronauer A, Schmidhalter U, Heuwinkel H (2012) Evaluation of Agricultural Feedstock-Robust near Infrared Calibrations for the Estimation of Process Parameters in Anaerobic Digestion. J near Infrared Spectrosc 20:465–476. https://doi.org/10.1255/jnirs.1013

Laera A, Shakeri Yekta S, Hedenström M et al (2019) A simultaneous assessment of organic matter and trace elements bio-accessibility in substrate and digestate from an anaerobic digestion plant. Bioresour Technol 288:121587. https://doi.org/10.1016/j.biortech.2019.121587

Lashermes G, Nicolardot B, Parnaudeau V et al (2009) Indicator of potential residual carbon in soils after exogenous organic matter application. Eur J Soil Sci 60:297–310. https://doi.org/10.1111/j.1365-2389.2008.01110.x

Lee J-C, Kim H-W (2018) Convergence of direct-transesterification and anaerobic digestion for improved bioenergy potentials of microalgae. J Clean Prod 178:749–756. https://doi.org/10.1016/j.jclepro.2018.01.036

Lesteur M, Bellon-Maurel V, Gonzalez C et al (2010) Alternative methods for determining anaerobic biodegradability: A review. Process Biochem 45:431–440. https://doi.org/10.1016/j.procbio.2009.11.018

Lesteur M, Latrille E, Maurel VB et al (2011) First step towards a fast analytical method for the determination of Biochemical Methane Potential of solid wastes by near infrared spectroscopy. Bioresour Technol 102:2280–2288. https://doi.org/10.1016/j.biortech.2010.10.044

Li M, Xia T, Zhu C et al (2014a) Effect of short-time hydrothermal pretreatment of kitchen waste on biohydrogen production: Fluorescence spectroscopy coupled with parallel factor analysis. Bioresour Technol 172:382–390. https://doi.org/10.1016/j.biortech.2014.09.040

Li X, Dai X, Takahashi J et al (2014b) New insight into chemical changes of dissolved organic matter during anaerobic digestion of dewatered sewage sludge using EEM-PARAFAC and two-dimensional FTIR correlation spectroscopy. Bioresour Technol 159:412–420. https://doi.org/10.1016/j.biortech.2014.02.085

Li L, Peng X, Wang X, Wu D (2018a) Anaerobic digestion of food waste: A review focusing on process stability. Bioresour Technol 248:20–28. https://doi.org/10.1016/j.biortech.2017.07.012

Li X, Mei Q, Yan X et al (2018b) Molecular characteristics of the refractory organic matter in the anaerobic and aerobic digestates of sewage sludge. RSC Adv 8:33138–33148. https://doi.org/10.1039/C8RA05009K

Li L, Wang Y, Zhang W et al (2020) New advances in fluorescence excitation-emission matrix spectroscopy for the characterization of dissolved organic matter in drinking water treatment: A review. Chem Eng J 381:122676. https://doi.org/10.1016/j.cej.2019.122676

Liao X, Zhu S, Zhong D et al (2014) Anaerobic co-digestion of food waste and landfill leachate in single-phase batch reactors. Waste Manag 34:2278–2284. https://doi.org/10.1016/j.wasman.2014.06.014

Liu R, Hao X, van Loosdrecht MCM et al (2019) Dynamics of humic substance composition during anaerobic digestion of excess activated sludge. Int Biodeterior Biodegradation 145:104771. https://doi.org/10.1016/j.ibiod.2019.104771

Ma Y, Gu J, Liu Y (2018) Evaluation of anaerobic digestion of food waste and waste activated sludge: Soluble COD versus its chemical composition. Sci Total Environ 643:21–27. https://doi.org/10.1016/j.scitotenv.2018.06.187

Madsen M, Holm-Nielsen JB, Esbensen KH (2011) Monitoring of anaerobic digestion processes: A review perspective. Renew Sust Energ Rev 15:3141–3155. https://doi.org/10.1016/j.rser.2011.04.026

Mákadi M, Tomócsik A, Orosz V (2012) Digestate: a new nutrient source—review. In: Kumar DS (ed) Biogas. InTech, New York, pp 296–310

Mallet A, Péréme M, Awhangbo L et al (2021) Fast at-line characterization of solid organic waste: Comparing analytical performance of different compact near infrared spectroscopic systems with different measurement configurations. Waste Manag 126:664–673. https://doi.org/10.1016/j.wasman.2021.03.045

Maqbool T, Quang VL, Cho J, Hur J (2016) Characterizing fluorescent dissolved organic matter in a membrane bioreactor via excitation–emission matrix combined with parallel factor analysis. Bioresour Technol 209:31–39. https://doi.org/10.1016/j.biortech.2016.02.089

Marcato C-E, Mohtar R, Revel J-C et al (2009) Impact of anaerobic digestion on organic matter quality in pig slurry. Int Biodeterior Biodegradation 63:260–266. https://doi.org/10.1016/j.ibiod.2008.10.001

Martínez EJ, Gil MV, Fernandez C et al (2016) Anaerobic Codigestion of Sludge: Addition of Butcher's Fat Waste as a Cosubstrate for Increasing Biogas Production. PLoS ONE 11(4):e0153139. https://doi.org/10.1371/journal.pone.0153139

Martin-Mata J, Marhuenda-Egea FC, Moral R et al (2015) Characterization of Dissolved Organic Matter from Sewage Sludge using 3D-Fluorescence Spectroscopy and Chemometric Tools. Commun Soil Sci Plant Anal 46:188–196. https://doi.org/10.1080/00103624.2014.988590

Martín-Ramos P, Martín-Gil J (2020) Facile Monitoring of the Stability and Maturity of Compost Through Fast Analytical Instrumental Techniques. In: Meghvansi MK, Varma A (eds) Biology of Composts. Springer International Publishing, Cham, pp 153–179

Maynaud G, Druilhe C, Daumoin M et al (2017) Characterisation of the biodegradability of post-treated digestates via the chemical accessibility and complexity of organic matter. Bioresour Technol 231:65–74. https://doi.org/10.1016/j.biortech.2017.01.057

McKnight DM, Boyer EW, Westerhoff PK et al (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol Oceanogr 46:38–48. https://doi.org/10.4319/lo.2001.46.1.0038

Meder R, Beets P, Oliver G (2007) Multivariate analysis of IR, NIR, and NMR spectra of soil samples from different land use conversions: Native forest, pasture, and plantation forest. NZ J Forest Sci 37:289–305

Mei X, Tang J, Zhang Y (2020) Sludge stabilization: Characteristics of the end-products and an alternative evaluative methodology. Waste Manag 105:355–363. https://doi.org/10.1016/j.wasman.2020.02.027

Meissl K, Smidt E (2007) High Quality Composts by Means of Cocomposting Of Residues from Anaerobic Digestion. Compost Science & Utilization 15:78–83. https://doi.org/10.1080/1065657X.2007.10702316

Mesquita DP, Quintelas C, Amaral AL, Ferreira EC (2017) Monitoring biological wastewater treatment processes: recent advances in spectroscopy applications. Rev Environ Sci Biotechnol 16:395–424. https://doi.org/10.1007/s11157-017-9439-9

Möller K, Müller T (2012) Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Eng Life Sci 12:242–257. https://doi.org/10.1002/elsc.201100085

Monlau F, Francavilla M, Sambusiti C et al (2016) Toward a functional integration of anaerobic digestion and pyrolysis for a sustainable resource management. Comparison between solid-digestate and its derived pyrochar as soil amendment. Appl Energy 169:652–662. https://doi.org/10.1016/j.apenergy.2016.02.084

Morán Vieyra FE, Palazzi VI, Sanchez de Pinto MI, Borsarelli CD (2009) Combined UV–Vis absorbance and fluorescence properties of extracted humic substances-like for characterization of composting evolution of domestic solid wastes. Geoderma 151:61–67. https://doi.org/10.1016/j.geoderma.2009.03.006

Mottet A, Ramirez I, Carrère H et al (2013) New fractionation for a better bioaccessibility description of particulate organic matter in a modified ADM1 model. Chem Eng J 228:871–881. https://doi.org/10.1016/j.cej.2013.05.082

Mukherjee S, Weihermueller L, Tappe W et al (2016) Microbial respiration of biochar- and digestate-based mixtures. Biol Fertil Soils 52:151–164. https://doi.org/10.1007/s00374-015-1060-x

Muller M, Jimenez J, Antonini M et al (2014) Combining chemical sequential extractions with 3D fluorescence spectroscopy to characterize sludge organic matter. Waste Manag 34:2572–2580. https://doi.org/10.1016/j.wasman.2014.07.028

Ni Z, Liu J, Zhang M (2017) Short-term pre-aeration applied to the dry anaerobic digestion of MSW, with a focus on the spectroscopic characteristics of dissolved organic matter. Chem Eng J 313:1222–1232. https://doi.org/10.1016/j.cej.2016.11.020

Nimptsch J, Woelfl S, Kronvang B et al (2014) Does filter type and pore size influence spectroscopic analysis of freshwater chromophoric DOM composition? Limnologica 48:57–64. https://doi.org/10.1016/j.limno.2014.06.003

Panuccio MR, Papalia T, Attinà E et al (2019) Use of digestate as an alternative to mineral fertilizer: effects on growth and crop quality. Archives of Agronomy and Soil Science 65:700–711. https://doi.org/10.1080/03650340.2018.1520980

Papa G, Scaglia B, Schievano A, Adani F (2014) Nanoscale structure of organic matter could explain litter decomposition. Biogeochemistry 117:313–324. https://doi.org/10.1007/s10533-013-9863-z

Patel-Sorrentino N, Mounier S, Benaim JY (2002) Excitation–emission fluorescence matrix to study pH influence on organic matter fluorescence in the Amazon basin rivers. Water Res 36:2571–2581. https://doi.org/10.1016/S0043-1354(01)00469-9

Peng H, Zhao Z, Xiao H et al (2019) A strategy for enhancing anaerobic digestion of waste activated sludge: Driving anodic oxidation by adding nitrate into microbial electrolysis cell. J Environ Sci 81:34–42. https://doi.org/10.1016/j.jes.2019.02.009

Penghe Z, Yuling L, Chuanchuan D, Pengliang W (2020) Study on Dissolution Characteristics of Excess Sludge by Low-Temperature Thermal Hydrolysis and Acid Production by Fermentation. ACS Omega 5:26101–26109. https://doi.org/10.1021/acsomega.0c03606

Pichler M, Knicker H, Kögel-Knabner I (2001) Solid-state (13) C NMR spectroscopic, chemolytic and biological assessment of pretreated municipal solid waste. J Ind Microbiol Biotechnol 26:83–89. https://doi.org/10.1038/sj/jim/7000059

Pigoli A, Zilio M, Tambone F et al (2021) Thermophilic anaerobic digestion as suitable bioprocess producing organic and chemical renewable fertilizers: A full-scale approach. Waste Manag 124:356–367. https://doi.org/10.1016/j.wasman.2021.02.028

Piterina AV, Barlett J, Pembroke JT (2009) 13C-NMR Assessment of the Pattern of Organic Matter Transformation during Domestic Wastewater Treatment by Autothermal Aerobic Digestion (ATAD). Int J Environ Res Public Health 6:2288–2306. https://doi.org/10.3390/ijerph6082288

Pognani M, D'Imporzano G, Scaglia B, Adani F (2009) Substituting energy crops with organic fraction of municipal solid waste for biogas production at farm level: A full-scale plant study. Process Biochem 44:817–821. https://doi.org/10.1016/j.procbio.2009.03.014

Pognani M, Barrena R, Font X et al (2010) Monitoring the organic matter properties in a combined anaerobic/aerobic full-scale municipal source-separated waste treatment plant. Bioresour Technol 101:6873–6877. https://doi.org/10.1016/j.biortech.2010.03.110

Polak J, Sułkowski WW, Bartoszek M et al (2007) Spectroscopic study of the effect of biological treatment on the humification process of sewage sludge. J Mol Struct 834–836:229–235. https://doi.org/10.1016/j.molstruc.2006.12.065

Provenzano M (2011) Qualitative Characterization and Differentiation of Digestates from Different Biowastes Using FTIR and Fluorescence Spectroscopies. J Environ Prot 02:83–89. https://doi.org/10.4236/jep.2011.21009

Provenzano MR, Malerba AD, Pezzolla D, Gigliotti G (2014) Chemical and spectroscopic characterization of organic matter during the anaerobic digestion and successive composting of pig slurry. Waste Manag 34:653–660. https://doi.org/10.1016/j.wasman.2013.12.001

Provenzano MR, Cavallo O, Malerba AD et al (2016) Co-treatment of fruit and vegetable waste in sludge digesters: Chemical and spectroscopic investigation by fluorescence and Fourier transform infrared spectroscopy. Waste Manag 50:283–289. https://doi.org/10.1016/j.wasman.2016.02.026

Provenzano MR, Cavallo O, Malerba AD et al (2018) Unravelling (maize silage) digestate features throughout a full-scale plant: A spectroscopic and thermal approach. J Clean Prod 193:372–378. https://doi.org/10.1016/j.jclepro.2018.05.081

Qu H, Guo X, Chen Y et al (2017) Characterization of Dissolved Organic Matter from Effluents in a Dry Anaerobic Digestion Process Using Spectroscopic Techniques and Multivariate Statistical Analysis. Waste Biomass Valor 8:793–802. https://doi.org/10.1007/s12649-016-9613-2

Ramdani A, Dold P, Gadbois A et al (2012) Characterization of the heterotrophic biomass and the endogenous residue of activated sludge. Water Res 46:653–668. https://doi.org/10.1016/j.watres.2011.11.030

Roccotelli A, Araniti F, Tursi A et al (2020) Organic Matter Characterization and Phytotoxic Potential Assessment of a Solid Anaerobic Digestate Following Chemical Stabilization by an Iron-Based Fenton Reaction. J Agric Food Chem. https://doi.org/10.1021/acs.jafc.0c03570

Rodríguez FJ, Schlenger P, García-Valverde M (2016) Monitoring changes in the structure and properties of humic substances following ozonation using UV–Vis, FTIR and 1H NMR techniques. Sci Total Environ 541:623–637. https://doi.org/10.1016/j.scitotenv.2015.09.127

Rodríguez-Abalde Á, Gómez X, Blanco D et al (2013) Study of thermal pre-treatment on anaerobic digestion of slaughterhouse waste by TGA-MS and FTIR spectroscopy. Waste Manag Res 31:1195–1202. https://doi.org/10.1177/0734242X13507312

Rosadi MY, Yamada T, Hudori H et al (2020) Characterization of dissolved organic matter extracted from water treatment sludge. Water Supply 20:2194–2205. https://doi.org/10.2166/ws.2020.120

Sanchez NP, Skeriotis AT, Miller CM (2013) Assessment of dissolved organic matter fluorescence PARAFAC components before and after coagulation–filtration in a full scale water treatment plant. Water Res 47:1679–1690. https://doi.org/10.1016/j.watres.2012.12.032

Sarker TC, Incerti G, Spaccini R et al (2018) Linking organic matter chemistry with soil aggregate stability: Insight from 13C NMR spectroscopy. Soil Biol Biochem 117:175–184. https://doi.org/10.1016/j.soilbio.2017.11.011

Schievano A, Adani F, Tambone F et al (2009) WHAT IS THE DIGESTATE? Anaerobic digestion: opportunities for agriculture and environment [s.l]: Regione Lombardia, 9788890374609

Shakeri Yekta S, Hedenström M, Stehr JE et al (2018) Pretreatment of anaerobic digester samples by hydrochloric acid for solution-state 1H and 13C NMR spectroscopic characterization of organic matter. Chemosphere 199:201–209. https://doi.org/10.1016/j.chemosphere.2018.02.015

Shakeri Yekta S, Hedenström M, Svensson BH et al (2019) Molecular characterization of particulate organic matter in full scale anaerobic digesters: An NMR spectroscopy study. Sci Total Environ 685:1107–1115. https://doi.org/10.1016/j.scitotenv.2019.06.264

Shao Z-H, He P-J, Zhang D-Q, Shao L-M (2009) Characterization of water-extractable organic matter during the biostabilization of municipal solid waste. J Hazard Mater 164:1191–1197. https://doi.org/10.1016/j.jhazmat.2008.09.035

Shao L, Wang T, Li T et al (2013) Comparison of sludge digestion under aerobic and anaerobic conditions with a focus on the degradation of proteins at mesophilic temperature. Bioresour Technol 140:131–137. https://doi.org/10.1016/j.biortech.2013.04.081

Smernik RJ, Oliver IW, McLaughlin MJ (2004) Changes in the Nature of Sewage Sludge Organic Matter During a Twenty-One-Month Incubation. J Environ Qual 33:1924–1929. https://doi.org/10.2134/jeq2004.1924

Smidt E, Parravicini V (2009) Effect of sewage sludge treatment and additional aerobic post-stabilization revealed by infrared spectroscopy and multivariate data analysis. Bioresour Technol 100:1775–1780. https://doi.org/10.1016/j.biortech.2008.10.003

Somers MH, Jimenez J, Azman S et al (2021) Ultrasonication affects the bio-accessibility of primary dairy cow manure digestate for secondary post-digestion. Fuel 291:120140. https://doi.org/10.1016/j.fuel.2021.120140

Spaccini R, Piccolo A (2008) Spectroscopic Characterization of Compost at Different Maturity Stages. CLEAN – Soil. Air, Water 36:152–157. https://doi.org/10.1002/clen.200720012

Spencer RGM, Butler KD, Aiken GR (2012) Dissolved organic carbon and chromophoric dissolved organic matter properties of rivers in the USA. J Geophys Res Biogeosci. https://doi.org/10.1029/2011JG001928

Sri Bala Kameswari K, Kalyanaraman C, Umamaheswari B, Thanasekaran K (2014) Enhancement of biogas generation during co-digestion of tannery solid wastes through optimization of mix proportions of substrates. Clean Techn Environ Policy 16:1067–1080. https://doi.org/10.1007/s10098-013-0706-3

Stockl A, Lichti F (2018) Near-infrared spectroscopy (NIRS) for a real time monitoring of the biogas process. Bioresour Technol 247:1249–1252. https://doi.org/10.1016/j.biortech.2017.09.173

Stutzenstein P, Bacher M, Rosenau T, Pfeifer C (2018) Optimization of Nutrient and Carbon Recovery from Anaerobic Digestate via Hydrothermal Carbonization and Investigation of the Influence of the Process Parameters. Waste Biomass Valor 9:1303–1318. https://doi.org/10.1007/s12649-017-9902-4

Su L, Chen M, Wang S et al (2021) Fluorescence characteristics of dissolved organic matter during anaerobic digestion of oil crop straw inoculated with rumen liquid. RSC Adv 11:14347–14356. https://doi.org/10.1039/D1RA01176F

Tallou A, Pedrero Salcedo F, Haouas A et al (2020) Assessment of biogas and biofertilizer produced from anaerobic co-digestion of olive mill wastewater with municipal wastewater and cow dung. Environ Technol Innov 20:101152. https://doi.org/10.1016/j.eti.2020.101152

Tambone F, Genevini P, D'Imporzano G, Adani F (2009) Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour Technol 100:3140–3142. https://doi.org/10.1016/j.biortech.2009.02.012

Tambone F, Scaglia B, D'Imporzano G et al (2010) Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81:577–583. https://doi.org/10.1016/j.chemosphere.2010.08.034

Tambone F, Adani F, Gigliotti G et al (2013) Organic matter characterization during the anaerobic digestion of different biomasses by means of CPMAS 13C NMR spectroscopy. Biomass Bioenergy 48:111–120. https://doi.org/10.1016/j.biombioe.2012.11.006

Tambone F, Terruzzi L, Scaglia B, Adani F (2015) Composting of the solid fraction of digestate derived from pig slurry: Biological processes and compost properties. Waste Manag 35:55–61. https://doi.org/10.1016/j.wasman.2014.10.014

Tambone F, Orzi V, Zilio M, Adani F (2019) Measuring the organic amendment properties of the liquid fraction of digestate. Waste Manag 88:21–27. https://doi.org/10.1016/j.wasman.2019.03.024

Tan F, Zhu Q, Guo X, He L (2021) Effects of digestate on biomass of a selected energy crop and soil properties. J Scie Food Agric 101:927–936. https://doi.org/10.1002/jsfa.10700

Tang Y, Li X, Dong B et al (2018) Effect of aromatic repolymerization of humic acid-like fraction on digestate phytotoxicity reduction during high-solid anaerobic digestion for stabilization treatment of sewage sludge. Water Res 143:436–444. https://doi.org/10.1016/j.watres.2018.07.003

Tay B-Y, Wang C, Stubbs LP et al (2011) Deuterated dimethyl sulfoxide as a good NMR solvent for the characterization of alkali metal cyclopentadienides, amides, alkoxides and phenoxides. J Organomet Chem

696:3431–3435. https://doi.org/10.1016/j.jorganchem.2011.07.038

Teglia C, Tremier A, Martel J-L (2011) Characterization of Solid Digestates: Part 1, Review of Existing Indicators to Assess Solid Digestates Agricultural Use. Waste Biomass Valor 2:43–58. https://doi.org/10.1007/s12649-010-9051-5

Veeken AHM, Adani F, Nierop KGJ et al (2001) Degradation of Biomacromolecules during High-Rate Composting of Wheat Straw-Amended Feces. J Environ Qual 30:1675–1684. https://doi.org/10.2134/jeq2001.3051675x

Wan S, Xi B, Xia X et al (2012) Using fluorescence excitation–emission matrix spectroscopy to monitor the conversion of organic matter during anaerobic co-digestion of cattle dung and duck manure. Bioresour Technol 123:439–444. https://doi.org/10.1016/j.biortech.2012.04.001

Wang S, Yuan R, Chen H et al (2020) Effect of sulfonamides on the dissolved organic matter fluorescence in biogas slurry during anaerobic fermentation according to the PARAFAC analysis. Process Saf Environ Protect 144:253–262. https://doi.org/10.1016/j.psep.2020.07.033

Wang X, Muhmood A, Lyu T et al (2021) Mechanisms of genuine humic acid evolution and its dynamic interaction with methane production in anaerobic digestion processes. Chem Eng J 408:127322. https://doi.org/10.1016/j.cej.2020.127322

Ward AJ (2016) Near-Infrared Spectroscopy for Determination of the Biochemical Methane Potential: State of the Art. Chemical Eng Technol 39:611–619. https://doi.org/10.1002/ceat.201500315

Wu H, Zhao Y, Long Y et al (2011) Evaluation of the biological stability of waste during landfill stabilization by thermogravimetric analysis and Fourier transform infrared spectroscopy. Bioresour Technol 102:9403–9408. https://doi.org/10.1016/j.biortech.2011.07.029

Wu D, Li L, Zhao X et al (2019) Anaerobic digestion: A review on process monitoring. Renew Sust Energ Rev 103:1–12. https://doi.org/10.1016/j.rser.2018.12.039

Wu Y, Du H, Li F et al (2020) Effect of Adding Drinking Water Treatment Sludge on Excess Activated Sludge Digestion Process. Sustainability 12:6953. https://doi.org/10.3390/su12176953

Xia T, Huang H, Wu G et al (2018) The characteristic changes of rice straw fibers in anaerobic digestion and its effect on rice straw-reinforced composites. Ind Crops Prod 121:73–79. https://doi.org/10.1016/j.indcrop.2018.04.004

Xiao K, Abbt-Braun G, Horn H (2020) Changes in the characteristics of dissolved organic matter during sludge treatment: A critical review. Water Res 187:116441. https://doi.org/10.1016/j.watres.2020.116441

Yang G, Li Y, Zhen F et al (2021) Biochemical methane potential prediction for mixed feedstocks of straw and manure in anaerobic co-digestion. Bioresour Technol 326:124745. https://doi.org/10.1016/j.biortech.2021.124745

Yang H, Mouazen AM (2012) Vis/near and mid-infrared spectroscopy for predicting soil N and C at a farm scale. In: Infrared Spectroscopy—Life and Biomedical Sciences. IntechOpen, pp 185–210. https://doi.org/10.5772/36393

Zeng Z, Zheng P, Ding A et al (2017) Source analysis of organic matter in swine wastewater after anaerobic digestion with EEM-PARAFAC. Environ Sci Pollut Res 24:6770–6778. https://doi.org/10.1007/s11356-016-8324-3

Zhang Y, Yin Y, Feng L et al (2011) Characterizing chromophoric dissolved organic matter in Lake Tianmuhu and its catchment basin using excitation-emission matrix fluorescence and parallel factor analysis. Water Res 45:5110–5122. https://doi.org/10.1016/j.watres.2011.07.014

Zhang Y, Zhang P, Guo J et al (2013) Spectroscopic analysis and biodegradation potential study of dissolved organic matters in sewage sludge treated with high-pressure homogenization. Bioresour Technol 135:616–621. https://doi.org/10.1016/j.biortech.2012.09.034

Zhang Z, Guo L, Wang Y et al (2017) Degradation and transformation of extracellular polymeric substances (EPS) and dissolved organic matters (DOM) during two-stage anaerobic digestion with waste sludge. Int J Hydrog Energy 42:9619–9629. https://doi.org/10.1016/j.ijhydene.2017.02.201

Zhang Y, Xu S, Cui M, Wong JWC (2019) Effects of different thermal pretreatments on the biodegradability and bioaccessibility of sewage sludge. Waste Manag 94:68–76. https://doi.org/10.1016/j.wasman.2019.05.047

Zhen G, Lu X, Li Y-Y, Zhao Y (2014) Combined electrical-alkali pretreatment to increase the anaerobic hydrolysis rate of waste activated sludge during anaerobic digestion. Appl Energy 128:93–102. https://doi.org/10.1016/j.apenergy.2014.04.062

Zheng W, Lü F, Phoungthong K, He P (2014) Relationship between anaerobic digestion of biodegradable solid waste and spectral characteristics of the derived liquid digestate. Bioresour Technol 161:69–77. https://doi.org/10.1016/j.biortech.2014.03.016

Zsolnay Á (2003) Dissolved organic matter: artefacts, definitions, and functions. Geoderma 113:187–209. https://doi.org/10.1016/S0016-7061(02)00361-0

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Prediction of organic matter accessibility and complexity in anaerobic digestates

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ABSTRACT

Further characterization to properly assess the fate of organic matter quality during anaerobic digestion and organic carbon mineralization in soils is required. Organic matter quality based on its accessibility and complexity was employed to successfully classify 28 substrate/digestate pairs through principal components and hierarchical clustering analysis. The two first components explained 58.02% of the variability and four main groups were separated according to the feedstock type. A decrease in the accessibility (16–66%) and an increase in the complexity (34–98%) of the most accessible fractions was noticed. Besides, an increase of non-biodegradable compounds (17–66%) was globally observed after anaerobic digestion. The observed trends in the conversion of organic matter during anaerobic digestion have allowed to fill the gap in the modeling of the anaerobic digestion process chain. Indeed, partial least squares regressions have accurately predicted the organic matter quality of digestates from their inputs ($R^2 = 0.831$, $Q^2 = 0.593$) although the digester operational conditions (temperature and hydraulic retention time) were non-explicative enough. As a novel approach, the predicted digestate quality was used to feed a partial least squares regression model previously developed to predict organic carbon mineralization in soil. The combined models have predicted experimental organic carbon mineralization in soil ($R^2 = 0.697$) with a model quality similar to the model for organic carbon mineralization in soil ($R^2 = 0.894$). This is the first study that has successfully conceived an additional step in the prediction of organic matter fate from raw substrate before anaerobic digestion to soil carbon mineralization.

1. Introduction

The current waste management model has started to evolve towards more sustainable and resource recovery strategies (Fonoll et al., 2016; Vidal-Antich et al., 2021). Anaerobic Digestion (AD) is a biological process widely used to convert the organic matter (OM) present in different wastes into methane (Fernández-Domínguez et al., 2020; Vinardell et al., 2021), along with the production of both OM and nutrient-rich by-product called digestate (Fernandez-Bayo et al., 2018; Guo et al., 2018). Nowadays, digestates represent alternative fertilizers used in agriculture either as organic amendment or fertilizer depending on the process, post-treatment and substrate type (Akhiar et al., 2017; Guilayn et al., 2020). However, digestate efficiency as organic amendment mainly depends on their OM stability (Kögel-Knabner, 2002), which remains a topic of ongoing research.

A need for accurate OM characterization added to the strict

Abbreviations: 3D, three dimension; AD, anaerobic digestion; BMP, biochemical methane potential; C, carbon; C_bio, biodegradable carbon; COD, chemical oxygen demand; DOM, Dissolved Organic Matter; EPS, extracellular polymeric substances; FCI, fluorescence complexity index; HRT, Hydraulic Retention Time; HCA, Hierarchical Clustering Analysis; NEOM, Non-Extractable Organic Matter; NMR, nuclear magnetic resonance; OM, organic matter; PCA, Principal Components Analysis; PEOM, Poorly Extractable Organic Matter; PLS, Partial least squares; Q^2, Percent of variation of Y predicted by model in cross-validation, R^2, Correlation coefficient from PLS; REOM, Readily Extractable Organic Matter; RMSE, Root Mean Square Error, RMSE_CV, Root Mean Square Error for Cross Validation; RMSEP, RMSE calculated on validation dataset; PC1, Principal Component 1; PC2, Principal Component 2; Pf(i), fluorescence proportion for a zone (i); SEOM, Slowly Extractable Organic Matter; SPOM, Extractable Soluble from Particulate Organic Matter; T, Temperature; TS, total solids; Vf (i), fluorescence volume for a zone (i); VS, volatile solids.

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limitations by legislation on contaminants, such as heavy metals for the agricultural reuse of specific feedstock, are points of main consideration to enhance digestate management (Khakbaz et al., 2020). Digestate stability has to be properly assessed before land application (Tambone et al., 2013; Maynaud et al., 2017). Digestates often acquire higher biological stability and nutrient availability (particularly nitrogen) than raw substrates, enhancing the interest for land use (Provenzano et al., 2011). During AD, recalcitrant compounds are concentrated since the labile organic structures are preferentially degraded (Insam et al., 2015). Nonetheless, this stabilization could occur due to the interaction of different factors, including (i) degradation and solubilization of simple compounds, (ii) molecular complexification, (iii) complex microbial-related products release from biomass growth and decay (Aemig et al., 2016).

For a suitable agronomic valorization of digestates, evaluating the OM fate during AD and soil carbon (C) mineralization is a meaningful aspect to reduce associated environmental impacts (e.g. C loss, greenhouse gases emission) or favor ecosystem service like soil C sequestration that contributes to climate change mitigation (Minasny et al., 2017). Thus, wider efforts are needed to better understand how OM from non-digested substrate is transformed into digestate OM (Shakeri Yekta et al., 2019; Tambone et al., 2015).

Standard methods to measure the biodegradability of organic wastes through biodegradability assays are laborious and time-consuming. At least, 30 and 90 days are required for anaerobic biodegradation potential tests and aerobic biodegradation during soil incubations, respectively (Jimenez et al., 2017). Hence, biochemical fractionation is a much more time-saving method to characterize the structural nature of organic wastes and assess waste biodegradability using successive chemical extractions (Teglia et al., 2011a). The Soest (1963) fractionation method was widely applied to associate OM composition with biodegradability (Fernandes et al., 2009; Gunaseelan, 2007; Triolo et al., 2012). Based on this fractionation method, the Iroc indicator (i.e. indicator of residual organic C in soils) has been developed as a proxy of the potentially remaining OM after application in soils for various organic amendments (Lashermes et al., 2009). Moreover, it was successfully used to predict the long term evolution of OM in soils after repeated applications of these organic amendments (Levavasseur et al., 2021). However, biodegradable substrates and digestates were not included in the panel of OM used for the development of the Iroc indicator. In addition, the Soest fractionation method presented limitations in predicting the biodegradability for a wide range of organic residues using biomethane potential tests (BMP) (Mottet et al., 2010). Therefore, less aggressive methods were requested as a more representative way for anaerobic biodegradability prediction (Bareha et al., 2018; Mottet et al., 2013).

With this purpose, Jimenez et al. (2015) suggested a less aggressive fractionation method coupled with 3D fluorescence on the extracted fractions. These authors successfully classified sixty organic residues based on their OM quality, which is defined by the OM accessibility (i.e. compounds availability to microorganisms for intra or extra-cellular degradation) and complexity (i.e. molecules structure) of organic waste. This methodology is a promising approach compared with single spectroscopic techniques. Indeed, it has allowed an accurate assessment of the OM quality (Muller et al., 2014; Zhang et al., 2019) and has defined new inputs for AD modeling approaches (Jimenez et al., 2020). Interestingly, in Jimenez et al. (2017), the OM quality of 82 samples, comprising organic waste of different origin, was used to accurately predict both BMP and potential C mineralization in soil using Partial Least Square (PLS) regression. Similarly, Bareha et al. (2018) have properly evaluated the correlation between substrates organic nitrogen accessibility indicators with C biodegradability using an extracellular polymeric substances (EPS) fractionation method modified from Jimenez et al. (2015) in PLS regressions. The accessibility and complexity of a treated substrate will probably vary during AD. However, no studies have been focused before on the prediction of digestate OM quality from their corresponding inputs. This approach would make it possible to further predict the potential C mineralization of digestates in soil as described in Jimenez et al. (2017). Therefore, models could be an interesting alternative to fill this gap, saving time and providing new tools to improve AD performance.

Feedstock typology stands as a key factor to understand the final OM quality of digestates (Rocamora et al., 2020). Indeed, Fourier-transform infrared spectroscopy (FTIR) analysis showed that the main spectroscopic features in digestates depend on the composition of the initial biomass (Provenzano et al., 2011). Nevertheless, structural changes of labile and recalcitrant fractions of OM were reported comparing the nuclear magnetic resonance (NMR) spectra of different substrates and their subsequent digestates (Laera et al., 2019; Shakeri Yekta et al., 2019). However, the OM quality evolution due to AD has not been evaluated for a broad range of feedstock. Nonetheless, it could provide relevant information for different sectors implementing AD (e.g. farms, wastewater treatment plants, solid waste treatment plants).

This study aims to assess the OM accessibility and complexity evolution during AD for a wide variety of feedstocks. To this end, 28 substrate/digestate pairs including different feedstock nature were classified using statistical analyses to evaluate how both feedstock type and AD parameters influenced OM quality in the final digestate. Finally, a model based on linear regression was proposed to predict digestate OM quality from their inputs. The predicted quality was used to feed the PLS model for C biodegradability in soil proposed by Jimenez et al. (2017) to determine the digestates organic C mineralization in soil after land spreading.

2. Material and methods

2.1. Substrate/digestate pairs

Twenty-eight substrate/digestate pairs (a total of 56 samples) were collected from different waste treatment plants in France. The selected organic substrates, including diverse types of feedstocks and origins, were anaerobically digested in laboratory or full-scale reactors under different digester operational conditions (Table 1). The studied samples comprised 6 digestate types from the digestate fertilizing-value typology reported in Guilayn et al. (2019). Each sample was characterized in terms of accessibility and complexity as assessed by the biochemical fractionation method coupled with 3D fluorescence developed in Jimenez et al. (2015) and described below. Biochemical fractionation was conducted on the freeze-dried particular matter and fluorescence on the aqueous phase.

2.2. Fractionation method

Following the reported methodology in Jimenez et al. (2017), all the fresh samples were centrifuged (ca. 18600*g* for 30 min) at 4 °C as a first step. The aqueous phase, namely dissolved organic matter (DOM), was separated. Total Solids (TS) and Volatile Solids (VS) on the raw sample as in the resulting particulate phase were determined (APHA, 2005). Then, the particulate phase was freeze-dried and ground (1 mm) and a quantity of 0.5 g of freeze-dried sample was subjected to the successive chemical extractions (30 mL of each extractant). After every extraction stage, the sample was centrifuged (18,600*g*, 20 min, 4 °C) and the liquid phase was filtered (0.45 μm) and kept for analyses. All tests were carried out in duplicate. The resulting fractions from the fractionation method, ordered from more to less accessible, were defined as: (1) Extractable Soluble from Particulate Organic Matter (SPOM), (2) Readily Extractable Organic Matter (REOM), (3) Slowly Extractable Organic Matter (SEOM), (4) Poorly Extractable Organic Matter (PEOM), (5) Non-Extractable Organic Matter (NEOM). Chemical oxygen demand (COD) and 3D fluorescence spectroscopy were performed on the four liquid extracted fractions (DOM, SPOM, REOM, PEOM). Regarding the fractionation method reproducibility, the measurement error is below 5%

Table 1
Summary of the raw substrate/digestate pairs type, origin and digester operational conditions.

Type of feedstock[a]	Digestate type[b]	Number of substrate/digestate pairs	Scale	Origin	T (°C)	HRT (d)
Cow manure	4	5	Industrial	Farms	35–41	56–75
Cow manure mixtures:		2				
CM + Straw		1	Industrial	Farms	35–37	56
50% CM/S + 23% HM/S + 27% Slu		1	Industrial	Farms	35–37	56
Straw	1	1	Lab scale	Farms	–	–
Biowaste	2	1	Industrial	Municipal solid waste plant	55	21
Biowaste mixtures:		1				
50% BW + 20% CS + 30% AIW	5	1	Industrial	Municipal solid waste plant	37	90
Municipal Waste	5	4	Industrial	Municipal solid waste plant	55	20–28
Pig Slurry	3	1	Industrial	Farms	38	60
Pig Slurry mixtures:		6				
45% PS + 40% PsluAI + 15% others	3	1	Lab scale	Farms	38	60
93% PS + 7% Cow Food	3	1	Lab scale	Farms	38	24
93% PS + 7% Horse Food	3	1	Lab scale	Farms	38	24
80% PS + 20% Maize Silage	1	1	Lab scale	Farms	38	25
62% PS + 38% CM	1	1	Lab scale	Farms	38	24
80% PS + 20% BW	3	1	Lab scale	Farms	38	21
Sludge	2	7	Industrial	Wastewater treatment plant	37	15–25

[a] Type of feedstock: AIW: agroindustrial waste; BW: biowaste; CM: cow manure; CS: cow slurry; HM: horse manure; PS: pig slurry; PSluAI: primary sludge from agroindustry; S: slurry; Slu: Sludge.

[b] Digestate type based on Guilayn et al. (2019): (1) Fibrous feedstock (2) Sewage sludge, Biowaste, food agroindustrial residues (FAI) mono/co-digestion; (3) Organic fraction of municipal solid waste (OFMSW), Food waste (FW), FAI, PS mono/co-digestion; (4) Manure/other co-digestion; (5) OFMSW and BW mono/co-digestion; (6) Fibrous feedstock: Cattle manure, green waste, silage.

(data not shown).

COD was determined on the extracts in duplicate using Aqualytic® Vario COD kits (0–1500 mg O_2/L) analyzed using an ultraviolet (UV) spectrophotometer MultiDirect from Aqualytic®. Samples were diluted with deionized water if required. Units were in mgO_2/L and mg O_2/g TS for the liquid and solid phases, respectively.

2.3. Fluorescence spectroscopy analysis

The 3D fluorescence spectroscopy analyses were conducted on the obtained liquid fractions using a spectrofluorimeter Perkin Elmer LS55. The excitation wavelengths varied from 200 to 600 nm and the values were recorded every 0.5 nm between 200 and 600 nm, with increments of 10 nm. The resulting spectra were divided into seven different zones (I-VII) corresponding to a biochemical family with common complexity level for spectra interpretation, as described by Jimenez et al. (2014) and He et al. (2011): I, protein-like (Tyrosine); II, protein-like (Tryptophane), III, protein-like (Tyrosine, Tryptophane and microbial products); IV, fulvic acid-like; V, inner filter, glycolated protein-like; VI, melanoidin-like and lignocellulose-like; VII, humic acid-like. Afterward, the obtained data were processed to calculate the fluorescence volume of each zone V_f (i) and then the fluorescence proportion of each zone P_f (i) as described in Eqs. (1) and (2), respectively.

$$V_f(i)\left(U.A./mgCOD\cdot\ L^{-1}\right) = V_{f\_raw}(i)\bigg/COD_{sample} \times 1\bigg/\frac{S(i)}{\sum_{i=1}^{7}S(i)} \tag{1}$$

$$P_f(i)(\%) = \frac{V_f(i)}{\sum_{i=1}^{7}V_f(i)} \times 100 \tag{2}$$

where:

V_f (i) is the normalized volume of a zone i (U.A./mg O_2 L^{-1}) V_{f_raw} is the raw fluorescence volume of a zone i (U.A./mg O_2 L^{-1}) COD_{sample} is the COD concentration of the sample (mg O_2 L^{-1}) S(i) is the area of a zone i (nm^2) P_f (i) is the fluorescence proportion of a zone i (%)

Moreover, a fluorescence complexity index (FCI) was calculated between the proportions of fluorescence volumes of the most complex molecules zones (IV-VII) and the less complex molecules zones (I-III), as stated in Eq. (3) (Aemig et al., 2016).

$$FCI = \frac{\sum_{i=4}^{7}V_f(i)}{\sum_{i=1}^{3}V_f(i)} \tag{3}$$

2.4. Statistical analysis

Principal component analysis (PCA), Hierarchical Clustering Analysis (HCA) and their corresponding plots were carried out in R (4.0.3) (R Development Core Team, 2021). The displayed groups for the PCA individuals were determined by HCA clustering. Both analyses were conducted on a total of 56 samples (28 substrate/digestate pairs) considered individually. A Euclidean distance matrix with center-scaled variables was calculated before HCA analysis and the number of groups was determined heuristically. PCA was performed on center-scaled variables. A total of 34 variables for each sample was evaluated. Six variables were related to the accessibility of the sample and corresponded to the biochemical fractions (DOM, SPOM, REOM, SEOM, PEOM, NEOM) and 28 variables were linked to the complexity of the sample (seven fluorescence variables for each extracted fraction). Tukey's methodology was followed for the displayed boxplots.

In order to determine a linear prediction model of digestate quality from substrates quality, PLS regressions were performed using SIMCA software from UMETRICS. In its simplest form, a linear model specifies the linear relationship between a dependent (response) variable Y, and a set of X predictor variables, the X's. Cross-validation was then performed to test the model's quality. K-fold cross-validation was used. The database was divided into 7 blocks and some samples were selected as validation samples. This step was repeated. The mean and standard deviation of the scores were calculated to estimate the bias and the variance of validation performance. The validation step was performed on 5 samples not used in the calibration step among the 28 samples, chosen as representative of each type of digestate: Sludge_2_D, PS + Cow Food_D, PS + Cow Manure_D, MW4_D and CM3_D.

The parameters from the PLS models used to assess model quality are the following:

Correlation coefficient: R^2.

Root Mean Square Error (RMSE): used as an accuracy measurement of differences between predicted values and measured model values.

RMSE_CV: which is the RMSE for the cross-validation and was applied as prediction model error.

RMSEP: RMSE calculated on the validation dataset.

Q^2: percentage of variation of Y predicted by the PLS model according to cross-validation. This parameter indicates how well the model predicts the data. A large Q^2 (>0.5) indicates good predictivity.

The PLS model was based on 33 variables: the fractionation percentage of COD (DOM + SPOM, REOM, SEOM, PEOM, NEOM) and the fluorescence percentage of the seven zones coming from each fraction. The PLS model coefficients are presented in Supplementary Material. The same X-Variables as Jimenez et al. (2017) model have been used. Thus, DOM and SPOM fractions have been pooled and only SPOM fluorescence percentage has been considered. To combine the PLS model for C biodegradability in soil from Jimenez et al. (2017) and the digestate quality PLS model, experimental data obtained from soil C mineralization tests as described in Jimenez et al. (2017) have been used. Fourteen digestates have been tested among the 28 samples previously defined. Table 2 presents the digestates considered and their respective biodegradable C percentage.

3. Results and discussion

3.1. Substrates and digestates classification

PCA and HCA analyses were conducted on 33 variables describing the accessibility and complexity of the OM for the 56 samples considered individually. Scores and loadings from PCA are presented in Fig. 1a and 1b, respectively. HCA clusters are illustrated in Fig. 1c. The PCA analysis has showed that the first two components explained 58.02% of the total variance, meaning that the samples were rather well described by the used characterization data. These results are in accord with previous studies focusing on organic waste classification (Jimenez et al., 2015) or the characterization of post-treated digestates (Maynaud et al., 2017). From the HCA, four main groups were identified by different colors (A, B, C, D) in PCA and HCA plots (Fig. 1a and 1c).

The Component 1 (PC1) was described by the complexity of the molecules, from the simplest samples on the right part of the loadings plot (mainly fluorescences zones II-III, related to simple sugars/proteins and microbial products with low complexity) to the most complex samples on the left part (mainly fluorescences zones VI-VII, linked to humic acids, lignocellulose and melanoidin). Hence, PC1 was significantly ($p\text{-value} < 0.05$) correlated to simple and intermediate fluorescence zones, such as REOM_I_II, SEOM_I_II and PEOM_I_IV variables ($R^2 = 0.77$, 0.67 and 0.66, respectively). Meanwhile, it was anti-correlated ($p\text{-value} < 0.05$) to complex fluorescence zones (VI-VII), particularly REOM_I_VII, REOM_I_VI and SEOM_I_VI variables ($R^2 = -0.94$, -0.90 and -0.87, respectively).

The second component (PC2) was explained by the complexity added to the accessibility of the sample. A significant ($p\text{-value} < 0.05$) and positive correlation was observed between REOM_C ($R^2 = 0.74$) and fluorescence zones from I to III (SPOM_I_III, REOM_I_I and SEOM_I_I, with $R^2 = 0.78$, 0.67 and 0.65, respectively). Contrarily, a significant ($p\text{-value} < 0.05$) and negative correlation was defined between PEOM_C ($R^2 = -0.79$) and fluorescence zones of intermediate complexity as REOM_I_IV, SEOM_I_IV, SEOM_I_V ($R^2 = -0.95$, -0.85, and -0.85, respectively). Biochemical fractionation variables were among the main variance contributors for PC2 which allowed to identify the most accessible samples on the top (essentially due to REOM) from the less accessible samples (mainly PEOM) on the bottom. DOM fraction was not correlated with SPOM/REOM/SEOM nor to PC1 but to a lesser extent ($R^2 = -0.59$ and $p\text{-value} < 0.05$). SEOM was positively correlated with the most accessible fractions (SPOM and REOM) while PEOM was negatively correlated with REOM and SEOM, as previously stated by Jimenez et al. (2015) and Aemig et al. (2016). Zhang et al. (2019) also showed that SEOM fraction shared protein-like compounds fluorescent peaks with SPOM and REOM fractions whereas PEOM presented a humic-like compound peak as the main peak. Indeed, NMR spectroscopy on the solid fraction showed that SEOM was mainly composed of proteins (Laera et al., 2019). NEOM fraction was non-explicative enough in this study.

Fig. 1c shows the four groups established from the HCA analysis based on the extracted fractions and their complexity of each sample: (A) pig slurry and slurry mixtures with primary sludge, agro-industrial waste or biowaste, (B) manure, fibers and municipal solid waste, (C) pig slurry mixtures with fiber or food wastes, and (D) sludge. All the groups were strictly related to the feedstock type, highlighting its influence on organic waste classification, which is supported by other authors (Akhiar et al., 2021; Bareha et al., 2018; Guilayn et al., 2019). Groups B, C, and D came from a different main cluster than Group A, probably due to a higher DOM content related to complex fluorescence zones (VI-VII) that could arise from the degradation of refractory compounds of other fractions (Zhang et al., 2019). The high reported complexity in animal slurries could be related to particular recalcitrant alkyl-C (e.g. sterols, lipids, cutin) (Tambone et al., 2019). Group B was defined by PEOM (poorly accessible C) and fluorescence zones IV and V. Indeed, these types of substrates are characterized to present high C/N and TS besides complex proteins and humic/fulvic acids (Akhiar et al., 2017). Finally, Groups C and D were related to low complexity zones (I-III) while sludge showed higher fluorescence proportions on zones III than pig slurry mixtures. In fact, zone III corresponds to protein content and microbial by-products, typically related to activated sludge metabolism and growth/decay (Fang et al., 2015). These results have been also reported by Zhang et al. (2019), who showed that the characteristic presence of protein-like organics (zones II and III) was in the SPOM and REOM fractions of sludge.

Interestingly, a broad variance on pig slurry mixtures clustering from fluorescence zones I-III to VI-VII was found depending on the co-substrate added (e.g. pairs of pig slurry + fiber co-substrate were clustered in Group C but spatially distributed close to Group B, which is mainly composed of fiber-rich samples). Notwithstanding that the percentage of co-substrate in raw mass was mainly below 20%, the addition of a co-substrate seems to influence their classification and should be considered to properly classify organic wastes (see Table 1).

Most of the digestates were clustered together with their substrates, confirming that the main OM complexity and accessibility prevailed after AD (Provenzano et al., 2014). Nevertheless, within the same cluster, variations in the classification between substrates and digestates have been observed. Since the OM accessibility and complexity conversion after AD remains unclear, it is discussed in the following section.

Table 2
Biodegradable carbon percentage after soil incubation of several studied digestates coming from Jimenez et al. (2017) data.

Digestate name	C_bio (%C)
Sludge2_D	39%
Sludge7_D	31%
CM3_D	15%
MW1_D	25%
MW3_D	32%
MW4_D	19%
MW5_D	27%
PS + BW_D	46%
PS + Cow Food_D	43%
PS + Cow Manure_D	24%
PS + Horse Food_D	35%
PS + Maize Silage_D	44%
BW_D	24%
PS + PrimSluAI_D	31%

3.2. OM accessibility and complexity: Revealed groups characterization

3.2.1. OM accessibility and fluorescence complexity index of substrates

The proportion of total COD in the biochemical fractions and the FCI (defined in Section 2.3) of substrates profiles have been evaluated to define each group and are summarised in Fig. 2.

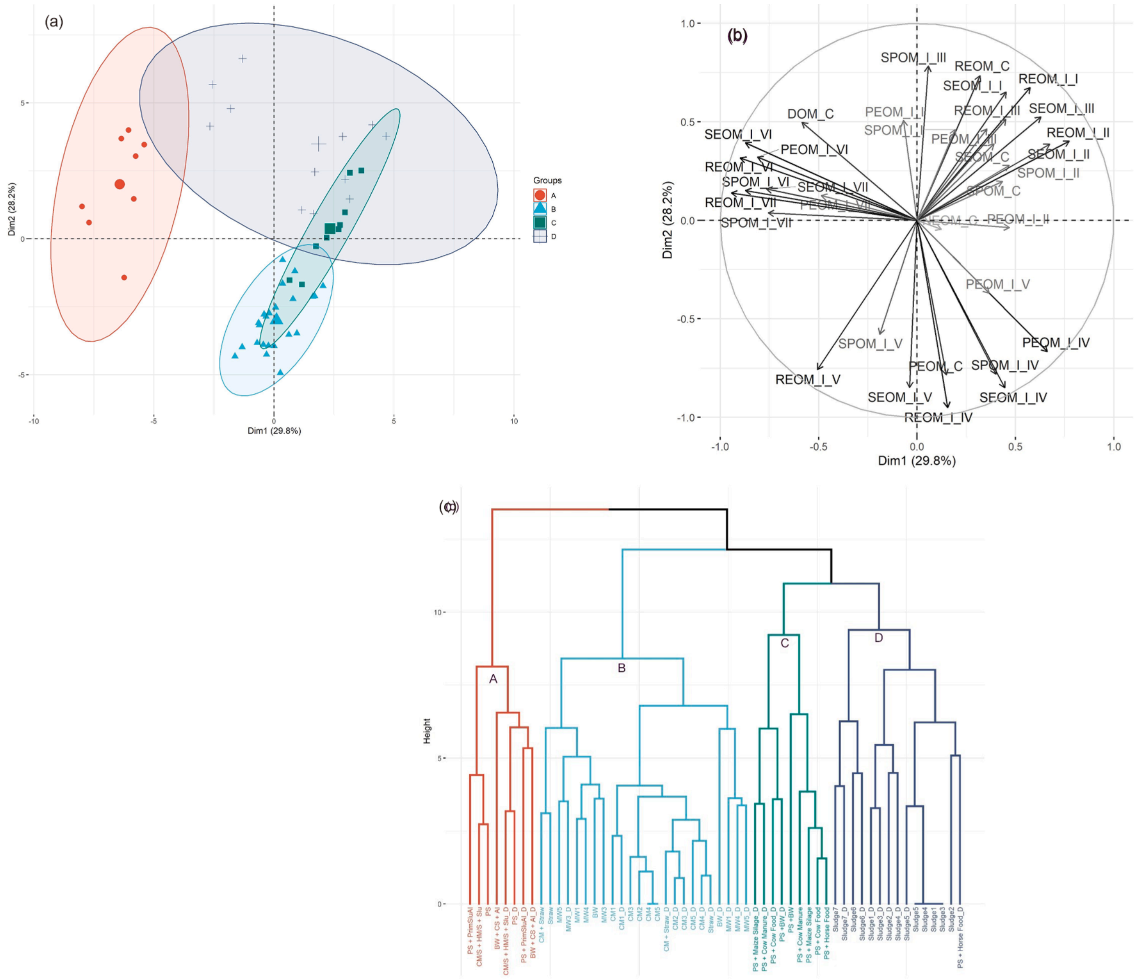

Fig. 1. Scores plot (a) and loadings (b) obtained from the PCA analysis, and (c) HCA analysis for the 56 samples studied. PCA individuals are distinguished by shape and color according to the HCA revealed groups (A-D). Ellipses show 95% confidence intervals. Loadings intensity color (plot b) is related to the variables contribution, from low (1) to high (4). Groups: (A) pig slurry and slurry mixtures with primary sludge, agro-industrial waste or biowaste, (B) manure, fibers and municipal solid waste, (C) pig slurry mixtures with fiber or food wastes, and (D) sludge.

The proportions of COD for each extracted fraction varied between groups according to the different origins of the sample (Fig. 2). The largest proportion of COD for Group A was in the DOM fraction (30–55%), indicating a notable content of water-soluble organic substances in slurries, such as simple sugars (e.g. sucrose, glucose and fructose), proteins (mainly globular protein), volatile fatty acids or soluble recalcitrant compounds. High DOM fraction content was also reported by Laera et al. (2019) (76% of total COD) for a substrate from a household, slaughterhouse and industrial waste co-digestion plant. For Groups B and C, PEOM fraction was the main COD fraction extracted and ranged between 22 and 58% of total COD. Indeed, these results are consistent since the PEOM fraction is rich in hemicellulose, cellulose-like, starch and certain proteins (Laera et al., 2019), which characterizes the fibrous feedstocks of Groups B and C. Finally, Group D extracted COD was dominated by the NEOM fraction (23–33%). Similar values of NEOM (30–40%) have been reported by different authors for sludge samples (Jimenez et al., 2015; Maynaud et al., 2017).

Comparing the accessibility between groups, Group D had the highest SEOM (27%) and SPOM (22%) fractions probably due to high complex protein content (mainly fibrous proteins) and simple sugars/proteins in sludge samples. Group C presented the highest REOM (25%) and NEOM (39%) fractions probably because of the protein and lipid content provided by food wastes. Group B presented the highest PEOM fraction (58%), while the NEOM fraction was the second extracted fraction for all the groups (except for Group D), indicating that all wastes had a considerable amount of non-extractable OM. Whilst, SPOM and REOM fractions showed low percentages of the total extracted COD for all the groups.

Regarding the FCI of the substrates, Groups A and B had higher FCI in all fractions compared with Groups C and D (Fig. 2). Indeed, Group C and D were mainly characterized by fluorescence regions I to III likely they have less complex proteins/lignified compounds than Groups A and B. Only the FCI of the SPOM fraction of Group C (1.03–1.50) was higher than the one of the other Groups. The highest FCI for all groups was for Groups A and B and corresponded to the less accessible fractions (SEOM and PEOM), as reported by Muller et al. (2014). Concerning the FCI of each group, the FCI of the SEOM fraction was the highest for the Group A (3.60). In fact, this fraction targets recalcitrant compounds such as humic-like acids, fulvic-like acids and complex proteins, which could explain that Group A was previously defined by complex fluorescence zones (VI-VII) (see Fig. 1). The FCI of the PEOM fraction showed the highest values for Group B and D (2.94 and 1.97, respectively) whereas Group C displayed slight differences in the FCI for all the extracted fractions.

Fluorescence spectroscopy was also performed on some DOM fractions samples (PS, CM/S and HM/S + Slu, BW + CS + AI, Sludge_6 and Sludge_7). However, this data was not considered in this study because (i) all the DOM fractions in the dataset were not analyzed and (ii) Jimenez et al. (2017) did not consider it in their model. Nonetheless, the fluorescence percentage for zone I to VII of SPOM and DOM of these 6

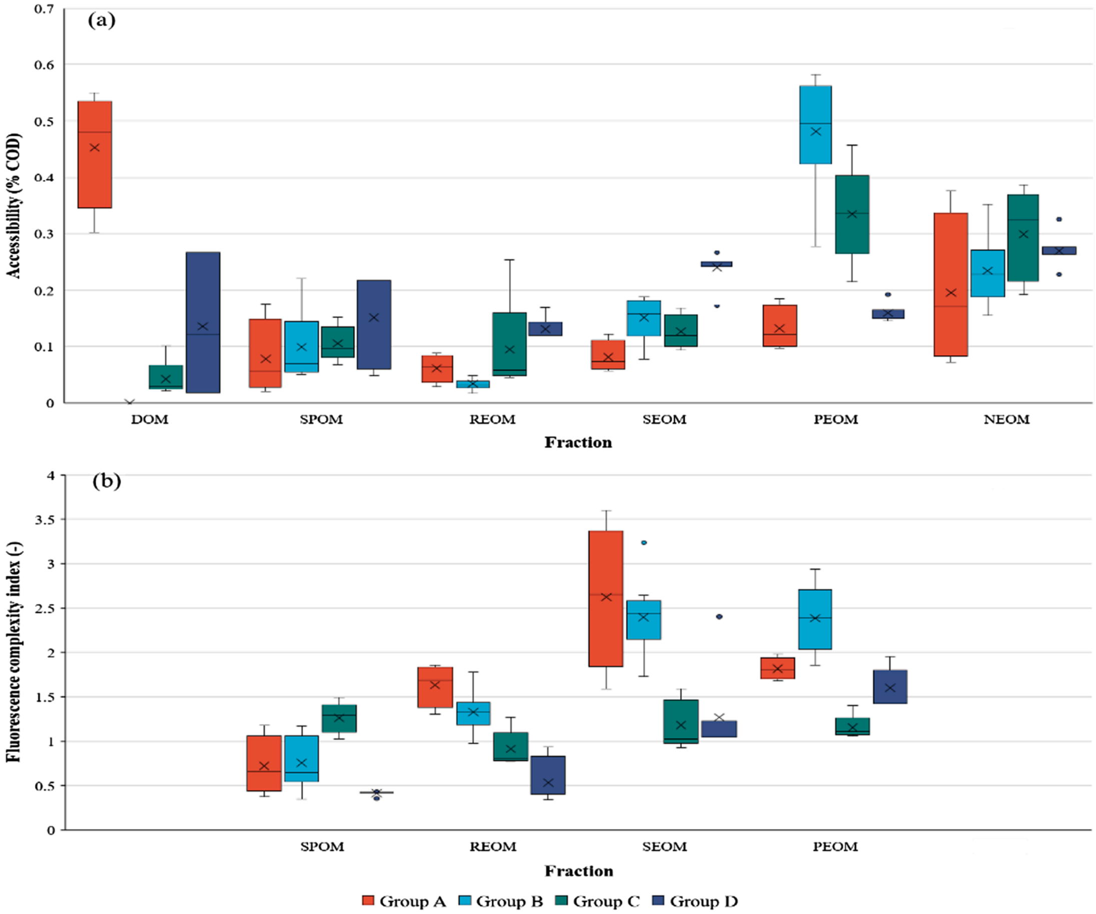

Fig. 2. Accessibility (a) and fluorescence complexity index (b) boxplots of substrates for the HCA revealed groups. Groups: (A) pig slurry and slurry mixtures with primary sludge, agro-industrial waste or biowaste, (B) manure, fibers and municipal solid waste, (C) pig slurry mixtures with fiber or food wastes, and (D) sludge.

samples were similar for the evaluated substrates. Zhang et al. (2019) reported similar results for sewage sludge. Therefore, DOM fluorescence was not relevant for an accurate prediction of the final C mineralization in soil concerning the present study (see Supplementary Material).

3.2.2. OM accessibility and fluorescence complexity index conversion after AD

The anaerobic biodegradability of OM also depends on the chemical nature of the compounds, therefore, the more accessible fractions will not strictly be the more biodegradable fractions (Bareha et al., 2019; Mottet et al., 2010). However, simpler compounds (e.g. soluble sugars or proteins) will be extracted in the most easily extractable fractions, while lignocellulose-like compounds are normally present in the last extracted fractions. To evaluate these considerations, Table 3 shows the proportions of COD for each extracted fraction and the FCI evolution between the substrates and their corresponding digestates after AD. Meanwhile, Fig. 3 displays the proportions of COD for each extracted fraction (described in Section 2.2) and FCI profiles for the digestates samples of each group.

From the four groups evaluated, general assumptions can be drawn regarding the influence of AD in the OM quality of digestates. The SPOM and REOM fractions have decreased after AD whereas the FCI increased for all feedstock groups (except SPOM and REOM complexity of Group C which decreased). Aemig et al. (2019) have also reported the highest biodegradation yields for the most accessible fractions (74% and 69% for SPOM and REOM, respectively) after sewage sludge AD. Thus, SPOM and REOM have shown the lowest percentage of extracted COD (<10% of total COD) for all digestates. Indeed, the sum of SPOM + REOM in digestates ranged between 3 and 27% of the total COD samples (data not shown). Low extracted COD proportions for the most accessible fractions were also found in Bareha et al. (2018) and Laera et al. (2019), where the sum of SPOM, REOM and SEOM was below the 10% of total COD.

Table 3
Proportions of COD for each extracted fraction and fluorescence complexity index evolution after AD in the different groups. The results display the relative percentage of the increase or decrease as expressed in Tambone et al. (2013).

	Accessibility (% in COD)						Fluorescence complexity index (-)			
Group	DOM	SPOM	REOM	SEOM	PEOM	NEOM	SPOM	REOM	SEOM	PEOM
A	–	**–**	**–**	+	++	++	++++	++	+	++
B	N.A.	**–**	**–**	–	–	++	++	++	0	+
C	++++	—	**–**	++	–	+	–	–	+++	–
D	–	**–**	**–**	–	+	++	++	++	0	+++

Relative conversion ranges*:* +: 0 to 25%; ++: 25 to 50%; +++: 50 to 75%; ++++: 75 to 100%; -: 0 to -25%; –: -25 to -50%; —: -50 to -75%; ——: -75 to -100%. Relative percentage = (final value in the digestates - initial value in the substrates)/initial value in the substrates) × 100. N.A. = no presence of DOM for Group B (solid digestates).

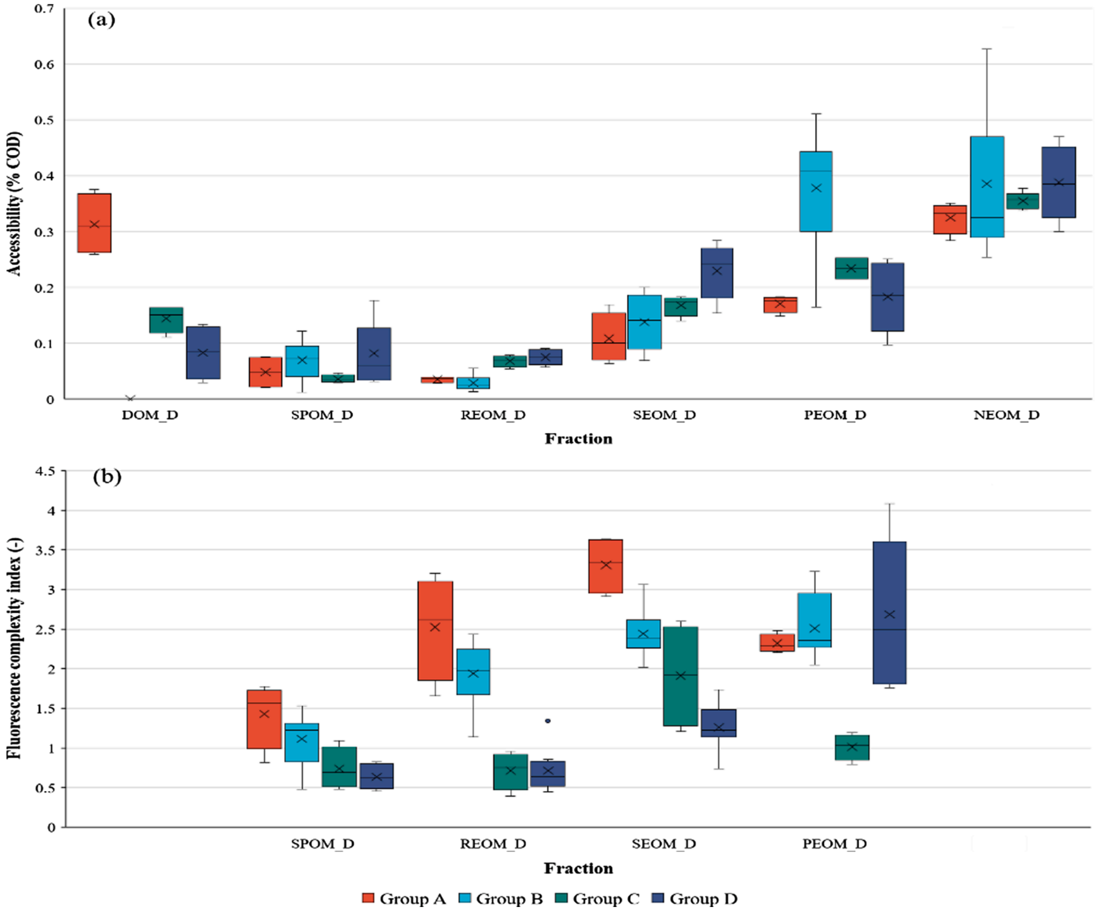

Fig. 3. Accessibility (a) and fluorescence complexity index (b) boxplots of digestates for the HCA revealed groups. Groups: (A) pig slurry and slurry mixtures with primary sludge, agro-industrial waste or biowaste, (B) manure, fibers and municipal solid waste, (C) pig slurry mixtures with fiber or food wastes, and (D) sludge.

Similarly, the sum of SPOM + REOM only accounted for the 2.7–10.6% of the total COD of digestates OM by Maynaud et al. (2017). In accordance with the present study, Aemig et al. (2019) and Zhang et al. (2019) have also reported an increase of the FCI for SPOM and REOM fractions since simple fluorescence peaks remarkably decreased after anaerobic digestion.

Regarding the DOM fraction, Group A kept the highest COD content in DOM (26–38%) after AD. This could suggest that, DOM fraction could contain non-biodegradable compounds or that AD could produce accessible but complex molecules (Jimenez et al., 2015; Lashermes et al., 2009), which has also been shown during composting (Peltre et al., 2011). Indeed, Zhang et al. (2019) showed that refractory organic compounds (i.e. complex compounds) could be accumulated in DOM due to solubilization. In the studied dataset, the DOM fraction has been displayed to be variable among the groups. Actually, SPOM and DOM digestates spectra have been observed to present differences, although without a significative general trend (see Supplementary Material).

The SEOM fraction was kept similar or increased depending on the group. Laera et al. (2019) have also noticed a SEOM fraction increase from 7 to 15% after AD. These authors have associated it with the concentration of protein from the growth/decay of microorganisms and to a preferable consumption of simple soluble compounds. Similarly, the SEOM reduction was smaller compared with SPOM and REOM fractions for sewage sludge and cow manure digestion, as they are expected to have more complex and therefore less degradable compounds (Somers et al., 2021; Zhang et al., 2019). The FCI of SEOM has tended to increase in the digestate of Groups A and C. Meanwhile, the FCI of SEOM of Group B and D remained constant, whilst the SEOM accessibility decreased, probably due to the biodegradation of non-fluorescent compounds, as previously reported by Aemig et al. (2019).

PEOM fraction has also varied among feedstock types. The Groups with high initial PEOM fraction (B and C) has shown a decrease while an increase was observed for the Groups with low initial PEOM fraction (A and D). Similar findings were previously reported in Bareha et al. (2018) and Aemig et al. (2016). These authors have reported a PEOM fraction decrease for cow manure (i.e. rich in PEOM) and an increase for sewage sludge (i.e. poor in PEOM), respectively. Therefore, higher degradation rates of PEOM fraction (cellulose and hemicellulose) during AD could be expected when other simpler molecules are missing (Tambone et al., 2013). Biodegradable cellulose/hemicellulose in the PEOM fraction of Groups B and C have probably contributed to higher biodegradability of the PEOM fraction. However, the PEOM fraction of Groups A and D could contain non-identified complex compounds (e.g. complex proteins) that increase the recalcitrance to AD. The FCI of PEOM has increased for all groups (except for Group C). In contrast, Aemig et al. (2016) have reported no evolution on the FCI for the less accessible fractions during sewage sludge digestion. Nevertheless, a slight increase of the fluorescence intensity of humic-like compounds peaks in PEOM fraction after AD (suggesting poor biodegradability and high complexity of this fraction), was reported as a possible re-polymerization of humic acid (Tang et al., 2018; Zhang et al., 2019).

The NEOM fraction has increased for all groups after AD and represented between 25 and 63% of the total COD in digestates. This lignocellulose-type fraction is concentrated after AD due to its recalcitrance and poor biodegradability in anaerobic conditions (Usman Khan and Kiaer Ahring, 2021). Thus, digestates are expected to be enriched in recalcitrant compounds, enhancing their suitability as soil amendment compared to the raw substrates (Jimenez et al., 2017; Pognani et al., 2010; Shakeri Yekta et al., 2019; Teglia et al., 2011b).

The obtained results have shown that the OM conversions shared a similar pattern regardless of the feedstock type: (i) an increase of complexity for the majority of the fractions, (ii) a global decrease of accessibility of the most accessible fractions (SPOM and REOM), and (iii) an increase of the non-extractable organic matter (NEOM) fraction. Nonetheless, it should be noted that the discussed evolution of the OM quality does not distinguish between the contribution due to the exogenous OM (i.e. substrate) and the endogenous OM (i.e. microbial-related products). The contribution of each compartment will be prioritized in forthcoming studies.

The accessibility conversion pattern is in agreement with Aemig et al. (2016), who have also shown a decrease for the most accessible fractions (named DOM, S-EPS and RE-EPS) during AD (22–65%, 49–50%, 23–35%, respectively), whereas NEOM relatively increased. Similarly, cow manure AD was evaluated by Somers et al. (2021) and a significant decrease in DOM, SPOM and REOM fractions of 24%, 62%, and 61%, was associated with methane production and hydrolysis of organic matter. Laera et al. (2019) have also noted a remarkable decrease from 76 to 28% for the DOM fraction between raw and digested mixtures of household, slaughterhouse and industrial wastes. Besides, an increase from 9% to 47% for PEOM + NEOM was also reported by these authors. Moreover, Zhang et al. (2019) have also showed a decrease after AD from 46.74% to 39.42% for DOM, SPOM and REOM fractions and the FCI increased for all the extracted fractions. Furthermore, the FCI of the REOM fraction was increased by 34% during cow manure AD (Somers et al., 2021) whereas a non-significant increase in the FCI of SEOM and PEOM during sludge and cow manure AD was stated by Aemig et al. (2019; Aemig et al. (2016)a2016) and Somers et al. (2021), respectively. Therefore, general trends on OM accessibility and complexity evolution during AD have been observed. Additionally, the prediction of OM accessibility and complexity of digestates from their inputs have been further assessed.

3.3. Prediction of digestate OM quality from substrate OM quality

3.3.1. PLS model for the prediction of digestate OM quality

PLS regression was applied on 28 observations (substrate/digestate pair) split in two datasets: a calibration dataset (23 samples) and a validation dataset (5 samples). Seven models were tested (Table 4). Model n°1 used the substrate fractions DOM + SPOM, REOM, SEOM and PEOM as X-variables to predict the digestate fractions (i.e. DOM + SPOM, REOM, SEOM and PEOM). NEOM fraction was not included as this fraction is calculated by difference. Model n°2 was similar to Model n°1 with the addition of the reactor temperature (T) as X-Variable. Model n°3 was the Model n°1 with the addition of Hydraulic Retention Time (HRT) as X-Variables and Model n°4 merged Models n°2 and 3.

Model n°5 used both fractions and fluorescence percentage in each fraction measured in the substrates (32 variables) as X-variables to predict the same 32 Y-variables in the digestates. Models n°6, 7 and 8 were based on Model n°5 with the addition of the T, HRT and both variables as X-variables, respectively. Table 4 presents the quality parameters of each model. The errors of calibration, cross-validation and prediction using cross-validation methods (RMSE, RMSE_CV, and RSMEP) of each Y-variable in each model are specified in the Supplementary Material.

According to Table 4, all the models have shown good quality performances with $Q^2 > 0.5$ and correlation coefficients of prediction were between 0.691 and 0.832. Models n°5 to 8 had better correlation coefficients than Models n°1 to 4 and smaller calibration errors (cf. RMSE in Supplementary Material). Indeed, the addition of fluorescence variables from Model n°1 to n°5 improved the prediction of the 32 variables with 7 components. Overall, for Model n°5, R^2 was 0.831 and specific R^2 for each fraction prediction was 0.869, 0.910, 0.736 and 0.882 for respectively DOM + SPOM, REOM, SEOM and PEOM. Furthermore, the prediction error RMSEP was lower in Model n°5 for the fractions prediction than Model n°1 (see Supplementary Material).

Table 4
Quality parameters of the PLS models.

Model	Variables		Model quality			
	X	Y	Components number	R^2X	R^2Y	Q^2
Model n°1	DOM + SPOM; REOM; SEOM; PEOM	DOM + SPOM; REOM; SEOM; PEOM	2	0.892	0.691	0.621
Model n°2	DOM + SPOM; REOM; SEOM; PEOM;T	DOM + SPOM; REOM; SEOM; PEOM	3	0.919	0.763	0.681
Model n°3	DOM + SPOM; REOM; SEOM; PEOM; HRT	DOM + SPOM; REOM; SEOM; PEOM	3	0.922	0.757	0.639
Model n°4	DOM + SPOM; REOM; SEOM; PEOM; HRT;T	DOM + SPOM; REOM; SEOM; PEOM	4	0.938	0.775	0.587
Model n°5	DOM + SPOM; REOM; SEOM; PEOM;Pf_i	DOM + SPOM; REOM; SEOM; PEOM;Pf_i	7	0.953	0.831	0.593
Model n°6	DOM + SPOM; REOM; SEOM; PEOM;Pf_i; T	DOM + SPOM; REOM; SEOM; PEOM;Pf_i	7	0.950	0.832	0.588
Model n°7	DOM + SPOM; REOM; SEOM; PEOM;Pf_i; HRT	DOM + SPOM; REOM; SEOM; PEOM;Pf_i	7	0.951	0.829	0.580
Model n°8	DOM + SPOM; REOM; SEOM; PEOM;Pf_i; T;HRT	DOM + SPOM; REOM; SEOM; PEOM;Pf_i	7	0.949	0.830	0.576

The Model n°2 and 4 had the best performances among the models using only fractions and operational parameters as X-Variables. Indeed, the addition of T (Model n°2) and the addition of HRT (Model n°3) as X-variables increased R^2Y but needed a component addition. However, HRT addition did not improve the quality parameters R^2Y and Q^2 (Model n°4 in Table 4). This trend was not the same in the case of Models n°6 to 8 in comparison with Model n°5 without operational parameters. Indeed, despite a little increase of R^2Y when T was added as X-variables (Model N°6), the prediction accuracy (Q^2) was decreased when HRT and T were added. The results obtained in Model n°2 have shown that T was anti-correlated (p-value < 0.05) with SEOM ($R^2 = -0.593$) and, less significantly with DOM + SPOM ($R^2 = -0.313$). However, reactors T were mainly mesophilic except for MW digestates (55 °C). These digestates were associated with lower DOM + SPOM and SEOM fractions than the others. Consequently, relying on our dataset, the impact of T was related to the substrate type. To test the impact of T alone, it would have been interesting to get substrate/digestate pairs from various feedstocks at both mesophilic and thermophilic temperature.

Similarly, the obtained results in Model n°3 have shown that HRT was negatively correlated with REOM ($R^2 = -0.628$) and, positively correlated with PEOM ($R^2 = 0.397$). However, low HRT values were mainly found for sludge digestion whereas high HRT values were associated with cow manure digestates. Sludge digestate groups were related to a high value of REOM and SEOM fractions whereas cow manure digestates were characterized by high values of PEOM and low values of DOM + SPOM. Again, it was not possible to distinguish the effect of HRT from substrate impact on digestate quality prediction. Moreover, in the Model n°4, weight coefficients showed that HRT and T were considered as the least important variables for quality prediction (Supplementary Material).

As the operational conditions have been determined as non-explicative enough in this study, and considering the high quality of the prediction, Model n°5 was selected. Besides, the predicted variables of digestates quality are necessary for the PLS model for C biodegradability in soil. To go further, the impact of the most significant X-variables affecting the prediction of Y-variables derived from the calculation of the weight of each variable for Model n°5 (without T and HRT) was analyzed (data not shown).

The characteristics of input OM remarkably influenced the prediction of the digestate OM characteristics, meaning that the main pattern of accessibility present in feedstock input remained in digestate OM after AD. The variables of the most complex fluorescence zones from substrate impacted significantly the digestate quality. The recalcitrant compounds contained in the substrate were preserved in their subsequent digestate, as possible humus precursors (Guilayn et al., 2020; Tambone et al., 2010). Nonetheless, for the prediction of the simpler fluorescence zones, other factors such as: (i) solubilisation/complexification of biodegradable/non-biodegradable compounds, (ii) preferential compounds degradation, (iii) hydrolysis, (iv) prevalence of recalcitrant compounds, (iv) compounds contribution from other fractions are possible hypothesis that contribute to the explanation of the resulting prediction. Therefore, this is the first approach to understand how OM quality varies with AD based on accessibility and complexity for a wide range of feedstock. The addition of the T and the HRT as X-variables to predict OM quality and accessibility confirmed that the operational conditions were not informative enough for the studied dataset. To properly evaluate the impact of the operational conditions on the prediction of digestate quality, the digestion of the same substrate subjected to different T or HRT should be conceived in future investigations.

3.3.2. *Coupling digestate quality prediction with PLS model for carbon biodegradability in soil prediction*

To validate the digestate quality PLS model found, the PLS model for C biodegradability in soil was applied on the digestate quality predicted by Model n°5. Among all the samples that were used for the digestate quality model, 14 samples were incubated in soil and biodegradable carbon (C_bio) was obtained after 91 days (Table 2).

First, the PLS model for C biodegradability in soil was tested. A comparison between the 14 predicted values of proportion of biodegradable C in the digestates and experimental data obtained through soil incubation was plotted in Fig. 4a. Results have shown that the PLS model for C biodegradability in soil was successfully able to predict the biodegradable organic C of the 14 digestates ($R^2 = 0.739$) with low bias. Then, Model n°5 was combined with the PLS model for C

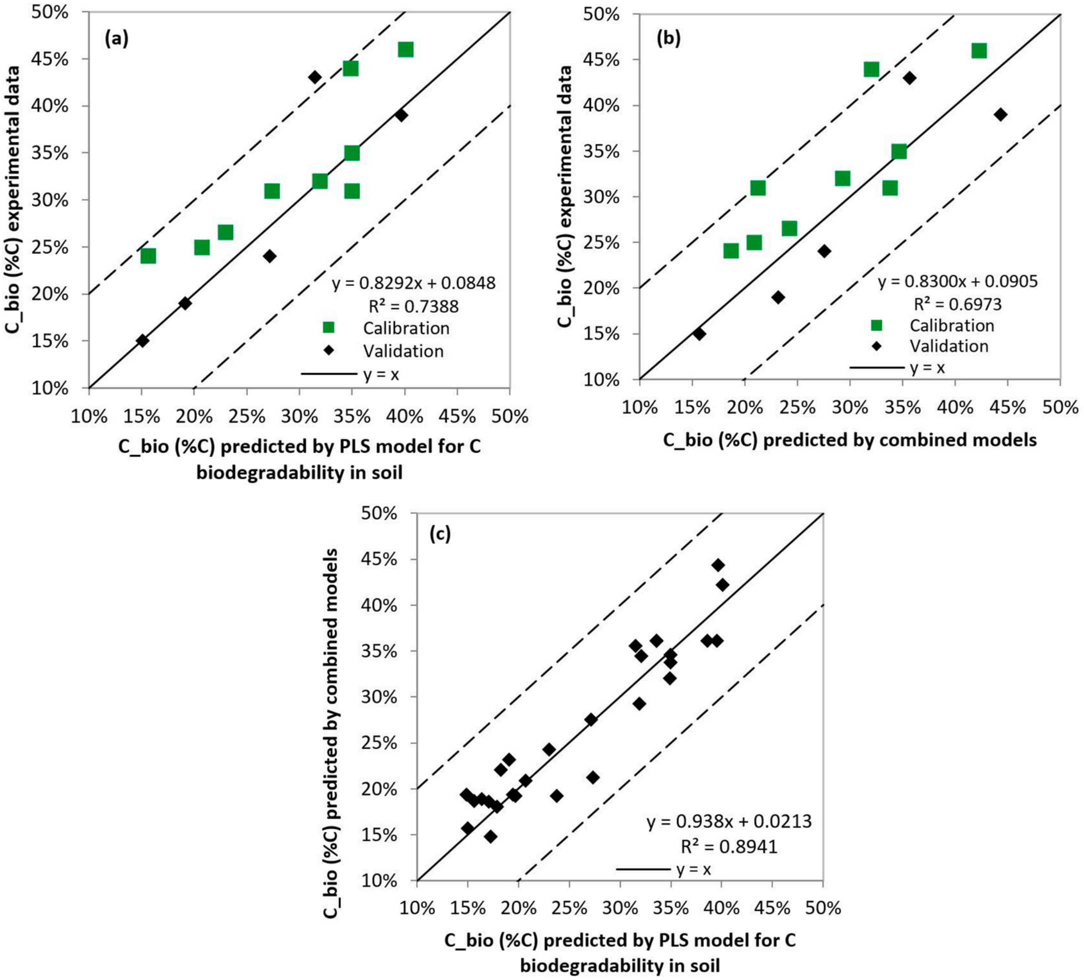

Fig. 4. Validation of model PLS: comparison between experimental data from biodegradable organic carbon tests on soil with PLS model for C biodegradability in soil (a) and combined models (b); and comparison between combined models and PLS model for C biodegradability in soil (c).

biodegradability in soil to predict C_bio. Fig. 4b shows that the C_bio prediction was not altered by the models' combination. Indeed, the combined models were able to predict the experimental data (R^2 = 0.697) with a similar bias as in Jimenez et al. (2017). The 5 validation samples that were used for Model n°5 were plotted in black in Fig. 4a and b. Prediction error of C_bio obtained by the combined models ranged between 1% and 7%, with R^2 = 0.828, which represents a high quality of prediction. Therefore, the reproducibility of the model was confirmed. Moreover, the model validation was performed with external data not included in the dataset used for model calibration. Finally, the 28 pair samples were used to compare both models' predictions as presented in Fig. 4c. Results showed that C_bio prediction by the combined models is quite similar to C_bio prediction by Jimenez et al. (2017) (R^2 = 0.894).

Thus, Model n°5 provided an extra step on OM fate prediction from raw substrate to soil C mineralization to better understand AD influence on OM accessibility and complexity before land disposal. Additionally, the present study related multiple aspects of scientific interest such as waste characterization, anaerobic transformation processes of OM and soil C mineralization and supposes an innovative approach to enhance the modeling of the AD process chain. Nonetheless, future actions could be addressed to improve the models' application when specific substrates are subjected to different AD operational conditions and soil typologies.

4. Conclusions

The prediction of digestate OM quality from their input was evaluated. PCA and HCA analysis have allowed to classify 28 substrate/digestate pairs covering a wide diversity of OM. This classification was based on the extracted fractions from the OM (accessibility) and their complexity assessed by 3D fluorescence. Substrates and their respective digestates were clustered together according to the feedstock type. Nonetheless, common trends on the conversions of OM quality were observed, indicating potential for the prediction of digestate quality of the entire dataset regardless of the feedstock type. Thus, this study proposed a digestate quality PLS model that accurately predicted (Q^2 = 0.593) the digestates OM quality from the substrate OM characteristics. However, future investigations should be focused on subjecting the same substrate to different T or HRT to properly evaluate the impact of operational conditions on the prediction of digestate quality. The predicted digestate OM characteristics validated the prediction of their biodegradability in soils using the PLS model for C biodegradability in soil previously developed. This work performed the combination of both models. OM conversion during AD and soil C mineralization was precisely predicted using a rapid analysis indicator (biochemical fractionation and 3D fluorescence). Such combined models brought a major contribution in the modeling of the AD process chain favoring the development of decision-making tools to properly manage the digestates.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2021.10.004.

References

Aemig, Q., Chéron, C., Delgenès, N., Jimenez, J., Houot, S., Steyer, J.-P., Patureau, D., 2016. Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in sludge organic matter pools as a driving force of their fate during anaerobic digestion. Waste Manage. 48, 389–396. https://doi.org/10.1016/j.wasman.2015.11.045.

Aemig, Q., Doussiet, N., Danel, A., Delgenès, N., Jimenez, J., Houot, S., Patureau, D., 2019. Organic micropollutants' distribution within sludge organic matter fractions explains their dynamic during sewage sludge anaerobic digestion followed by composting. Environ. Sci. Pollut. Res. 26 (6), 5820–5830. https://doi.org/10.1007/s11356-018-4014-7.

Akhiar, A., Battimelli, A., Torrijos, M., Carrere, H., 2017. Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. Waste Manage. 59, 118–128. https://doi.org/10.1016/j.wasman.2016.11.005.

Akhiar, A., Guilayn, F., Torrijos, M., Battimelli, A., Shamsuddin, A.H., Carrère, H., 2021. Correlations between the composition of liquid fraction of full-scale digestates and process conditions. Energies 14, 971. https://doi.org/10.3390/en14040971.

APHA, 2005. Standard Methods for the Examination of Water and Waste-Water, 22nd ed. Washington, DC.

Bareha, Y., Girault, R., Guezel, S., Chaker, J., Trémier, A., 2019. Modeling the fate of organic nitrogen during anaerobic digestion: development of a bioaccessibility based ADM1. Water Res. 154, 298–315. https://doi.org/10.1016/j.watres.2019.02.011.

Bareha, Y., Girault, R., Jimenez, J., Trémier, A., 2018. Characterization and prediction of organic nitrogen biodegradability during anaerobic digestion: a bioaccessibility approach. Bioresour. Technol. 263, 425–436. https://doi.org/10.1016/j.biortech.2018.04.085.

Fang, F., Hu, H.-L., Qin, M.-M., Xue, Z.-X., Cao, J.-S., Hu, Z.-R., 2015. Effects of metabolic uncouplers on excess sludge reduction and microbial products of activated sludge. Bioresour. Technol. 185, 1–6. https://doi.org/10.1016/j.biortech.2015.02.054.

Fernandes, T.V., Klaasse Bos, G.J., Zeeman, G., Sanders, J.P.M., van Lier, J.B., 2009. Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass. Bioresour. Technol. 100 (9), 2575–2579. https://doi.org/10.1016/j.biortech.2008.12.012.

Fernandez-Bayo, J.D., Yazdani, R., Simmons, C.W., VanderGheynst, J.S., 2018. Comparison of thermophilic anaerobic and aerobic treatment processes for stabilization of green and food wastes and production of soil amendments. Waste Manage. 77, 555–564. https://doi.org/10.1016/j.wasman.2018.05.006.

Fernández-Domínguez, D., Astals, S., Peces, M., Frison, N., Bolzonella, D., Mata-Alvarez, J., Dosta, J., 2020. Volatile fatty acids production from biowaste at mechanical-biological treatment plants: Focusing on fermentation temperature. Bioresour. Technol. 314, 123729. https://doi.org/10.1016/j.biortech.2020.123729.

Fonoll, X., Astals, S., Dosta, J., Mata-Alvarez, J., 2016. Impact of paper and cardboard suppression on OFMSW anaerobic digestion. Waste Manage. 56, 100–105. https://doi.org/10.1016/j.wasman.2016.05.023.

Guilayn, F., Jimenez, J., Martel, J.-L., Rouez, M., Crest, M., Patureau, D., 2019. First fertilizing-value typology of digestates: a decision-making tool for regulation. Waste Manage. 86, 67–79. https://doi.org/10.1016/j.wasman.2019.01.032.

Guilayn, F., Rouez, M., Crest, M., Patureau, D., Jimenez, J., 2020. Valorization of digestates from urban or centralized biogas plants: a critical review. Rev. Environ. Sci. Biotechnol. 19 (2), 419–462. https://doi.org/10.1007/s11157-020-09531-3.

Gunaseelan, V.N., 2007. Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition. Bioresour. Technol. 98, 1270–1277. https://doi.org/10.1016/j.biortech.2006.05.014.

Guo, X., Li, C., Zhu, Q., Huang, T., Cai, Y., Li, N., Liu, J., Tan, X., 2018. Characterization of dissolved organic matter from biogas residue composting using spectroscopic techniques. Waste Manage. 78, 301–309. https://doi.org/10.1016/j.wasman.2018.06.001.

He, X.-S., Xi, B.-D., Wei, Z.-M., Jiang, Y.-H., Yang, Y.u., An, D.a., Cao, J.-L., Liu, H.-L., 2011. Fluorescence excitation–emission matrix spectroscopy with regional integration analysis for characterizing composition and transformation of dissolved organic matter in landfill leachates. J. Hazard. Mater. 190 (1-3), 293–299. https://doi.org/10.1016/j.jhazmat.2011.03.047.

Insam, H., Gómez-Brandón, M., Ascher, J., 2015. Manure-based biogas fermentation residues – friend or foe of soil fertility? Soil Biol Biochem. 84, 1–14. https://doi.org/10.1016/j.soilbio.2015.02.006.

Jimenez, J., Gonidec, E., Cacho Rivero, J.A., Latrille, E., Vedrenne, F., Steyer, J.-P., 2014. Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: towards ADM1 variables characterization. Water Res. 50, 359–372. https://doi.org/10.1016/j.watres.2013.10.048.

Jimenez, J., Aemig, Q., Doussiet, N., Steyer, J.-P., Houot, S., Patureau, D., 2015. A new organic matter fractionation methodology for organic wastes: bioaccessibility and complexity characterization for treatment optimization. Bioresour. Technol. 194, 344–353. https://doi.org/10.1016/j.biortech.2015.07.037.

Jimenez, J., Lei, H., Steyer, J.-P., Houot, S., Patureau, D., 2017. Methane production and fertilizing value of organic waste: organic matter characterization for a better prediction of valorization pathways. Bioresour. Technol. 241, 1012–1021. https://doi.org/10.1016/j.biortech.2017.05.176.

Jimenez, J., Charnier, C., Kouas, M., Latrille, E., Torrijos, M., Harmand, J., Patureau, D., Spérandio, M., Morgenroth, E., Béline, F., Ekama, G., Vanrolleghem, P.A., Robles, A., Seco, A., Batstone, D.J., Steyer, J.-P., 2020. Modelling hydrolysis: simultaneous versus sequential biodegradation of the hydrolysable fractions. Waste Manage. 101, 150–160. https://doi.org/10.1016/j.wasman.2019.10.004.

Khakbaz, A., Nobili, M.D., Mainardis, M., Contin, M., Aneggi, E., Mattiussi, M., Cabras, I., Busut, M., Goi, D., 2020. Monitoring of heavy metals, eox and las in sewage sludge for agricultural use: a case study. Detritus 160. 10.31025/2611-4135/2020.13993.

Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem. 34, 139–162. https://doi.org/10.1016/S0038-0717(01)00158-4.

Laera, A., Shakeri Yekta, S., Hedenström, M., Buzier, R., Guibaud, G., Dario, M., Esposito, G., van Hullebusch, E.D., 2019. A simultaneous assessment of organic matter and trace elements bio-accessibility in substrate and digestate from an anaerobic digestion plant. Bioresour. Technol. 288, 121587 https://doi.org/10.1016/j.biortech.2019.121587.

Lashermes, G., Nicolardot, B., Parnaudeau, V., Thuriès, L., Chaussod, R., Guillotin, M.L., Linères, M., Mary, B., Metzger, L., Morvan, T., Tricaud, A., Villette, C., Houot, S., 2009. Indicator of potential residual carbon in soils after exogenous organic matter application. Eur. J. Soil Sci. 60, 297–310. https://doi.org/10.1111/j.1365-2389.2008.01110.x.

Levavasseur, F., Mary, B., Houot, S., 2021. C and N dynamics with repeated organic amendments can be simulated with the STICS model. Nutr. Cycl. Agroecosystems 119, 103–121. https://doi.org/10.1007/s10705-020-10106-5.

Maynaud, G., Druilhe, C., Daumoin, M., Jimenez, J., Patureau, D., Torrijos, M., Pourcher, A.-M., Wéry, N., 2017. Characterisation of the biodegradability of post-treated digestates via the chemical accessibility and complexity of organic matter. Bioresour. Technol. 231, 65–74. https://doi.org/10.1016/j.biortech.2017.01.057.

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59–86. https://doi.org/10.1016/j.geoderma.2017.01.002.

Mottet, A., François, E., Latrille, E., Steyer, J.P., Déléris, S., Vedrenne, F., Carrère, H., 2010. Estimating anaerobic biodegradability indicators for waste activated sludge. Chem. Eng. J. 160, 488–496. https://doi.org/10.1016/j.cej.2010.03.059.

Mottet, A., Ramirez, I., Carrère, H., Déléris, S., Vedrenne, F., Jimenez, J., Steyer, J.P., 2013. New fractionation for a better bioaccessibility description of particulate organic matter in a modified ADM1 model. Chem. Eng. J. 228, 871–881. https://doi.org/10.1016/j.cej.2013.05.082.

Muller, M., Jimenez, J., Antonini, M., Dudal, Y., Latrille, E., Vedrenne, F., Steyer, J.-P., Patureau, D., 2014. Combining chemical sequential extractions with 3D fluorescence spectroscopy to characterize sludge organic matter. Waste Manage. 34, 2572–2580. https://doi.org/10.1016/j.wasman.2014.07.028.

Peltre, C., Thuriès, L., Barthès, B., Brunet, D., Morvan, T., Nicolardot, B., Parnaudeau, V., Houot, S., 2011. Near infrared reflectance spectroscopy: A tool to characterize the composition of different types of exogenous organic matter and their behaviour in soil. Soil Biol. Biochem. 43, 197–205. https://doi.org/10.1016/j.soilbio.2010.09.036.

Pognani, M., Barrena, R., Font, X., Scaglia, B., Adani, F., Sánchez, A., 2010. Monitoring the organic matter properties in a combined anaerobic/aerobic full-scale municipal source-separated waste treatment plant. Bioresour. Technol. 101, 6873–6877. \10.1016/j.biortech.2010.03.110.

Provenzano, M.R., Iannuzzi, G., Fabbri, C., Senesi, N., 2011. Qualitative characterization and differentiation of digestates from different biowastes using FTIR and fluorescence Spectroscopies. J. Environ. Prot. 2, 83–89. https://doi.org/10.4236/jep.2011.21009.

Provenzano, M.R., Malerba, A.D., Pezzolla, D., Gigliotti, G., 2014. Chemical and spectroscopic characterization of organic matter during the anaerobic digestion and successive composting of pig slurry. Waste Manag. 34, 653–660. https://doi.org/10.1016/j.wasman.2013.12.001.

Rocamora, I., Wagland, S.T., Villa, R., Simpson, E.W., Fernández, O., Bajón-Fernández, Y., 2020. Dry anaerobic digestion of organic waste: A review of operational parameters and their impact on process performance. Bioresour. Technol. 299, 122681 https://doi.org/10.1016/j.biortech.2019.122681.

Shakeri Yekta, S., Hedenström, M., Svensson, B.H., Sundgren, I., Dario, M., Enrich-Prast, A., Hertkorn, N., Björn, A., 2019. Molecular characterization of particulate organic matter in full scale anaerobic digesters: an NMR spectroscopy study. Sci. Total Environ. 685, 1107–1115. https://doi.org/10.1016/j.scitotenv.2019.06.264.

Soest, V., J, P., 1963. Use of detergents in the analysis of fibrous feeds. 2. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Agric. Chem. 46, 829–835.

Somers, M.H., Jimenez, J., Azman, S., Steyer, J.-P., Baeyens, J., Appels, L., 2021. Ultrasonication affects the bio-accessibility of primary dairy cow manure digestate for secondary post-digestion. Fuel 291, 120140. https://doi.org/10.1016/j.fuel.2021.120140.

Tambone, F., Adani, F., Gigliotti, G., Volpe, D., Fabbri, C., Provenzano, M.R., 2013. Organic matter characterization during the anaerobic digestion of different biomasses by means of CPMAS 13C NMR spectroscopy. Biomass Bioenergy 48, 111–120. https://doi.org/10.1016/j.biombioe.2012.11.006.

Tambone, F., Terruzzi, L., Scaglia, B., Adani, F., 2015. Composting of the solid fraction of digestate derived from pig slurry: Biological processes and compost properties. Waste Manage. 35, 55–61. https://doi.org/10.1016/j.wasman.2014.10.014.

Tambone, F., Orzi, V., Zilio, M., Adani, F., 2019. Measuring the organic amendment properties of the liquid fraction of digestate. Waste Manag. 88, 21–27. https://doi.org/10.1016/j.wasman.2019.03.024.

Tang, Y., Li, X., Dong, B., Huang, J., Wei, Y., Dai, X., Dai, L., 2018. Effect of aromatic repolymerization of humic acid-like fraction on digestate phytotoxicity reduction during high-solid anaerobic digestion for stabilization treatment of sewage sludge. Water Res. 143, 436–444. https://doi.org/10.1016/j.watres.2018.07.003.

Development Core Team, R., 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Teglia, C., Tremier, A., Martel, J.-L., 2011a. Characterization of solid digestates: Part 1, review of existing indicators to assess solid digestates agricultural use. Waste Biomass Valorization 2, 43–58. https://doi.org/10.1007/s12649-010-9051-5.

Teglia, C., Tremier, A., Martel, J.-L., 2011b. Characterization of solid digestates: Part 2, Assessment of the quality and suitability for composting of six digested products. Waste Biomass Valorization 2, 113–126. https://doi.org/10.1007/s12649-010-9059-x.

Triolo, J.M., Pedersen, L., Qu, H., Sommer, S.G., 2012. Biochemical methane potential and anaerobic biodegradability of non-herbaceous and herbaceous phytomass in biogas production. Bioresour. Technol. 125, 226–232. https://doi.org/10.1016/j.biortech.2012.08.079.

Usman Khan, M., Kiaer Ahring, B., 2021. Improving the biogas yield of manure: effect of pretreatment on anaerobic digestion of the recalcitrant fraction of manure. Bioresour. Technol. 321, 124427 https://doi.org/10.1016/j.biortech.2020.124427.

Vidal-Antich, C., Perez-Esteban, N., Astals, S., Peces, M., Mata-Alvarez, J., Dosta, J., 2021. Assessing the potential of waste activated sludge and food waste co-fermentation for carboxylic acids production. Sci. Total Environ. 757, 143763 https://doi.org/10.1016/j.scitotenv.2020.143763.

Vinardell, S., Astals, S., Koch, K., Mata-Alvarez, J., Dosta, J., 2021. Co-digestion of sewage sludge and food waste in a wastewater treatment plant based on mainstream anaerobic membrane bioreactor technology: a techno-economic evaluation. Bioresour. Technol. 330, 124978 https://doi.org/10.1016/j.biortech.2021.124978.

Zhang, Y., Xu, S., Cui, M., Wong, J.W.C., 2019. Effects of different thermal pretreatments on the biodegradability and bioaccessibility of sewage sludge. Waste Manage. 94, 68–76. https://doi.org/10.1016/j.wasman.2019.05.047.

Valorization of digestates from urban or centralized biogas plants: a critical review

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REVIEW PAPER

Valorization of digestates from urban or centralized biogas plants: a critical review

F. Guilayn · M. Rouez · M. Crest · D. Patureau · J. Jimenez

Abstract Direct landspreading of anaerobic digestates is the most common digestate management strategy. Nevertheless, digestate post-treatment can be unavoidable, especially for environmental services providers operating large-scale anaerobic digestion (AD) facilities. This review aims to assess the technical feasibility of achieving value-added products from digestates from urban and/or centralized AD plants (UC-AD). An exhaustive effort was dedicated to identifying and clarifying the available processing technologies and specific issues that can be related to UC-AD digestates. The valorization options were classified according to the final product destination. The result is a useful information source for assessing digestate valorization pathway given a local market and context. Agriculture was the first destination to be considered, as it allows a more direct closing of nutrient and carbon cycles. Several processes exist either for concentrating desirable characteristics of digestates, enhancing organic matter stability or producing pure and reformulated fertilizers. Thermal conversion processes are either under development or full-scale demonstration. They allow to valorize the solids through the production of biofuels and/or biochar and in the coming future, to start a whole biorefinery system. Similarly, biomass harvesting processes such as microalgae are under upscaling, enabling to valorize the nutrients of the digestate liquid phase while producing renewable biomass from sunlight. Several value-added products were already obtained in laboratory to pilot conditions from UC-AD digestates, for example, biopesticides, biosurfactants and composite materials. Adding to technical challenges, the quality variation of digestates, regulation barriers, public acceptance and the difficult access to new markets are among the main obstacles to UC-AD digestates valorization into value-added products.

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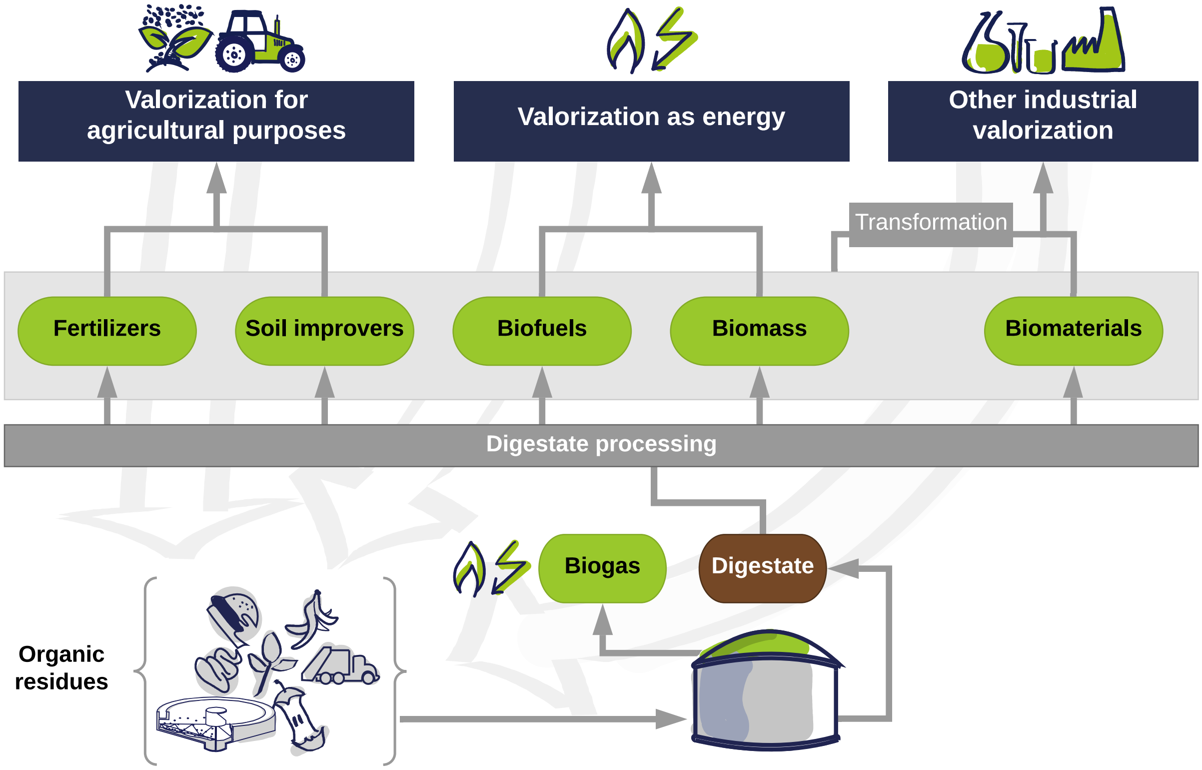
Graphic abstract



Keywords Anaerobic digestion · Biogas effluent · Digestate valorization · Biogas slurry · Nutrient recovery · Resource recovery · Circular economy · Upcycling · Biowaste · Food waste · Sewage sludge · Wastewater treatment plant

Abbreviations

ABP	Animal by-products
AC	Activated carbon
AD	AnAerobic digestion
DM	Dry matter
FAO	Food and Agriculture Organization of the United Nations
GCV	Gross calorific value
HLS	Humic-like substances
HM	Heavy metals
MBT	Mechanical biological treatment
MSW	Municipal solid waste
NA	Not available
NUE	Nutrient uptake efficiency
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
OM	Organic matter
OMP	Organic micropollutants
RT	Retention time
SS	Sewage sludge
TAN	Total ammoniacal nitrogen
TSS	Total suspended solids
UC-AD	Urban or centralized AD plants
TKN	Total Kjhedal nitrogen
VFA	Volatile fatty acids
VS	Volatile solids
WWTP	Wastewater treatment plant
ww	Wet weight

1 Introduction and objectives of the review

Anaerobic digestion (AD) of organic residues is a well-developed and growing solution for both waste upcycling and green energy production. The European Biogas Association defines digestate as the solid or liquid material from controlled anaerobic fermentation processes of biodegradable material (European

Biogas Association 2015). For the treatment of organic residues, AD present several advantages, such as:

- Waste stabilization with mass and volume reduction (Möller et al. 2010; Kothari et al. 2014);
- Increased recycling of organic matter and nutrients, thus promoting the conservation of natural resources (Drosg et al. 2015);
- A cost-effective (Kothari et al. 2014) and relatively simple technology capable of processing a wide range of substrates (Ağdağ and Sponza 2005; Appels et al. 2008; Capson-Tojo et al. 2016);
- Resulting valuable products: biogas, a renewable energy source and *digestate*, recognized by its application as a fertilizer and/or soil improver.

This review is oriented to digestates from urban and/or centralized AD plants (UC-AD). These plants are here defined as those receiving mostly urban or industrial inputs (such as sewage/industrial sludge, food/household/commercial waste, municipal solid waste, among other). They are usually not inserted in the context of farms though in many cases the centralized plants[1] do receive agricultural inputs. In opposition, agricultural digestates receive, essentially, agricultural residues, livestock manure/slurry and/or energy crops. Compared to agricultural digestates, UC-AD digestates present particular issues and more challenging management, which will be addressed in this review.

As in the context of sewage sludge management in wastewater treatment plants (WWTP), the choice of the digestate treatment pathway on biogas plants should be evaluated in an early stage of AD projects conception. For the definition of a treatment line/process, the designers should consider the economic and environmental aspects of the local legislation, as well as the quality requirements from the possible consumers and disposal sites nearby the plant. However, compared to digestates, sewage sludges have characteristics that can be more easily generalized. Actually, the word *digestate* refers to a set of very heterogeneous matter (Guilayn et al. 2019a). Digestate processing equipment are being adapted from previous technologies developed for other substrates such as raw manure, wastewater and sewage sludge (Al Seadi et al. 2013). As AD is being intensively encouraged, policymakers, public and private environmental sectors can deal with a difficult decision-making scenario.

A significant number of digestate-dedicated scientific reviews have been already published. Some of them are digestate post-treatment inventories, including commercial solutions and promising techniques (Fuchs and Drosg 2013; Sheets et al. 2015). Other reviews have more special focus; such as the effects of AD on the digestate characteristics (Möller and Müller 2012), valorization of digestates from the organic fraction of municipal solid waste[2] (OFMSW) (Logan and Visvanathan 2019), valorization of agricultural digestates (Monlau et al. 2015), the risks associated to the agricultural spreading of digestates (Nkoa 2014), characterization methods (Teglia et al. 2010), nutrient recovery techniques (Makádi et al. 2012; Lebuf et al. 2013; Lin et al. 2015; Tao et al. 2016; Vaneeckhaute et al. 2017), algae production (Uggetti et al. 2014; Monlau et al. 2015), energy valorization and anaerobic biorefinery (Uggetti et al. 2014; Sawatdeenarunat et al. 2016). However, to the best of the authors' knowledge, no review has a special focus on urban and centralized AD facilities, the broad possibility of value-added end-products and their technical feasibility. This review aims to fill up this gap, while:

- Applying a reverse approach, giving insights and assessing the technical feasibility of achieving value-added products with existing and developing technologies.
- Classifying the valorization options according to the final product (instead of process), for providing a piece of practical information for urban and centralized AD plants needing to better valorize digestates.

Before addressing the valorization techniques, more detailed contextualization is provided: first a global UC-AD digestate production prospection (Sect. 2), then a review of digestate properties (Sect. 3). The latter includes the effects of AD on digestate fertilizing value and innocuity. Following (Sect. 4), the specific challenges of UC-AD digestate

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[1] Internally defined as large scale AD plants receiving a wide diversity of waste streams and with an installed capacity superior to 30–50 kt/y.

[2] OFMSW: not source-separated. Defined operationally by the authors as the organic material obtained after mixed collection of municipal solid waste and separation by mechanical biological treatments (MBT).

valorization are discussed. Finally (Sect. 5), the two objectives above are addressed throughout a critical review on UC-AD digestate valorization backed up by both scientific and technical expertise from co-authors counting with academic and industrial backgrounds. Attached to this review: (i) a detailed summary of digestate processing technologies (Appendix A in ESM), which includes its by-products, advantages, bottlenecks and readiness levels; and, (ii) the methodology used for gathering an initial scientific publication library along with a short bibliometric study on digestate valorization technologies (Appendix B in ESM).

2 Digestate production: situation and prospection

Currently, the world's biggest economies present either important biogas sectors or specific policies that will boost centralized and urban AD (Table 1). Those might include direct economic incentives or indirect legislation such as the international trend for minimizing organic waste landfilling and incineration. Besides, AD is more than a trending solution to agricultural issues in both developed and developing countries. It is also between the most suitable solutions to treat and recover the increasing volumes of urban residues such as food waste (Capson-Tojo et al. 2016). There is certainly a trend for increasingly global production of UC-AD digestates,thus raising the concerns around its destination.

3 Digestate properties

3.1 Fertilizing and amendment value

The same AD process mechanisms make both agricultural and UC-AD digestates recognized as fertilizers, despite the possible different associated risks. The effects of AD on the fertilizing and amendment value of digestates are summarized in Table 2.

Due to a conversion of the more easily biodegradable organic matter into biogas (mostly CH_4 and CO_2), AD increases the biological stability of the digested material and the concentration of recalcitrant OM such as lignin and humic-like substances. Humification degree is therefore increased during AD, which was demonstrated by several advanced techniques (Massaccesi et al. 2013). Some authors suggest the occurrence of humification processes during AD (Polak et al. 2005; Brunetti et al. 2012). In any case, AD engenders an improvement in the organic soil amendment value of the input residues.

OM abatement also implies the reduction of dry matter (DM) and viscosity (Möller et al. 2008; Möller and Müller 2012). DM reduction is about 25% (Smith et al. 2010). Final DM values greatly depend on the applied process moisture. Moreover, as small-sized particles are first consumed, AD tends to lead to better dewatering properties of the digestate if compared to the raw material (Hjorth et al. 2010).

In AD, organic matter conversion rates may greatly vary (13–65% (Monlau et al. 2015), 20–95% (Möller and Müller 2012)), depending mostly on the type of substrate fed to the digester, AD parameters (organic loading rate, retention time and temperature) and the resulting performance (Bauer et al. 2009). Easily biodegradable inputs such as food waste, some types of OFMSW, animal slurries and cereal grains, will tend to induce greater OM reduction (Fuchs and Drosg 2013). On the contrary, since lignocellulosic material is more difficulty digested (Labatut et al. 2011), fibrous lignocellulosic material such as litter bed, silages and cattle manure will lead to lower DM and OM reduction and digestates with a greater VS/DM ratio if not pre-treated (Möller and Müller 2012).

For application as a soil amendment, the C/N ratio of organic material must be compatible with the soil microorganism's requirements. This is necessary to avoid excessive N release (from OM decomposition, if the C/N is too low) or, in the opposite scenario, soil-N immobilization (microbiological uptake, if the C/N is too high). Following OM mineralization, a significant fraction of the input C is converted to CO_2 and CH_4 (biogas), the C/N ratio is thus reduced during AD (Möller et al. 2008). For the same configuration, higher retention times will lead to greater C consumption thus lower C/N ratios on digestate. Desired digestate C/N stability thresholds are reported between 10 and 20 (Teglia et al. 2010). This value can be highly variable depending on the digestate type, ranging from about 3 to 20 (Guilayn et al. 2019a).

Digestates inherit the nutrients content from the AD feedstock. However, due to the mass reduction (OM biodegradation), digestate nutrients concentrations tend to increase. Nonetheless, the fertilizer value of a given product not only depends on the total nutrient

Table 1 AD situation and prospect in some of the world's biggest economies

Country/region	Remarkable information	References
European Union	World's biggest producer of electricity from biogas (half of the world's production) AD boosted through extensive national support schemes such as feed-in-tariffs Over 16.000 AD plants (2017), Germany is the leading country with 8000 agricultural plants and over 1200 sludge digestion plants (2015) European policies favor organic waste prevention while encouraging recovery techniques such as AD over landfilling	Council Directive (1975), Deremince and Königsberger (2017), Liebetrau et al. (2017), Scarlat et al. (2018)
United States of America	1480 organic waste AD plants producing biogas With proper support, over 11,000 existing sites could host an AD process to generate biogas in farms or WWTP The US is currently the second-fastest-growing market for renewable energies after China	USDA, U.S. EPA and U.S. DOE (2014)
China	One of the first countries to have implemented massive AD policies AD is now being applied for almost a century Over 40 million of familiar AD units (agricultural) Large-scale engineered biogas installations are also numerous, estimated above 100,000 in 2014 AD tends to be boosted with the Chinese Medium-and-Long Term Development Plan for Renewable Energy. This plan aims 8000 new large-scale biogas plants and almost doubling the number of domestic digesters	Scarlat et al. (2018)
Japan	At least 63 AD plants treating animal waste to produce electricity and/or heat. Food waste AD is also reported 300 Japanese WWTP had AD tanks in 2010 Among national incentives, Japan government established a generous feed-in tariff system back in 2012 aiming to boost renewable electricity injection in the grid	Ministry of Land Infrastructure Transport and Tourism (2013), Yokoyama and Matsumura (2015)
India	With the implementation of a National Biogas and Manure Management Program back in the 1980s': the country counted with 4.75 million household digesters in 2014 and had an estimated potential for additional 12 million Biogas production from centralized AD is reported to be growing fast	Ministry of New and Renewable Energy (2014)
Brazil	Biorefinery pioneer with the bioethanol production from sugarcane after the National Alcohol Program in the 1970s' Biogas technologies are underdeveloped. A recent survey in Brazil has listed only about 130 AD plants producing more than 1250 m^3 of biogas/day Important regulatory landmarks should boost AD in the coming years, such as the recent resolution authorizing biomethane injection in the natural gas grid	Mariani (2015), ANP (2017)

content. It also depends on the nutrient availability to the plants and their uptake efficiency, which also tends to be enhanced with AD. During the digestion process, the organic fraction of nutrients is mineralized and released from complex organic compounds, thus increasing N, P, K, Ca and Mg accessibility. This effect is highly relevant for organic N and organic P, and notably the first (Möller and Müller 2012; Al Seadi

Table 2 Summary of AD effects on inputs and resulting digestate quality

Characs.	Effect	Resulting change	Main mechanisms	References
DM	Decrease	− 1.5% to − 5.5% (absolute)	Destruction of biodegradable matter	Möller et al. (2008), Pognani et al. (2009), Vaneeckhaute et al. (2013a)
VS or OM	Decrease	− 5 to –15% DM (absolute)	OM conversion to biogas	Möller and Müller (2012)
Viscosity	Decrease	NA	Destruction of small particles	Carballa et al. (2009), Hjorth et al. (2010), Möller and Müller (2012)
Dewater-ability	Possible increase	NA	Prior destruction of small particles that are commonly slow-settling/non-filterable	Carballa et al. (2009), Hjorth et al. (2010)
Biodegrad.	Decrease	Highly depends on RT	OM conversion to biogas	Teglia et al. (2011), Menardo et al. (2011b)
pH	Increase	+ 0.5 to + 2 units	VFA degradation, ammoniacal nitrogen release	Kiely et al. (1997), Möller and Müller (2012), Batstone et al. (2015)
Fibers content	Increase	Final lignin content about 10 to 20% DM basis	Lignocellulose is poorly biodegradable	Pognani et al. (2009), Menardo et al. (2011b), Dabert (2015), Sambusiti et al. (2015, 2016), Cavalli et al. (2016)
Amend. value	Increase	Highly variable. No consensus on indicators.	Increase in concentration of recalcitrant OM such as humus precursors	Pognani et al. (2009), Tambone et al. (2009)
Nutrient availability	May vary, overall positive effect for N, P and K	Highly variable	Increase if organic N and P mineralization Decrease if formation of Ca/Mg/Fe–P compounds	Möller and Müller (2012), Bachmann et al. (2016)
TAN	Increase	+ 25 to 55%	Mineralization of proteins and amino acids. Released TAN is partially used for biomass growth	Möller and Müller (2012)
Total N	From concentration increase to possible significant losses	Usually little/no change	Increase: mass reduction due to OM conversion Loss: struvite incrustation/settling and ammonia volatilization	Möller et al. (2008, 2010), Banks et al. (2011), Möller and Müller (2012), Zirkler et al. (2014)
Total P, K, Ca, Mg	From concentration increase to possible significant losses	Usually little/no change	Increase: mass reduction due to OM conversion Loss: precipitation and incrustation inside the digester	Marcato et al. (2008), Schievano et al. (2011), Banks et al. (2011), Zirkler et al. (2014)
S	No change/possible loss	Usually little/no change	Degradation of OM containing S, reduction to H_2S	Schievano et al. (2011), Zirkler et al. (2014)
C/N	Decrease	− 3 to − 5 units	C mineralization	Möller and Müller (2012)
Heavy metals	Increase of concentration Reduced bioaccessibility	Highly variable	Concentration: digestate mass and volume reduction Accessibility: biological uptake and pH increase reducing metals solubility	Appels et al. (2008), Marcato et al. (2009), Stefaniuk et al. (2015)
Heavy metals	Possible loss of Cd and Zn	Highly variable	Precipitation of Cd and Zn sulfides, which has low solubility and a high density	Zirkler et al. (2014)

Table 2 continued

Characs.	Effect	Resulting change	Main mechanisms	References
Bad odor	Decrease	up to—98%	VFA degradation	Powers et al. (1999), Hjorth et al. (2009)
Pathogens	Partial to complete kill	30% up to > 99% abatement	Temperature and time; competition for substrate with the adapted AD microorganisms	Carballa et al. (2009), Bonetta et al. (2014)
OMP	Highly variable	Highly variable	Highly compound-dependent	Brändli et al. (2007), Govasmark et al. (2011), Stasinakis (2012), De Moor et al. (2013), Mailler et al. (2014, 2017)

AD anaerobic digestion, *Bio-degrad* biodegradability, *Characs* characteristics, *DM* dry matter, *NA* not available, *OM* organic matter, *OMP* organic micropollutants, *RT* retention time, *TAN* total ammoniacal nitrogen, *VFA* volatile fatty acids, *VS* volatile solids

et al. 2012; Mehta and Batstone 2013; Bachmann et al. 2016). However, the pH rise may also lead to the formation of low plant-available compounds such as calcium phosphates (Wahal et al. 2010; Möller and Müller 2012; Mehta and Batstone 2013).

Along with the OM digestion, highly biodegradable material tends to produce digestates with a higher Total Ammoniacal Nitrogen to Total Nitrogen (TAN/TN) ratio and the opposite for fibrous materials. N-rich digestates are greatly associated with animal waste such as pig slurry and poultry manure, slaughterhouse waste, sewage sludge, cereal residues and some agri-industrial residues. Some specific biowaste and many fibrous materials (e.g. maize silage, cattle manure) are related to lower N-content digestates (Benoît et al. 2014). In fact, the protein content on the feedstock is the greatest source of the N and S contents of digestates (Straka et al. 2007; Möller and Müller 2012; Park and Kim 2016).

Throughout the AD process, several mechanisms promote a pH increase from about 0.5 to 2 units (Möller and Müller 2012). Final pH in digestates ranges from 7 to 9 (internal databases). In usual conditions, the pH increase is driven by the consumption of volatile fatty acids (VFA) and the production of ammonium (Kiely et al. 1997; Möller and Müller 2012; Batstone et al. 2015). Moreover, increasing the pH buffering capacity by adding an alkali is usually performed in AD operation, in order to avoid the inhibition of methanogenic archaea by acidification (Nguyen et al. 2015).

For storage and spreading purposes, the conversion of VFA and the destruction of odor components (e.g. benzaldehyde and phenols) are responsible for a significant decrement of odor nuisances in digestates if compared to raw inputs (Hjorth et al. 2009).

On the negative side, several mechanisms during AD may lead to serious nutrient losses in full-scale operational systems, even if many authors relate negligible unbalances. First of all, the mineralization of N during AD produces ammoniacal nitrogen, which can represent a + 10 to + 33% TAN share on TN (Möller and Müller 2012). Higher TAN concentrations coupled with a pH increase favors the conversion of NH_4^+ to free ammonia (NH_3). This effect results in an important increase of ammonia volatilization risk during the AD process or digestate storage, transport, spreading and processing (Nkoa 2014), but not systematically (Risberg et al. 2017). Also due to the pH rise, carbonates, phosphates and cations such as NH_4^+, K^+, Ca^{2+} and Mg^{2+} may precipitate and incrust (e.g. as struvite) inside the digester and equipment downstream (Marcato et al. 2008), which might represent non-negligible nutrient loss.

Globally, AD tends to significantly upgrade the fertilizing value of the input waste in both organic and mineral aspects. Resulting digestates may present nutrient uptake performances equivalent or better than those of industrial mineral fertilizers (Vaneeckhaute et al. 2013a, b), as well as amendment characteristics that can be similar to those of composts (Tambone et al. 2010). However, if no specific quality criteria are available for raw digestates, they can be outside the scope of standards and regulations conceived for conventional fertilizers and soil amendments. For example, for France and EU, Guilayn et al. (2019a) observed that raw digestates were too wet (low DM) for meeting "soil improvers" regulations and with poor nutrient content (wet weight basis) for meeting several "(organic-)fertilizer" regulations.

3.2 Digestate innocuity

The conversion of OM to biogas represents a mass loss that might concentrate not only the valuable nutrients but undesirable components such as heavy metals (HM) and organic micropollutants (OMP) (Lehmann et al. 2015). Nevertheless, a prevailing absence of exceeding legislation limits can be observed in the literature, in studies from several countries and for various types of digestates (Benoît et al. 2014; Zirkler et al. 2014; Dabert 2015; Koszel and Lorencowicz 2015). For HM, it was confirmed with a previously published meta-analytic study by the same authors of the present review (Guilayn et al. 2019a). Nevertheless, an important number of studies present concerns regarding the ecotoxicological effects and the environmental safety of digestates for land application. Gas emissions, heavy metals, ammonium toxicity, high salinity and pathogens are among the most frequent concerns. (Teglia et al. 2011; Alburquerque et al. 2012; Bonetta et al. 2014; Owamah et al. 2014; Tigini et al. 2016). The content of non-biodegradable compounds such as plastics, metals, glasses and stones, also called "physical/inert impurities", is often related to digestates from urban and commercial waste, including OFMSW, source-separated biowaste (such as household food waste) and depackaging unities (Dabert 2015, SUEZ expertise).

Regarding HM, several authors have reported hazardous characteristics of animal manures, sewage sludge and OFMSW (Zirkler et al. 2014; Dabert 2015). Indeed, animal manure is one of the main sources of HM because Cu, Zn and other heavy metals are widely applied in animal feed due to antimicrobial and growth-stimulating effects (Poulsen 1998). For example, copper sulfate is widely used on dairy disease-preventing footbaths. Crop residues may also be related to chemical contamination due to the application of pesticides, impurities of mineral fertilizers and the accumulation of heavy metals on the soil. Cadmium, for example, is a major contaminant issue in agriculture as it is easily assimilated by plants and it is between the most toxic heavy metals to humans. Cadmium soil contamination in fields is related to the application of P fertilizers from phosphate rocks, spreading of sewage sludge and atmospheric transport of mining dust (Van Bruwaene et al. 1984; Robson et al. 2014). In the case of OFMSW, a study has associated higher HM of Cr, Hg and Pb (Dabert 2015). Zirkler et al. (2014) reported higher levels of Zn, Pb, Cd and Ni for digestate with sewage sludge inputs compared to agricultural digestates. Interestingly, they report Cd and Zn loss which was associated with precipitation as sulfides.

Despite the concerns on total HM concentrations, several authors indicate that the chemical accessibility of HM is decreased with AD. Apart from biological accumulation (e.g. biofilm complexation, chelation or translocation to the cells' interior), the decreasing of heavy metals' accessibility is suggested to be driven by the pH increase that reduces the solubilities of metals and promotes precipitation processes with carbonates and sulfides (Bloomfield and McGrath 1982; Zandvoort et al. 2006; Marcato et al. 2009). Nevertheless, the HM bioavailability to plants will greatly depend on soil characteristics (Almeida et al. 2019). Little attention seems to be dedicated to plant growth experiments for assessing the actual plant HM uptake after spreading (Marcato et al. 2009).

As for HM, OMP concentrations on digestates will depend on the effective control on the use of such substances at the source of the AD feedstock and its respective production chain. Apart from sewage sludge (SS) and manure AD, little attention seems to be dedicated to the fate of OMP during AD. Papers dedicated on sewage sludge AD (Stasinakis 2012; Mailler et al. 2014, 2017) assessing pharmaceuticals, estrogens, flame retardants, phthalates, detergents, hydrocarbons, among others, concluded that the fate of these contaminants in AD is generally influenced by solids retention time (SRT), temperature, sludge composition, bioaccessibility of the compounds and the adaptation of the microbial biomass. Results are variable and highly compound-dependent.

Regarding biological contamination, weed seeds, crop disease spores, resistance genes, bacteria, viruses, fungi, and other pathogens present in the feedstock are partially eliminated during AD, depending on SRT and temperature for a possible full abatement (Al Seadi et al. 2012; Kjerstadius et al. 2013; Youngquist et al. 2016; Tian et al. 2016; Seruga et al. 2020). For safety reasons, even if some AD configurations are being demonstrated to insure sanitation, legislations as the European (The Commission of the European Communities 2011), for Animal By-Products (ABPs), may require the pasteurization of specific AD feedstocks (before AD) or the whole digestate, regardless the following digestate processing.

3.3 Time variability of digestate quality

AD feedstock may greatly vary on quality and quantity during an annual basis, which may impact the composition of digestate within the same biogas plant, especially if those variations provoke process instabilities. Zirkler et al. (2014) monitored 4 full-scale biogas plants in Germany during one year concluded that digestates from the same plant present important heterogeneity over time, discouraging studies bases on single samples.

It must be stated, however, that AD can be regarded as a tool for reducing the time variability of waste streams for better organic waste management within a territory. For example, a French long-term research project (Project DIVA, Dabert 2015) monitored 5 full-scale plants with diversified inputs and processes and noted a variability usually inferior to 10% and always inferior to 20% for all the monitored characteristics (including agri-value and OMP). In parallel, much greater variability in the feedstock was observed during the same period. Banks et al. (2011) collected AD input (mostly food waste) and output data for a mass balance study under a period of 426 days. They observed less variability of N, P and K output (digestate) than the input (food waste). These observations can be explained by an overall buffering effect of the AD plant.

Quality warranty is necessary for a trusted and legal marketing of bio-based products. For marketing purposes, it is usually attained by labeling the products based on existing quality criteria. However, UC-AD digestates are, most of the time, out of the current regulations conceived for composts or classical fertilizers (Guilayn et al. 2019a). Additionally, UC-AD digestates can present non-source separated inputs that are stricter regulated in the EU (Guilayn et al. 2019a). Both are reasons why digestate post-treatment might be necessary to ensure digestate valorization.

3.4 Types of UC-AD digestates

Supported by the works of Guilayn et al. (2019a), two great types of UC-AD digestates can be defined: those coming from wet and dry AD processes. This classification appeared as a first factor explaining the clustering analysis.

Except for sewage sludge mono-digestion, UC-AD digestates from wet AD processes are originated mainly from co-digestion plants and regional facilities, many times integrated to WWTP. In many cases, process water is used to dilute the inputs, which implies an increased capacity of digestate post-treatment downstream. A rising configuration is the dilution of inputs during some depackaging techniques of packed biowaste. Common inputs are sewage sludge, food waste, source-separated organic waste, OFMSW, food-agri industrial residues and even agricultural residues. The reactors are usually Continuously Stirred Tank Reactor (CSTR) working in mesophilic conditions.

According to Guilayn et al. (2019a) these digestates present a low C/N ratio (< 10 and usually < 8), moderate to low VS content (55–70%, DM basis) and high TN (50 up to > 150 g/kg DM) (Guilayn et al. 2019a). When presenting higher inputs of sewage sludge and other P-rich material such as pig slurry, they present a high P-content (20 ± 10 g/kg DM). When presenting great amounts of protein-rich feedstock they present a particularly high TAN/TN ($64 \pm 11\%$), which is regularly the case of food waste and livestock slurry.

UC-AD digestates from dry-AD plants are mostly originated from OFMSW and source-separated organic waste (biowaste). The digestion is performed in high-solids conditions (DM $> 15\%$ but up to 30–40%) (Li et al. 2011). Most of the centralized Dry-AD plants in Europe seem to be performed in continuous plug flow reactors but several commercial batch systems exist (Li et al. 2011; André et al. 2018). In dry conditions, the reduced content of water allows a better energy efficiency to heat the digesters. Dry-AD is thus commonly performed in thermophilic conditions (Li et al. 2011). The higher process temperature induces faster hydrolysis kinetics that compensates for the lower mass transfer rates and also results in greater pathogen inactivation. On a dry matter basis, they present lower VS (40–50% DM basis) and poorer nutrient content (< 50 g N/kg DM, < 10 g P/kg DM and < 25 g K/kg DM), which is probably linked to inputs. However, if comparing nutrient contents of dry and wet-AD UC-AD digestate on a wet weight basis, no clear difference can be observed (Guilayn et al. 2019a). Adding to that, digestates from Dry-AD were observed to present C/N ratio greater than 8–10, which can be necessary for several soil amendment regulation and quality criteria.

In both cases, a common inconvenience of UC-AD digestates is the presence of inert impurities that will affect negatively the processes downstream and the quality/acceptability of final products. The content of inert impurities greatly depends on the effectiveness of source-separation and/or on an effective removal/depackaging step before AD. Effective pre-treatment of OFMSW can be achieved to remove this kind of material, producing a digestate that can be processed to achieve quality standards for landspreading (SUEZ 2017a).

As a rule of thumb, UC-AD digestates present inferior content of fibers compared to agricultural digestates, as it can be observed in Table 3. Agricultural digestates may present a lignocellulosic content up to 875% OM due to large quantities of silage, manure and crop residues. In its turn, are mostly between 25 and 30%. This is strikingly important for the choice of post-treatment equipment and the possible valorization pathways, as discussed later.

It must be noticed, though, that UC-AD may also receive important volumes of (ligno-)cellulosic material such as green waste, paper waste, cardboards and even agricultural residues. All these inputs can be recalcitrant. As it can be observed in Table 3, the UC-AD digestates presenting the highest content of residual fibers are (i) a digestate from a biowaste greatly composed of green waste and (ii) an OFMSW digestate. The OFMSW from different locations are highly variable but can be composed of a significant amount of paper waste and cardboards (Demirbas 2006 (Turkey); Zhang and Banks 2013 (UK); Li et al. 2016 (USA)).

4 UC-AD digestates management: challenge, particularities and opportunities

Land spreading of digestates is the most common valorization route. However, for the providers of environmental service operating urban and regional facilities (non-agricultural), several legal, logistical and technical bottlenecks along with environmental concerns can be listed:

(i) Digestate spreading can be strictly controlled by environmental authorities. In some cases, it can be associated with important environmental risks including ammonia volatilization, pathogens, organic micropollutants and heavy metal content, over-fertilization, nutrient runoff, among other (Nkoa 2014);

(ii) Digestate spreading regulation is usually stricter for digestates originated from non-source-separated AD inputs. For example, in the new EU regulation on fertilizers (2019/1009), digestates are an authorized component material but excluding those from sewage sludge, OFMSW and other mixed-stream substrates as AD feedstock;

(iii) Production of UC-AD digestates is permanent and relatively constant on a yearly basis (in quantity), while the agricultural needs are highly seasonal. Digestates must thus be stored for a long time until the growing season, or even transported to distant regions (King et al. 2013; Gong et al. 2013);

(iv) Adding to the time-quantity issue, digestate characteristics might be highly time-variable (Zirkler et al. 2014), depending on feedstock variations and process performance stability. In this matter, UC-AD digestates may be more problematic than agricultural ones. In order to operate in full capacity and to maximize methane production, the operators may receive a very wide variety of organic waste streams within the territory (internal expertise). Quality variation may be problematic in terms of quality control for machinery operation, landspreading and marketing purposes (Dahlin et al. 2015);

(v) The production of digestate may exceed the capacity of the local available arable lands for receiving nutrients (Vaneeckhaute et al. 2013a; Nkoa 2014). Increasing land competition implies in high and growing digestate transportation costs. According to Dahlin et al. (2015) the digestate transport distance is reported to have doubled in the last years to distances reaching 150 km. Moreover, the number of large and centralized facilities exceeding local nutrient spreading capacity tends to grow since they present better economic feasibility.

Table 3 Fiber content of agricultural and UC-AD digestates (full-scale plants if not indicated). Ordered by HC + C

Main inputs as designed by the source	Type	Actual digestate destination	Innovative research: final product	Fraction	DM % ww	VS % DM	HC % VS	C % VS	HC + C % VS	LG % VS	Source
Three digestates mainly composed of cattle manure, cattle/swine slurry and silage	Agri	NA	Methane (i)	SF	12.4–29	83.6–89.7	13.9–26.3	36.1–43.1	56.9–63.2	21–27.9	Menardo et al. (2011a)
95% cattle manure, 5% millfeed (% ww)	Agri	Landspreading	NA	SF	26.5	72.8	23.8	34.3	58.1	14.3	Dabert (2015)
51% pig slurry, 49% FAI[a] (% ww)	Agri	Landspreading	NA	RW	6.4	70.9	20.3	24.2	44.5	16.9	Dabert (2015)
84% OFMSW, 16% source-separated urban biowaste	UC-AD	(Co-)composting	NA	RD	25.1	43.7	19.7	22.6	42.3	34.6	Dabert (2015)
9% groats, 29% olive oil cake, 57% triticale silage and 5% chicken manure[b]	Agri	Not available	Ethanol (ii)	SF	94.4[c]	89.9	19.4	22.7	42.0	35.3	Sambusiti et al. (2016)
95% cattle manure, 5% millfeed	Agri	Spreading	NA	RD	17.4	65.5	17.5	23.5	41.0	18.3	Dabert (2015)
86% biowaste[d], 14% FAI[e] (% ww)	UC-AD	(Co-)composting	NA	RD	19.3	53.1	8.6	32.3	40.9	30.5	Dabert (2015)
42% cattle slurry, 18% cattle manure, 17% pig slurry, 14% liquid FAI[f], 9% other[g]	Agri	Landspreading	NA	RW	5.7	68.9	13.2	23.6	36.8	17.1	Dabert (2015)
Source selected biowaste	UC-AD	(Co-)composting	Enzymes, biosurfactants, biopesticides (iii)	SF	24.4	63.0	16.0	16.5	32.5	27.8	Cerda et al. (2019)
59% OFMSW, 22% cow manure slurry, 18% agro-industrial waste and 2% energy crops (%ww)	UC-AD/Agri	NA	NA	RW	5.8	75.1	9.1	13.0	22.1	26.8	Pognani et al. (2009)
Source-separated food waste	UC-AD	NA	NA	RD	19.9	61.8	7.6	3.3	10.8	3.1	Tampio (2016)
Source-separated domestic food waste	UC-AD (lab-scale)	NA	NA	RW	6.7	67.7	5.0–6.0	3.6–5.3	8.6–11.3	2.6–2.8	Tampio (2016)
VW and waste activated sludge	UC-AD (pilot)	NA	NA	RD	34.2	69.9	6.7	2.5	9.2	3.2	Tampio (2016)

Table 3 continued

Main inputs as designed by the source	Type	Actual digestate destination	Innovative research: final product	Fraction	DM % ww	VS % DM	HC % VS	C % VS	HC + C % VS	LG % VS	Source
Source-separated OFMSW[h]	UC-AD	NA	NA	RD	32.2	58.7	4.8	3.0	7.8	2.1	Tampio (2016)

C cellulose, *DM* dry matter, *FAI* food/agri-industrial waste, *HC* hemicellulose, *LG* lignin, *NA* not available or not applicable, *OFMSW* organic fraction of municipal solid waste, *RD* wholraw digestate from dry-AD, *RW* whole raw digestate form wet-AD, *SF* solid fraction, *SOL* soluble matter, *UC-AD* urban and/or centralized AD plant, *VS* volatile solids, *ww* wet weight

[a] FAI: sludge from slaughterhouse, grease sludge, swine digestive contents, Millfeed, among other

[b] NOt clear if %ww

[c] Dried milled

[d] Biowaste: OFMSW, paper, cardboard, textile, green waste

[e] FAI: yogurt, dough, grease, among other

[f] FAI: no more information

[g] Other: cereals residues, silage, among other

[h] not clear since the original author also defined OFMSW as being originated by MSW mixed collection followed by MBT

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(i) Thermal post-treat. to produce CH4 (AD): successful

(ii) Post-treatment for bioethanol fermentation: successful (about 9% of whole digestate's COD)

(iii) Fermentation for producing biosurfactants and other value-added compounds: low soluble carbohydrate content thus poor yields

A holistic view of a biogas plant considering digestate treatment and destination is necessary to avoid inappropriate management that could lead to negative results in terms of carbon print, energy balance, economic viability and public acceptance of AD as a waste valorization option. As discussed, land spreading is not individually enough to overcome the digestate challenge, especially for UC-AD digestates. The application of digestate upgrading technologies is essential and such processes might have different and multiple objectives, including:

- Treating or removing undesired digestate characteristics as a waste/wastewater treatment strategy (e.g. chemical precipitation, nitrification/denitrification), prior to digestate valorization or simply for reaching disposal requirements,
- Reducing transportation costs by concentrating the valuable components and properties of the digestate (e.g. solid/liquid separation, thermal drying, membrane filtration, evapo-concentration);
- Producing relatively pure and value-added products (e.g. N-stripping and struvite crystallization);
- Ensuring controlled and homogenous quality of products over time;
- Increasing market acceptance and
- Creating/reaching new markets.

To achieve these objectives, compared to agricultural AD plants, UC-AD designers and operators may benefit from higher capital investment, economies of scale, proximity to industrial clusters and better synergy with industrial actors.

5 Achieving value-added products from non-agricultural digestates

The literature review on digestate valorization processes started by a systematic literature research and a bibliometric study presented on Appendix B in ESM. In brief, a research query was developed for identifying publications containing, in the title, multiple variations of the words "digestate" (e.g. biogas effluent/slurry, anaerobically digested) and "treatment" or "valorization" (e.g. processing, recovery, removal). This query resulted in 1362 papers that were manually verified, resulting in a first library of 520 publications. For complementing the review, this original library has been extensively expanded by looking into citations and by performing specific queries on the identified processes and products.

The valorization of digestates into value-added products was separated into three great categories: agriculture, energy and other industrial valorization. The products cited in the coming sections are summarized in Tables 4, 5, 6 and 7, with a focus on feasibility. No product prices were added either because of their incertitude for fertilizers (highly volatile N/P/K world commodity market) or due to the lack of established market and market prices for other components (notably the carbon content) and by-products.

This review classifies digestate products according to their destinations, not according to processes that generate them. Products and processes are thus repeated when necessary, with different focus depending on their destinations. A same processe can generate different products for different destinations. Processes and their general advantages and bottlenecks are thus introduced only once during the review, usually, the first timethey are cited but clearly indicated in the few exceptions. Digestate processing techniques are summarized in Appendix A in ESM, where inputs, outputs, advantages, digestate limiting (necessary) characteristics, bottlenecks, costs and readiness levels are included.

5.1 Agricultural valorization

The following sections summarize a wide range of options for generating value-added products from UC-AD digestates, aiming to reach the agricultural market. Agriculture is considered a priority since closing the food production loop is essential for meeting our civilization challenge of sustainable agriculture and food supply. Adding to that, returning organic matter to soil is one of the most promising climate change mitigation strategies (e.g. in the frame of the "4 per mille initiative") (Minasny et al. 2017).

As numerous reviews have addressed nutrient recovery from digestates (Lebuf et al. 2013; Romero Güiza et al. 2016; Vaneeckhaute et al. 2017; Monfet et al. 2018), our focus is to address the technical issues related to UC-AD digestates.

Agricultural valorization of digestates will be classified, in the following sections, into three product categories: N/P/K fertilizers (Sect. 5.1.1) and soil improvers & organo-mineral fertilizers (Sect. 5.1.2).

Table 4 Summary of agricultural valorization: value-added products from UC-AD digestates and qualitative considerations

Final product designations	Config.	TRL	Major product concerns, not including legislation[a]	References[b]
Liquid fraction, liquid phase, supernatant	Raw digestate mechanical separation	Commercial	- N-concentration → gaseous emissions, especially NH_3 and N_2O - Relative low nutrient concentrations- > short transportation -distances - Salinity - Chemical and organic contaminants - Pathogens - Market value can be < 0	Al Seadi et al. (2012), Nkoa (2014)
Concentrates, retentates	Pressure-driven membrane filtration of (pre-treated) LF	Commercial	Acidification needed to avoid N-loss thus N-depleted product	Vaneeckhaute et al. (2017)
(from membrane or evaporation processes)	(Vacuum)-evapoconcentration of LF or membrane retentates			
Adsorption products	Adsorption columns or batch reactions starting from LF	Not clear	Nutrient accessibility can be poor	Vaneeckhaute et al. (2017)
N/S solutions: Ammonium sulfate or ammonium nitrate	NH_3 from direct or indirect stripping processes (stripping columns, evaporators, dryers or composting)	Commercial	The resulting solution is often too acid and corrosive	Vaneeckhaute et al. (2017)
Struvite (magnesium ammonium phosphate, MAP)	Crystallization in raw digestate LF mainly	Commercial	- Nutrient accessibility can be low especially in alkaline soils - Heavy metal contents	Desmidt et al. (2015), Yetilmezsoy et al. (2017), Vaneeckhaute et al. (2017)
K-struvite	c.f. struvite	Not clear	c.f. struvite	Desmidt et al. (2015), Vaneeckhaute et al. (2017)
Ca-P, mainly calcium phosphate or hydroxyapatite	c.f. struvite	Commercial	c.f. struvite	Desmidt et al. (2015), Vaneeckhaute et al. (2017)
Incineration ashes	Combustion of dried digestate pellets	NA	No nitrogen P-accessibility tends to be poor	Ehmann and Lewandowski (2013), Christel et al. (2014)

Table 4 continued

Final product designations	Config.	TRL	Major product concerns, not including legislation[a]	References[b]
Microalgae and macrophyte biomass	Phototropic raceway pound with pre-treated LF	Mostly on pilot and demonstration	Simple use as fertilizer does not allow the economic feasibility of microalgae production. Biorefinery downstream allows several value-added products.	Mulbry et al. (2005), Uggetti et al. (2014), Pascual (2016), Xia and Murphy (2016)

Config. most common or most feasible configuration, *LF* liquid fraction, *NA* not available, *UC-AD* urban or centralized plants, *TRL* technology readiness level of product application

[a] See Appendix A in ESM for process limitations related to UC-AD digestates

[b] Prioritizing specific literature reviews on processes or products when available

The different options are summarized in Fig. 1 (N/P/K fertilizers) and Fig. 2 (soil improvers and organo-mineral fertilizers). Other agricultural products such as biopesticides are discussed in the "Industrial valorization" section.

According to studies from different countries, those are characteristics that the farmers are concerned when dealing with organic fertilizers and/or soil improvers (Tur Cardona et al. 2015; Dahlin et al. 2015; Case et al. 2017): nutrient accessibility (equivalency to traditional mineral fertilizers), consistency in the nutrient content, odor nuisances, capacity to enhance soil structure, salinity, pathogens, possible application with current available machinery and price (often free to farmers). Based on literature material, an effort has been made to address these points for the different products under the agriculture section.

5.1.1 N/P/K fertilizers

5.1.1.1 Liquid fraction from phase separation (dewatering) To begin with, a relatively simple dewatering process can be a tool to enhance digestate value. It produces a liquid fraction (LF) and a dewatered fraction also called "solid fraction" (SF). The most common phase separation equipment are screw presses and decanter centrifuges, either isolated or combined in this sequence (Guilayn et al. 2019b). Phases separation of digestates tends to concentrate nutrients into the LF and the organic matter into the SF, but the global mass balances are highly dependent on the separation technique whose choice depends on digestate characteristics thus AD input type (Guilayn et al. 2019b). Centrifuges present a much greater separation performance but are costly to operate and not adapted to digestates with big particles and long fibers (Guilayn et al. 2019b and internal industrial expertise). Globally, digestate LF present fertilizing properties equivalent/close to mineral fertilizers (Sigurnjak et al. 2017) and the SF is closer to organic amendments such as composts, but with a greater amount of nutrients (Tambone et al. 2015). The SF, as a product, will be discussed within the organic soil improvers Sect. 5.1.2.

In the case of certain separators, the separation of N from P can be a tool for a better nutrient management, following crop needs. N tends to concentrate into de liquid fraction, as TAN follows water. P tends to concentrate into the SF, as P is mostly present as or

Table 5 Summary of soil improvers and organic fertilizers for agricultural valorization: value-added products from digestates and qualitative considerations

Final product	Config.	Application	TRL	Major product concerns, not including legislation[a]	References[b]
Solid fraction fibers, cake[c]	SF issued from raw digestate mechanical separation	- Organic fertilizer - Litter bed - Culture support	Comm.	- C/N can be low - Inerts (stones, plastics, glass and metal parts) - Relative low nutrient concentrations- > short transportation distances - Not as stable as compost - Market value can be < 0	Teglia et al. (2010), Al Seadi et al. (2012)
(Enriched-) dried digestate	Dying of raw digestate or SF	- Organic fertilizer - Litter bed - Culture support	Comm.	- DM > 75–85% for to allowing stable long term storage - Bulk density > 300 kg/m^3 required for long-distance transportation (might require pelletizing to be achieved) - Acidification needed to avoid N-loss	Delfosse et al. (2011), Dahlin et al. (2015)
Compost	Composting digestate or SF with addition of bulking material	- Soil improver - Culture support	Comm.	- Market value can be close to 0 - Heavy metal contents - Inerts (stones, plastics, glass and metal parts)	Teglia et al. (2010), Dahlin et al. (2015)
Vermi-compost, vermicast	c.f. compost	- Organic fertilizer - Soil improver - Litter bed - Culture support	Comm.	- Heavy metals contents - Pathogens	Quintern and Morley (2017)
Biochar	Pyrolysis of dried digestate pellets	- Organic fertilizer - Soil improver	Comm.	- Nutrient accessibility can be low, decreasing with increasing temperatures - Currently too expensive for simple land spreading in agriculture	Christel et al. (2014), Al-Wabel et al. (2018)

Table 5 continued

Final product	Config.	Application	TRL	Major product concerns, not including legislation[a]	References[b]
Humic-like substances	Alkaline extraction from raw digestate, or composts, possibly followed by acidification and drying to isolate humic acids	- Soil improver - Biostimulant	Lab.	Research needed	Montoneri (2017)

Comm. commercial, *Config.* most common or most feasible configuration, *Lab.* laboratory conditions, *LF* liquid fraction, *NA* not available, *UC-AD* urban or centralized plants, *TRL* technology readiness level of product application

[a] See Appendix A in ESM for process limitations related to UC-AD digestates

[b] Prioritizing specific literature reviews on processes or products when available

[c] In literature, "fibers" are usually referred to as the solid fractions from agricultural digestates, while "cake" is mainly used to designate solid fractions from WWTP sludge digestate

adsorbed into particles, thus following DM distribution (Guilayn et al. 2019b, Hjorth et al. 2010). However, this N/P fractionation effect cannot be taken as a rule. It is highly depending on the matrix composition and separation performance (Guilayn et al. 2019b). Adding to that, the resulting LF present enhanced physical characteristics: from a slurry viscous digestate, one can produce a pumpable and injectable liquid fraction (Fuchs and Drosg 2013). More recently, a growing number of articles propose short-circuit urban farming to recycle urban waste digestates. For example, the LF can be diluted and used and applied to hydroponic systems for providing nutrients and even boosting plant yields (Krishnasamy et al. 2012; Stoknes et al. 2016; Antón et al. 2017; Fuldauer et al. 2018; Takemura et al. 2019).

Finally, phases separation is a common process before advanced post-treatments. These processes are oriented and more developed to the LF for two main reasons. Firstly, LF represents up to 90% of the absolute input mass (Guilayn et al. 2019b) and, as previously stated, it is where most of inorganic nutrients are distributed.

5.1.1.2 Membrane filtration From the separated LF, membrane filtration are fully commercial to further fractionate and concentrate the nutrient content of digestates until reuse (c.f. section 5.3) or disposal water is produced. They include microfiltration (0.1–2 µm), ultrafiltration (0.01–0.1 µm), nanofiltration (0.001–0.01 µm) and reverse osmosis (< 0.001 µm). However, they require low total suspended solids (TSS) concentrations to avoid fouling and damage (< 1% DM) (Frischmann 2012). Capital expenditures are high (up to 1.5 M€ for a 40 kt/y facility) as well as operational costs due to energy requirement, cleaning maintenances and membrane replacement (4 to 7 €/t). The investment on membrane filtration are normally limited to large facilities (> 10 to 15,000 t/y) with great local nutrient surpluses (Levasseur et al. 2017). Few full-scale/industrial pilot units treating digestates are reported in literature (Chiumenti et al. 2013b; Bolzonella et al. 2018; Adam et al. 2018).

5.1.1.3 Adsorption and ion exchange products A lot of attention has been dedicated to the selective separation of soluble nutrients through adsorption, mostly ammoniacal nitrogen and orthophosphates

Table 6 Summary of energy valorization: value-added products from UC-AD digestates and qualitative considerations

Final product	Config.	Application	TRL	Major product concerns, not including legislation[a]	References[b]
Dried pellets	Drying and pelletization of raw digestate or SF (mainly)	- Bio-fuel - Thermal conversion	Industrial pilot	High ashes content → low calorific value	Kratzeisen et al. (2010), Pedrazzi et al. (2015)
Bio-oil or bio-crude	- Pyrolysis of dried digestate pellets - Hydrothermal liquefaction of digestate	- Bio-fuel - Investigated to replace crude oil in cracking refineries	Industrial pilot	- Removal of tars - Poor quality for refining compared to crude oil	Balat et al. (2009), Yuste (2016)
Syngas	Pyrolysis or gasification of dried pellets	- Bio-fuel - Conversion to biomethanol, biodiesel - Fermentation to bioethanol or several other metabolites	Gasification: commercial	- Necessary gas conditioning, especially the removal of tars - Fermentation: low gas solubility	Balat et al. (2009)
Bio-methane	Post-treatment for digestate post-digestion or recirculation	Bio-fuel	Commercial	Minimum 35% methane in biogas needed for combustion in Stirling engines	Monlau et al. (2015), Sun et al. (2015)
Bio-ethanol	- Post-treatment for digestate fermentation - Syngas fermentation	Bio-fuel	Lab.	Research needed	Monlau et al. (2015)
Bio-hydrogen	- Post-treatment for digestate fermentation - Syngas purification	Bio-fuel	Lab.	Research needed	Uggetti et al. (2014)
Bio-diesel	- Microalgae harvesting and extraction - Syngas conversion	Bio-fuel	Industrial pilot	Research needed	Uggetti et al. (2014)

Config. most common or most feasible configuration, *Lab.* laboratory conditions, *SF* solid fraction, *UC-AD* urban or centralized AD plants, *TRL* technological readiness level of process and product application

[a] See Appendix A in ESM for process limitations related to UC-AD digestates

[b] Prioritizing specific literature reviews on processes or products when available

Table 7 Summary of industrial valorization: value-added products from UC-AD digestates and qualitative considerations

Final product	Config.	Application	TRL	Major product concerns, not including legislation[a]	References[b]
Reuse water	Membrane filtration or distillation after evaporation processes	Several (irrigation, livestock watering, industry)	Not clear	Salinity and suspended solids	Ayers et al. (1985), Chiumenti et al. (2013b), Adam et al. (2018)
Biopesticides	Raw digestate or SF as growing media after inoculation or intrinsic properties of digestates or digested compost	Plant protection	Lab.	Research needed	Raymond and Federici (2017), Cerda et al. (2019)
Duckweed or microalgae biomass	Phototropic raceway pound with pre-treated LF	Livestock feed	Not clear	Protein content	Uggetti et al. (2014), Vaneeckhaute et al. (2017)
Mushrooms	Composting digestate or SF with the addition of bulking material	Livestock feed	Not clear	R&D needed	Stoknes et al. (2013, 2016)
Earthworms	Vermi-composting	Livestock feed	Commercial	R&D needed	Edwards (1985), Quintern and Morley (2017)
Biochar (raw or activated)	Dried pellets pyrolysis or gasification	- Livestock feed additive - TAN adsorbant for litter beds - Replacing activated carbon for several purposes - Enhancement of biological processes (AD and composting)	Commercial (mostly wood biochar)	Biochar commercial production is still mainly derived from wood biomass	Schmidt (2012), Fagbohungbe et al. (2016), Wu et al. (2017), Hagemann et al. (2018)
Ashes	Incineration of dried pellets	Portland cement manufacturing (input in kilns) or cement additive	Not clear	R&D needed	Donatello and Cheeseman (2013)
Flame retardants	Struvite or	Wood composites and textile	NA	R&D needed	Yetilmezsoy et al. (2018), Guo et al. (2019)
Flame retardants	Ammonium sulfate	Wood and mulch protection	Theoretical possibility	R&D needed	George and Susott (1971), Hickman and Perry (1996)
Explosives	Ammonium nitrate from N-stripping processes	Mining and civil construction	Theoretical possibility	R&D needed	Meyer et al. (2007)
Humic-like substances	Raw digestate	- Biosurfactants - Bioplastic composite	Lab.	R&D needed	Montoneri (2017)

Config. most common or most feasible origin configuration, *Lab.* laboratory conditions, *LF* liquid fraction, *TRL* readiness level of process and product application

[a] See Appendix A in ESM for process limitations related to UC-AD digestates

[b] Prioritizing specific literature reviews on processes or products when available

adsorption. The concept consists on driving the effluent through adsorption columns, then recover the adsorbed nutrients. The most common adsorbents are natural zeolites, biochar, clays and resins (He et al. 2016). As some relatively low-cost adsorbents as zeolites and biochar are investigated, some studies propose the enriched adsorbent as a final fertilizing product after simple adsorption through batch reactions (Kizito et al. 2015). In a deep review of nutrient recovery options from digestates, Vaneeckhaute et al. (2017) pointed out that the recovery of ammoniacal nitrogen as N-rich zeolites is potentially the lowest-cost technology for N-recovery. This is not developed at full-scale and it is limited by the availability of the adsorbent at a low price and current limited performances. One of the main concerns around this topic is the plant availability (desorption) of the sorbed nutrients, which was not found within digestate studies. With ^{15}N labeled ammonia, Taghizadeh-Toosi et al. (2012) proved that ammonia adsorbed to wood biochar was accessible to plants.

Another low-cost adsorption strategy that has been investigated is an adsorption contact reaction followed by phase separation (Olga et al. 2014). However, it seems more appropriate to classify this strategy as a phase separation enhancement.

5.1.1.4 Evapo-concentration processes Concentrating LF, SF and membrane retentates can be a useful technique to reach fertilizer product categories (Guilayn et al. 2019a), while possibly complying to recognized ABP hygienization when thermal treatments are applied with temperatures above 70 °C and over > 1 h. LF and membrane retentates can be concentrated with evaporation to achieve up to 50–90% volume and mass reduction (Chiumenti et al. 2013a; Guercini et al. 2014). Vacuum-evaporation needs an input DM below 1 to 3% (Frischmann 2012; Levasseur et al. 2017). However, evaporators need the removal of large particles otherwise the fouling of heat exchangers will be a current operational problem (Vondra et al. 2018, internal industrial expertise).

In evaporation, the effect of ammoniacal nitrogen volatilization can be an option to recover N (next section). Countering this effect, another option is to acidify the input to pH 5 to 6, which may allow to

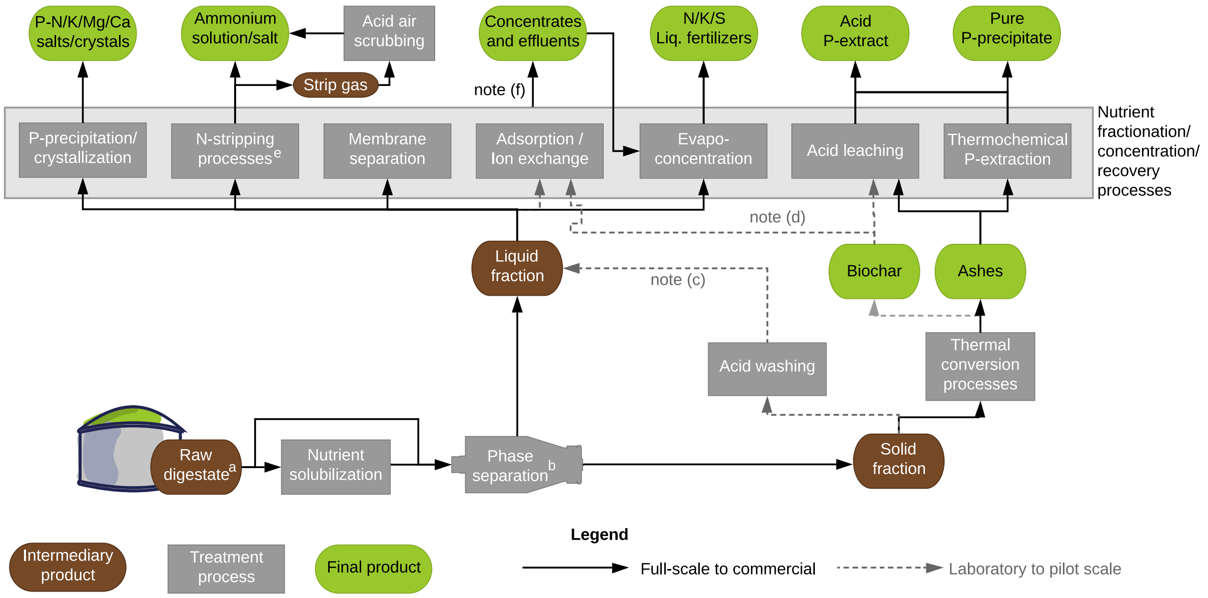

Fig. 1 Processes to produce N/P/K fertilizers from digestate. Major process inputs and outputs may be omitted. **a** Usually comprises a storage step. **b** Some plants or research might directly treat whole digestates, which depends mostly on suspended solids and rheology to match downstream equipment requirements. **c** The washing solution can be treated by most processes applicable to digestate LF (or mixed to it). **d** As the adsorbent. **e** N-stripping processes are here considered as those specifically designed for N-stripping (e.g. stripping towers), but also indirect processes such as thermal drying, evaporation and even composting. **f** concentrates and effluents from most of the previous processes

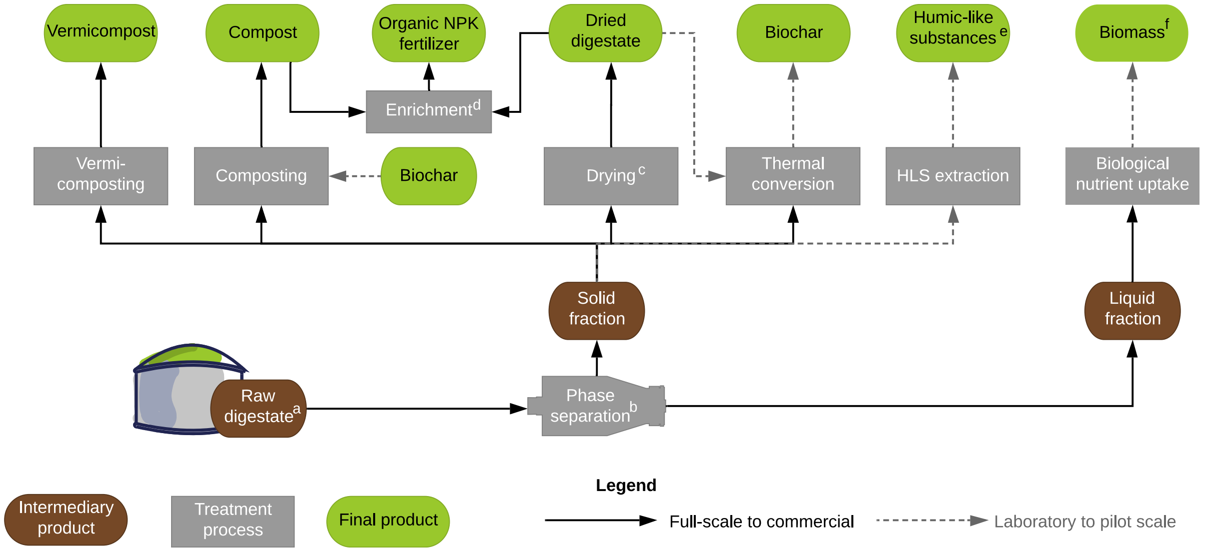

Fig. 2 Processes to produce soil improvers and organo-mineral fertilizers from digestates. Major process inputs and outputs may be omitted. **a** Usually comprises a storage step. **b** Some plants or research might directly treat whole digestates, which depends mostly on suspended solids and rheology to match downstream equipment requirements. **c** Thermal drying might include a pelletizing/granulation step downstream. **d** "Enrichment" refers to adding mineral nutrients (conventional or recovered from digestates) usually for matching specific formulations. **e** Humic-like substances are also applied as biostimulants, which must be differentiated from nutrient supply and soil amendment. **f** Most of publications are related to microalgae. As an agricultural product microalgae biomass is usually cited as a slow-release fertilizer

maintain more than 95% of the total N in the concentrated or dried product (Chiumenti et al. 2013a; Pantelopoulos et al. 2016).

For operators of UC-AD, simple digestate concentration processes such as phase separation and evaporation can be relatively low cost and robust solutions. However, they present a much smaller opportunity to create value and overcome the difficulties of reaching the fertilizer market from raw digestate. Advanced solutions such as membrane filtration can be used to further fractionate nutrients but will need economies in scale.

5.1.1.5 Nitrogen/sulfur solutions from ammoniacal nitrogen removal process Digestates present up to 80% of the nitrogen in digestates as ammoniacal nitrogen (Guilayn et al. 2019a) and an alkaline pH, which makes them particularly attractive for promoting N-stripping. Ammoniacal nitrogen is present in water as the equilibrium of two species: NH_3 and NH_4^+ (free ammonia and ammonium). N-stripping is achieved by favoring free ammonia in the equilibrium through pH and/or temperature increase while enhancing liquid/gas transfer. The temperature and pH increase tend both to promote hydrolysis of proteins, increasing ammoniacal nitrogen content, which might extend N-recovery efficiency beyond the original TAN content (Serna-Maza et al. 2015). In practice, alkalization is often performed with the addition of NaOH or KOH and/or through CO_2 stripping, which also enhances gas transfer.

Battista and Bolzonella (2019) conceived an innovative low-cost process based on solar energy. A greenhouse equipped with solar-powered fans promote digestate concentration ($> 60\%$ DM) and N-stripping process. The preliminary tests on OFMSW digestate resulted in over 95% stripping efficiency (TAN concentration reduction corrected for DM content) and about 40% NH_3 recovery (limited by the acid trap efficiency).

Many commercial solutions already exist. The main operational problem is scaling of stripping columns and corrosion (Hidalgo et al. 2015). Indeed, TSS must be low to avoid stripping column clogging. For this reason, stripping is often performed in the centrifuge liquid fraction or with screw press followed by an additional step as sieving or settling.

After N-stripping, N-recovery is performed through scrubbing in acidic solutions or water to produce ammonium sulfate, ammonium nitrate (Jamaludin et al. 2018) or ammonium water (Gasum 2016). Ammonium nitrate is a common fertilizer but usually submitted to stricter regulation due to its explosive ignition reaction (Lallanilla 2013). In vapor stripping, condensation can be used to form directly ammonia in water. Recent research has demonstrated the interest of using alternative and safer acids such as citric acid to produce ammonium citrate (Jamaludin et al. 2018).

Alternatively, hydrophobic membrane contactors can be placed directly into solution (raw digestate or liquid fraction) either in the operating digester (Lauterböck et al. 2012) or in a dedicated removal tank (Vanotti et al. 2017).

In any case, final ammonium sulfate solution, the most common product, is acidic and can present at up to 6–10% of TN which is diluted compared to commercial fertilizers (Vaneeckhaute et al. 2016; Bolzonella et al. 2018). It can be difficult to commercialize.

5.1.1.6 Precipitation/crystallization P-products The studies on precipitating nutrients from digestates are normally oriented to P-precipitation. This is due to unique features of P among the major macro-nutrients: (1) there is no volatile gaseous form of P in the global P cycle; (2) global production of P-fertilizers are concentrated into a very few counties and (3) it is estimated that we can reach a peak in P production still in this century. Additionally, as the quality of remaining phosphate rocks is rapidly degrading, the concentration of impurities such as heavy metals is increasing (Desmidt et al. 2015).

The most cited precipitation product that can be produced from digestates is magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$), known simply as struvite. Several commercial processes worldwide allow an efficient recovery of high-quality struvite crystals from phosphate rich effluents, including: PHOSPAQ^{TM} (Paques), AirPrex$^{®}$ (CentriSys), PEARL$^{®}$ (Ostara), NuReSys$^{®}$ (NuReSys), Phosphogreen^{TM} (SUEZ), among other. Other include K-struvite ($KMgPO_4 \cdot 6H_2O$), calcium phosphate and hydroxyapatite (Monballiu et al. 2019). Ca-P products seems to be considered less valuable due to an overall lower P-accessibility to plants (Cabeza et al. 2011).

High consumption of chemicals is one of the main disadvantages of (K-)struvite crystallization. Indeed, pH must be increased from about 7–8 to 9–10. As in N-stripping; alkali requirements can be reduced through CO_2 stripping (Fattah et al. 2010). Since the formation of struvite is a proton releasing reaction, constant addition of an alkali is necessary to keep a constant high pH. Additionally, from both economic and environmental perspectives, a great limitation of (K-)struvite recovery is the necessary source of Mg, which is also listed as critical raw material by the EU (European Comission 2011).

Any choice of a particular process and optimization criteria for struvite recovery should be assessed case-by-case. They have been extensively studied and reviewed (Kataki et al. 2016; Tao et al. 2016; Kataki and Baruah 2018). In brief, pH, temperature and the molar ratios of Mg^{2+}, NH_4^+, PO_4^{3-} and competitive ions such as Ca^{2+} must be carefully controlled to promote struvite supersaturation levels. Adding to that, to produce a pure product with proper crystal growth, TSS must be low (< 1 g/L) and the possible disturbing effect of dissolved OM on ionic dissociation must be considered (Capdevielle et al. 2015; Aleta et al. 2018). According to several technology providers, struvite feasibility need orthophosphate minimal concentration threshold of 50 to 70 mg P-PO_4^{3-}/L (P-REX 2017; SUEZ 2017b; PAQUES 2019), which is largely overpassed by most of UC-AD digestate liquid fractions (Akhiar et al. 2017). It must be noticed that either in R&D a full-scale operation, many large/pilot-scale producers of "struvite" do not actually have a precise control of struvite supersaturation and quality control (internal expertise). This fact can jeopardize struvite image, for example, if the product contains large amounts of Ca-P and it is tested for agricultural use while referred as "struvite".

Full-scale P-recovery as struvite is still mostly limited to WWTP, including the previously cited commercial processes. Struvite from sewage sludge digestate has been demonstrated to have very low concentrations of heavy metals and organic micropollutants (Uysal et al. 2010). The WWTP sector is promising because its economic feasibility does not rely on struvite sales. In WWTP, struvite crystallization reduces spontaneous incrustation problems and P-removal operational costs. In traditional WWTP configurations, about 20% of the P input load can be due to the return of digestate's centrate to head of the plant (Evans 2007).

Full-scale recovery of soluble phosphates through P-crystallization can achieve over 90% efficiency (Desmidt et al. 2015). Thus, nowadays, the limiting step for achieving great total recovery efficiencies relies in a solubilization step prior to crystallization. For this reason, several pre-treatments are proposed and the most addressed seems to be chemical/biological acidification (Braak et al. 2015; Szogi and Vanotti 2015; Vaneeckhaute et al. 2016; Piveteau et al. 2017; Guilayn et al. 2017). UC-AD digestates from co-digestion plants accepting high charges of effluents with high alkalinity and calcium content tend to produce low-quality struvite. Two examples of such co-substrates are pig slurry (Piveteau et al. 2017) and dairy manure (Tao et al. 2016). In such cases, the necessary steps of P dissolution and the following removal/chelation of competitive ions can thus hinder struvite recovery feasibility. The same applies for sewage sludge from WWTP with coagulation steps based on aluminum. They might contain Al-P salts that are highly difficult to solubilize (Braak et al. 2015).

Oliveira et al. (2018) are possibly the first to evaluate struvite recovery from OFMSW digestate. The procedure included dilution with distilled water or 1.1 M nitric acid (2.5 S/L ratio), followed by electrodialysis for recovering the negatively charged phosphates in the anode side and, finally, struvite precipitation. Under the best conditions, they have achieved 43% total P extraction and up to 100% precipitation.

Struvite is a slow-release mineral fertilizer. Research seems inconsistent about whether struvite needs an acidic soil or not to be equivalent or even better than conventional mineral fertilizers (Ackerman et al. 2013; Talboys et al. 2016). This controversy might reflect a product quality heterogeneity among studies.

Struvite selling prices are related from 40 up to $>$ 1400 €/t (Lebuf et al. 2012; Desmidt et al. 2015). In most of the cases, struvite price is considered below its nutrient market value calculated to be around 690 €/t in 2012 (Desmidt et al. 2015). The study of Yetilmezsoy et al. (2017), based on laboratory conditions, estimated a minimum sale price of 560 €/ton for a 6-year payback.

Vivianite ($Fe(II)_3(PO4)2\bullet 8H_2O$) recovery is a new promising solution whose R&D is being led by researchers of the Delft University of Technology. As iron coagulants are widely applied in WWTP, the reducing environment of AD favors the formation of Fe(II) and, more particularly, it can be bound with phosphates as vivianite. Prot et al. (2019) have demonstrated the feasibility of a magnetic separation system for recovering vivianite from sewage sludge digestate.

5.1.1.7 Ashes and biochar as source of mineral nutrients Thermal conversion processes such as incineration (combustion) or pyrolysis can be effective options for digestate valorization. Some definitions and operational aspects regarding thermal conversion of digestates will be discussed later in the section “Energy valorization”.

Ashes are the product resulting from incineration and char (referred as biochar when produced from renewable biomass) are the solid final products from carbonization processes such as pyrolysis. Biochars though can present itself a high content of measured ashes (up to 40–60%). The ash content is increased with increasing process temperatures (Neumann et al. 2016; Opatokun et al. 2017).

Thermal conversion processes concentrate the elements that are non-volatile at the applied temperature, that may reach more than 1000 °C for combustion. For this reason, P and K in digestate ashes can be as high as 25% and 15%, respectively (Kratzeisen et al. 2010). However, several studies indicate that the P in ashes present poor accessibility to plants. Cabeza et al. (2011) studied heavy metal depleted sewage sludge ashes and concluded that they are not effective P-fertilizers due to low P accessibility.

Biochar tends to present a better nutrient bioaccessibility than ashes. Christel et al. (2014) observed that after pyrolysis at $>$ 700 °C the biochar P content was not extractable with water. However, at lower temperatures biochar had more water-extractable P than ashes produced at the same combustion temperatures.

Commercial processes are already in place to recover high-grade phosphates from ashes through chemical or thermochemical extraction but are still not economically interesting (Vaneeckhaute et al. 2017).

5.1.2 Soil improvers and organo-mineral fertilizers

5.1.2.1 Biomass harvested from digestates as slow-release organo-mineral fertilizers The nutrients from digestates can be recovered through several biomass harvesting processes. As the nutrients are trapped within the biomass, these products are commonly referred as slow-release fertilizers

(Mulbry et al. 2005). The most common biomasses that can be produced from digestate nutrients are duckweed and microalgae. They are the only category of products under the "Soil improvers and organo-mineral fertilizers" section that can be originated from the liquid fraction of digestates. They greatly differ as the organic matter content is produced from photosynthesis rather than originated from the digestate.

Microalgae have been used in engineered ponds to produce biomass either in photoautotrophic, heterotrophic or mixotrophic systems (Xia and Murphy 2016). Given the species and environmental conditions, they can be used to selectively produce proteins (up to 60% in biomass), lipids (up to 60%), carbohydrates (up to 30%) and a large range of possible valuable and unique compounds (Uggetti et al. 2014). For UC-AD digestates, microalgae biomass concentration can reach up to 3 g/L and productivity up to 0.6 g/L .d (Xia and Murphy 2016).

Microalgae have been successfully harvested from digestate liquid fractions, but digestates present a series of negative effects that may jeopardize microalgae growth. The most recurrent are high turbidity and ammonia toxicity (Xia and Murphy 2016). Because of it, most of the studies use diluted digestates at ratios ranging from around 1:5 to 1:50 (Erkelens et al. 2014; Marcilhac et al. 2014, 2015; Koutra et al. 2017). No consensus on TAN inhibition concentrations could be found since it is highly depending on microalgae species and ionic strength. Other than dilution, recurrent considered treatments are N-stripping (Marazzi et al. 2017), adsorption (Marazzi et al. 2017) and coagulation (Chen et al. 2012). As summarized by Uggetti et al. (2014), other potential problems of digestates are the presence of heavy metals and OMP (potential biological toxicity), competitive microorganisms and pathogens, VFA (stimulation of competing heterotrophic bacteria) and long-chain fatty acids (potential toxicity).

Full-scale economic feasibility of microalgae still depends on strong price reduction in each step of the production chain, which also include the source of nutrients and water. Both can be replaced by digestates. The application of microalgae biomass as slow-release fertilizer can be efficient (Mulbry et al. 2005; Coppens et al. 2016), but the economic feasibility of microalgae harvesting will depend on the establishment of true biorefinery systems allowing to profit from the whole biochemistry of the biomass. Some insights on microalgae biorefinery are provided in "Energy valorization" and "Other industrial valorization possibilities".

Duckweed are small macrophytes from different species classified as the *Araceae* subfamily *Lemnoideae*. They have indeed been used for decades for wastewater treatment (Alaerts et al. 1996). In Europe, the LEMNA project claimed to build the first full-scale nutrient recovery plant based on duckweed (Pascual 2016). The plant will operate on pig slurry digestates and the output biomass is intended to be used as fertilizer and for feed (high protein content).

There is little research on duckweed applications for UC-AD digestates. This is probably because duckweed have extremely high land requirement. For example, given a maximum potential to remove 600 kg N/ha (Leng 1999), a small/medium-sized urban AD facility producing only 1 kt/y of digestate with a 10 gN/kg concentration would require at least 15 ha of duckweed ponds.

5.1.2.2 Solid fraction from phase separation (dewatering) The digestate dewatered fraction, or solid fraction (SF) of digestates is obtained from the same phase separation processes described previously for the LF (Sect. 5.1.1.1). As for the LF, the separation process enhances the biochemical and physical characteristics of the SF compared to the whole digestate. Indeed, after separation, an initially liquid to viscous digestates can be turned into a well-stackable product, presenting better handling properties, lower transportation costs and with a better bulking capacity for returning to soil (Fuchs and Drosg 2013). While the LF present properties closer to mineral fertilizers (Sigurnjak et al. 2017), the SF is more similar to organic amendments such as composts, but with a greater amount of nutrients (Tambone et al. 2015).

The literature results seem controversial about SF effectiveness as an amendment product compared to compost. For example, Tambone et al. (2015) concluded that digestate SF (from pig slurry, energy crops and agro-industrial residues) were already stable and further composting did not enhance remarkably its characteristics. Teglia et al. (2011) evaluated SF from WWTP sludge and food processing waste digestates. The authors concluded that the products were not stable and recommended composting as a post-treatment.

Again, phase separation is a key-enabling technology prior to OM valorization. In the case of SF, composting, thermal drying and thermal conversion are usually applied or investigated as following processes. They will be discussed and described in the following sections.

5.1.2.3 Dried digestates Dried digestates are produced from thermal drying process which usually require a DM concentration input higherthan 15%. To achieve this level, UC-AD digestates from wet AD processes might need a previous phase separation step. Some plants apply a recirculation of the dried product, which implies a loss of equipment capacity (Frischmann 2012).

As in evaporation process, thermal drying promotes ammoniacal nitrogen volatilization, which can be seen as an opportunity to recover N. In the opposite, in order to maintain up to 95% of the total N in the dried product, digestates can be previously acidified (Pantelopoulos et al. 2016).

Dried UC-AD digestates can be nutrient-rich, but it seems that there is no consensus for their applicability as organic soil amendments (stable OM). For example, Tambone et al. (2010) demonstrated that a dried WWTP sludge digestate presented much inferior amendment properties than composts. However, in the same study, the authors indicated that other different raw digestates (including OFMSW, animal slurry and FAI among inputs) were similar to composts as amendments, which should be expected to be maintained after thermal drying. Indeed, OM stability seems to be the more controversial digestate quality parameter around digestates (Alburquerque et al. 2012). It reflects the lack of consensus on the indicator to be used thus a lack of homogenized data for performing conclusive meta-analysis.

Many types of thermal dryers are available in the market. The most common for digestate treatment are rotary, disk and belt dryers. The latest have been the more indicated to digestates presenting large particles, which can be the case of UC-AD digestates (Arlabosse et al. 2010). All of them require high thermal energy, relevant capital investments (600 k€ for a 10 kt/y facility) and intensive air treatment for safe air disposal and to avoid atmosphere ignition. Indeed, an explosive atmosphere due to organic dust can be a constant threat for operators, especially in the case of mixing-drying equipment such as rotary driers (internal expertise). In some cases, the fire risk can be aggravated by intensive self-heating of dried digestate (internal expertise).

The quantity of digestate to be dried and the final moisture depend on the available heat from cogeneration. Dried products should achieve DM > 75–85% for allowing long term stability (VALDIPRO 2015; Dahlin et al. 2015). Energy consumption for thermal drying ranges from 1.2 to 1.3 kWh/kg of removed water. In biogas plants, the heat from cogenerators is normally not enough to dry the whole digestate flow. It usually allows drying less than half of the whole digestate flow (Bolzonella et al. 2018). In addition, raw dried digestate may present a low bulk weight (around 100 kg/m^3) making long-distance transportation costly (Dahlin et al. 2015). Long-distance transportation is indicated to require more than 300 kg/m^3. Pelletization/granulation can be used to increase dried digestate bulk weight up to 600 kg/m^3 (Dahlin et al. 2015). Pelletization/granulation also enhances handling ease of final product, making it more easily applicable with equipment designed for conventional mineral fertilizers. Nagy et al. (2018) performed an economic evaluation on the production of dried pellets and concluded that the production costs would difficultly justify a simple use in agriculture. In such cases, the economics will drive digestate dried pellets to be seen as an energy product more than a fertilizer.

5.1.2.4 Composts Composting is an aerobic process that decomposes the biodegradable fraction of the OM into CO_2 and microbial biomass. Digestates composting is a developed technology in agricultural, urban and industrial AD plants. It is commonly performed either in situ or ex situ. Due to the more favorable thermodynamics, the OM decomposition in the composting aerobic conditions can achieve greater extents than AD. The composted material is then cured (maturation phase) and grinded. The main operational parameters of industrial composting are the duration (from 3 weeks up to 1 or 2 months), C/N ratio (20 to 40), temperature, moisture (ideally around 50%) and aeration method and intensity (Epstein 2011). Composting is an intensive self-heating process. Temperatures can reach more than 70 °C within the piles. For this reason, under certain criteria, composting is authorized in the EU as a hygienization process for digestates containing Category 2 and 3 ABP (Amlinger and Blytt 2013).

For wet digestates, composting is conventionally performed on the SF after dewatering. Additionally, raw digestates or SF need, usually, co-substrates for one or more of these reasons: (i) lack of residual biodegradable OM allowing the bioprocess requirements for an effective temperature increase, (ii) digestates are normally too wet for composting and/or not sufficiently physically structured when dewatered, and (iii) C/N of digestates is too low for composting (Tremier et al. 2014; Zeng et al. 2014, 2015). Common co-substrates and bulking agents are green waste, wood chips, sawdust and recirculated compost grinding refuse (Epstein 2011, internal industrial expertise). An emerging composting technique useful for liquid residues such as UC-AD digestates from wet-AD consists of spreading and constantly turning the digestate into a saturated support/bulking material (Chiumenti 2015; Levasseur et al. 2017).

Due to heating, great water loss during composting represent a volume and mass reduction thus an advantage in terms of transportation costs. Compare to the compost feed, a massic reduction from about 30 to 50% can be achieved (Levasseur et al. 2017). However, for UC-AD, the need for co-substrates represent a substantial increase of final volume to be managed in comparison to initial digestate volume.

Moreover, eventual physical impurities and trace metals present in UC-AD digestates are not removed during the composting process. These contaminants can either be concentrated or "diluted" depending on water loss and co-substrate quantity and quality. It is consensual, though, that the bioaccessibility and solubility of heavy metals are highly limited with composting due to strong bonding to the compost organic matter matrix (Smith 2009).

UC-AD operators must consider that digestate composting is not always a low-cost process and that compost price is commonly low outside the retail marketing. According to the survey of Dahlin et al. (2015), composts are sold to agriculture from 0 to 7 €/t, which is far below other products that can be recovered from digestate. Prices up to 80 €/t could be achieved through the application of fine processing for meeting the horticultural market.

Due to longer RT and the co-substrate volume, the composting installations need as much as 4 times the surface of AD installations (internal industrial expertise). In the case of UC-AD land price is normally higher in urban/industrial areas than agricultural ones. Moreover, in urban areas, the composting facility are usually required to be enclosed. It is also the case in some countries as UK and Ireland for ABP processing regardless the zone where the installations are localized (Amlinger and Blytt 2013). In such cases, ventilation for air collection in a high surface and subsequent air treatment can be extremely costly. Adding to that, several composting regulators require industrial composters to apply forced aeration and/or mechanical turning such as the U.S. Environmental Protection Agency (Epstein 2011), which adds significant operational costs compared to the low-cost convective aeration composting.

5.1.2.5 Vermicomposts/Vermicast Although less developed than simple composting, digestate *vermicomposting* is already performed at industrial scale. Only in New Zealand, 200,000 tons of dewatered sludge from municipalities and industries was valorized through vermicomposting (Quintern and Morley 2017). Compared to composting, the main operational advantages are the promotion of aeration, turning and process acceleration by the earthworms. Additionally, it is reported to achieve greater volume reduction (65 to 85%) (Quintern and Morley 2017).

Digestate vermicomposting was demonstrated to achieve great pathogen reduction and compliance with spreading standards (Rajpal et al. 2014). However, it is often preceded by a short thermophilic composting phase. The composting phase allows high temperature securing hygienization and desactivation of weed seeds. Moreover, pre-composting allows the removal of ammoniacal nitrogen, a highly toxic compound to earthworms (Krishnasamy et al. 2014). As for composting, structuration is also important so digestates are usually vermicomposted with a bulking agent. With pre-composting, Krishnasamy et al. (2014) experimented food waste digestate vermicomposting and get the best results with 7:3 digestate:sawdust in 75 days. Tesfamichael and Stoknes (2017) reported a sample of a commercial vermicompost produced from SF of food waste digestate only (no co-substrate). The product is related to be high-quality: stable, structured and nitrified.

Vermicomposting is an emerging alternative for digestate composting allowing to add more value to digestates. However, little information is available in literature. The high ammoniacal nitrogen content of digestates is fatal to earthworms which can be an important cause of process disturbances. If pre-treated by composting, most of the exposed composting drawbacks need to be considered.

5.1.2.6 Biochar Biochars contain a significant amount of transformed and stabilized organic matter. It is seen much more as a soil improver than a mineral fertilizer. A food waste digestate biochar, for example, can present up to 61% volatile solids, 45% C and 6% N (Opatokun et al. 2016). Digestate biochars present a relatively high specific surface, pore volume and polar functional groups that confer it adsorbent properties (Inyang et al. 2012; Stefaniuk and Oleszczuk 2015; Opatokun et al. 2016; Wongrod et al. 2018; Jiang et al. 2018). Biochars are considered an emerging organic amendment and slow-release fertilizer with a large list of advantages to the soil and plants. These advantage include soil structuration, the reduction of greenhouse gases emissions, adsorption of contaminants and many of the benefits attributed to soil humus (Tan et al. 2017). The scientific literature is richer in biochars from wood and other carbon-rich residues such as rice straw (Tan et al. 2017). Nevertheless, the agronomic interest of biochars originated from different types of digestate, including industrial food waste had already been demonstrated (Opatokun et al. 2017).

Integrate thermal conversion with AD is a promising alternative. It allows enhancing the digestion and the AD plant energy efficiency while increasing the digestate value-chain. The produced digestate biochar can be used to increase soil fertility while promoting carbon sequestration for fighting climate change.

It is indicated though, that current biochar prices are over-expensive for field application. Nowadays, the main large crops present specific costs of over 1000 €/ha (Desbois and Legris 2007). The recommended biochar doses from 5 to more than 100 t/ha (Major 2010; Someus 2015) are extremely high considering biochar prices reported from 500 to over 1000 €/t (Jirka and Tomlinson 2014). The application frequency is not annual, but the farmers must calculate indirect long-term fertilization benefits thus long term payback periods. From an economic point-of-view, many other applications of biochars can be prioritized (Schmidt 2012), some of them being addressed in this review in next Sect. 5.3.

5.1.2.7 Humic-like substances for soil amendment or biostimulation Humic substances are complex organic compounds abundant in nature originated from the decomposition and reorganization of organic matter. This domain is still of a great interest pushed by the recognized importance of humic substances for understanding the fertility of natural soils, but also due to its remarkable applications on agriculture or horticulture as soil conditioners and biostimulants (Muscolo et al. 2013). Indeed, depletion of soil OM is a worldwide problem resulting from intensive agricultural systems with low reintroduction of stable organic matter. The benefits of adding humic-like substances (HLS) to soil are widely recognized, especially in the case of OM-poor soils (Lyons and Genc 2016).

For commercial purposes, HLS are commonly extracted from natural fossil sources such as leonardite and peat. They can be found, similarly, in anthropogenic organic material such as (vermi-)composts, digestates and landfill leachates (Atiyeh et al. 2002; Eyheraguibel et al. 2008; Morard et al. 2011; Calvo et al. 2014; Fascella et al. 2015, 2018; Silva and Brás 2016; Palumbo et al. 2018). Digestates from UC-AD have been successfully applied for the extraction of a pool of soluble organic compounds referred to as HLS, "soluble biopolymers", "biobased organic substances", "biowaste derived soluble substances", among other variants. To maximize the extraction of HLS from digestates, the digestate must undergo a strong alkaline treatment allowing to solubilize the humic-like acids (Salati et al. 2011; Fascella et al. 2015; Prevot et al. 2015; Montoneri 2017).

In one hand, the intrinsic lack of standards for the quality of HLS is one of the major problems of this sector for a wider agriculture adoption. In the other hand, it can be an opportunity for upcycling complex organic matrices such as digestates from UC-AD. For agriculture, another problem is the lack of consensus on application doses. The current doses recommended by product furnishers can be ineffective (Lyons and Genc 2016).

If doses are uncertain for conventional fossil HS, the scenario is even blurrier considering the small literature around digestate HLS as biostimulants. To the best of our knowledge, only Guilayn et al. (2020) compared HLS from different digestates (sludge and manure) at different doses for biostimulation.

Extraction costs of HLS from UC-AD digestates and other substrates were estimated from 100 to 500 USD/t in the frame of the BioChemEnergy project (Montoneri et al. 2011). As a reference, global wholesale internet prices of soluble dried humic acids extracted from leonardite range from 100 up to 1300 USD/t.

5.1.3 *Conclusions on agricultural valorization*

In UC-AD, effective fertilizers and soil improvers can be produced from digestates. Phase separation is both a valorization process itself and a key technology enabling more advanced treatments. From the LF, membrane filtration, evaporation, N-stripping and P-precipitation are full-scale feasible options allowing producing value-added products. From the SF, the most used processes are composting and thermal drying. Adding to that, thermal conversion for biochar production and HLS extraction seems promising solutions that need to be further investigated for product comprehension, process optimization and cost reduction.

Adding to the technical challenges, some important bottlenecks hamper the development of the agricultural valorization of UC-AD digestates: (i) in the case of non-source-separated AD feedstock, marketing of fertilizing products are usually more limited by regulatory frameworks; (ii) in the conventional agriculture, nutrient value depends on highly volatile global prices of N, P and K; (iii) there is still no clear market value to be associated for the OM content and quality; (iv) the presence of inert impurities separation (e.g. glass and plastics) in the case of poor source/post-separation can be a particular problem of UC-AD digestates.

5.2 Energy valorization

The following sections address the valorization of UC-AD digestates as energy products. Figure 3 present the most recurrent pathways in scientific literature.

5.2.1 *Biofuels from thermal conversion processes*

The European Biogas Association positioned itself against the promotion of digestate combustion mostly due to the interruption of the carbon cycle and the loss of nitrogen during drying (European Biogas Association 2013). However, as previously discussed, the nitrogen can be recovered after acid scrubbing and the waste treatment service itself (removing pollution) must not be neglected. The innocuity of digestates depend above all on the innocuity of the AD feedstock. Some organic waste innocuity will be a concern until the civilians and the production sectors do not provide safe residual streams and/or an effective waste source-separation. As this can take several decades, combustion and thermal conversion processes in such cases can be effective to destroy pollutants while recovering energy and biofuels.

Thermal conversion processes are usually classified as combustion, pyrolysis and gasification. Other variants include torrefaction, hydrothermal carbonization, vapothermal carbonization and hydrothermal liquefaction. All these processes share the principle of promoting an irreversible thermal decomposition of the organic matter, followed by different reactions of reorganization. They differ in terms of temperature range, oxygen level, pressure and water/vapor presence to drive the quality and yield of the different valuable products: heat and power, syngas, bio-oil and biochar. Beyond energy valorization, syngas and bio-oil (or biocrude) are investigated as inputs for complex biorefineries, based either on fermentation or thermochemical fractioning (Balat et al. 2009).

It must be highlighted that digestate biochar contain great amount of ashes (up to 40–60%), which is increased by higher process temperatures (Neumann et al. 2016; Opatokun et al. 2017). Indeed, digestate biochar from digestates is difficultly an energy product replacing charcoal. To illustrate it, solid fossil fuels present a gross calorific value (GCV) of 22–37 MJ/kg, while dry hardwood has a GCV of 18–19 MJ/kg (Osborn 1985). Peng et al. (2020) summarized biochar from different digestates and thermal conversion processes. They present GCV usually below 10 MJ/kg and only two values exceeding 25 MJ/kg (both from agricultural inputs). Digestate biochar applications in agriculture and industry as soil improver, adsorbent and bioprocess enhancer should be more suitable than energy valorizaiton.

Conventional combustion, pyrolysis and gasification processes are performed in dried digestate, usually pelletized (Opatokun et al. 2014; Wiśniewski et al. 2015; Gusiatin et al. 2016; Morero et al. 2017). The more recent hydrothermal conversion processes are similar to pyrolysis as they occur in the absence of oxygen. For wet biomasses as digestates, they present the important advantage of not requiring the intensive energy consuming drying pre-treatment (Mumme et al. 2011; Funke et al. 2013; Reza et al. 2016). Among all these processes, only gasification was found to be fully commercial on UC-AD digestates as several gasification installations are operating in the US for treating sewage sludge digestates (U.S. EPA 2012).

From a climate change and carbon cycle point-of-view, thermal conversion can be compared to AD but in a greater extent: a greater fraction of the carbon is converted to fuels that will eventually be combusted, but the remaining carbon is much more stable than previously. In soil, biochar is indicated to be stable for thousands of years, greatly contributing to the soil carbon sequestration strategy for fighting climate change (Vaccari et al. 2011).

5.2.2 *Biofuels from fermentation processes*

Lignocellulosic biomass is composed of carbohydrate polymers (cellulose and hemicellulose) structured in lignin (a phenolic polymer). AD alone is not able to break lignocellulose structure in order to access and monomerize its sugars. For this reason, the literature is abundant in pre-treatments and post-treatment to enhance methane yields. Adding to that, a lot of attention has been dedicated for valorizing the digestate residual OM as biofuels through fermentation.

In scientific literature, after several types of digestate post-treatment, two distinct approaches are commonly found to further produce fermentation biofuels from digestate: recirculation/post-digestion for biomethane production or alcoholic fermentation. These two options have been demonstrated to be interesting for agricultural digestates. These treatments are often alkaline, enzymatic or thermochemical (Monlau et al. 2015). Recently, Brémond et al. (2020) explored fungal treatment and had promising results. However, UC-AD digestates tend to present much lesser fibrous contents. As observed in Table 3, most of them are below 30% (hemicellulose plus cellulose, % of OM) while agricultural digestates can reach more than 60%. Unless the UC-AD receives important amounts of recalcitrant fibrous material, this valorization pathway tends to be ineffective. To integrate non-agricultural AD with the production of fermentation metabolites from non-fibrous residues, the best-known configuration is to perform a two-step process where intermediary fermentation products such as hydrogen and VFAs are recovered prior to the biogas-producing methanogenic reactor (Capson-Tojo et al. 2016).

Another approach linking digestate to fermentation biofuels is the use of digestate LF as a source of moisture and nutrients for fermentation, which will consist more of a nutrient recovery strategy rather than digestate OM valorization (Zhang et al. 2010; Bashiri et al. 2016).

5.2.3 *Biofuels from harvested biomass*

Biodiesel is produced from the transesterification of vegetable or animal lipids, oil and fats with an alcohol under the presence of a catalyzer. From UC-AD digestates, the best approach for producing biodiesel seems to be the production of lipid-accumulating biomass such as microalgae (Uggetti et al. 2014). Operational aspects and bottlenecks on microalgae harvesting have been previously discussed (Sect. 5.1.2.1). According to Shalaby (2014), algae can produce 30 time more lipids and oils than oilseed crops in terms of footprint. Uggetti et al. (2014) reported a lipid content between up to 60% of the biomass. Other than lipids to biodiesel, carbohydrate-rich microalgae have been studied for producing bioethanol through fermentation.Additionally, microalgae biomass can be used as inputs for the thermal conversion processes discussed above (Uggetti et al. 2014).

5.2.4 *Conclusion of energy valorization*

UC-AD digestates energy valorization via direct fermentative processes seems unlikely. Differently than agricultural digestates, UC-AD digestates tend to present a low content of lignocellulosic fibers that could be post-treated to release carbohydrates. Using the LF as a nutrient source for fermentation or to harvest biomass is possible but it does not consist of valorizing the energy contained in the digestate's residual OM.

Thermal conversion processes represent a huge potential to produce biofuels (and far beyond) from UC-AD digestates, regardless the presence of impurities and contaminants. Thermal conversion can enhance AD thermal efficiency but, from an energetic point-of-view, no discussion is provided about the

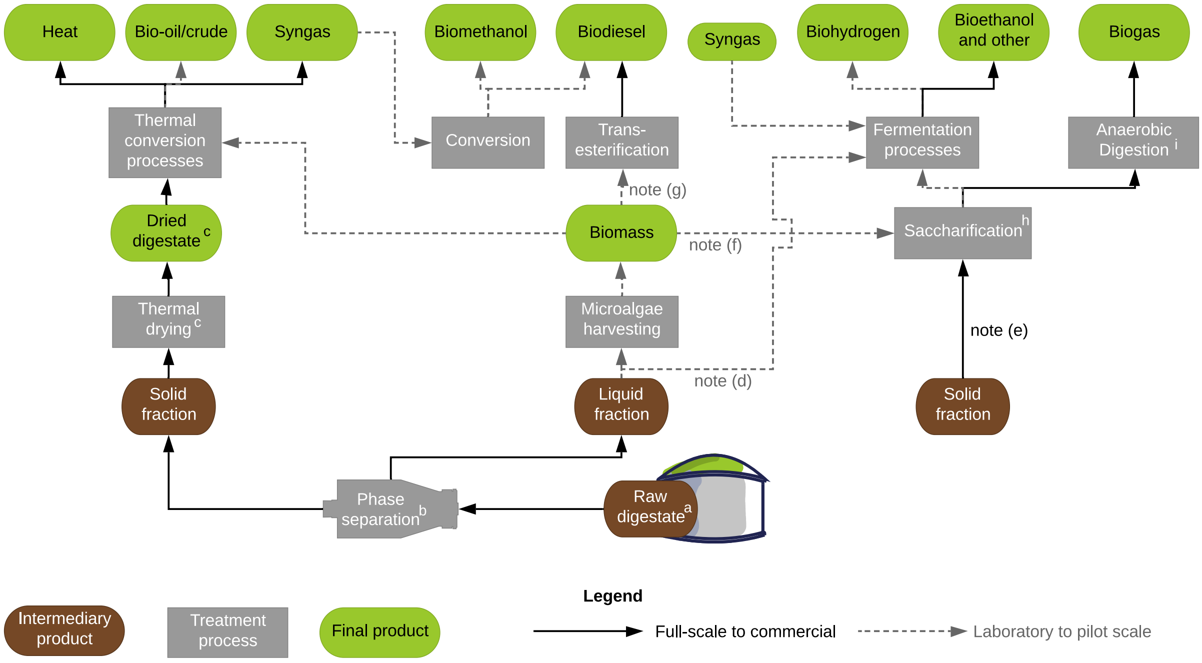

Fig. 3 Biofuels from digestate. Major process inputs and outputs may be omitted. **a** Usually comprises a storage step. **b** Some plants or research might directly treat whole digestates, which depends mostly on suspended solids and rheology to match downstream equipment requirements. **c** Thermal drying might include a pelletizing/granulation step downstream. **d** As the culture medium (source of nutrients and moisture). **e** If rich in residual fibers. **f** If rich in carbohydrates. **g** If rich in lipids. **h** Saccharification is referring to post-treatments aiming to release the carbohydrates from the structured organic matter. **i** Through a post-digester or simply recirculation

interest of including the AD step if the thermal conversion system is conceived from the beginning (Funke et al. 2013; Reza et al. 2016). Additionally, there is a lack of experience feedback when it comes to thermal conversion of digestates. Low development can be linked to the fact that thermal conversion processes require very high capital expenditures and an specific engineering expertise for operating machinery under high temperature and/or pressure.

5.3 Other industrial valorization possibilities

Little attention has been dedicated to industrial valorization of digestates beyond agriculture and energy. The following sections will describe few opportunities related in literature, along with some conceptual ideas.

5.3.1 Reuse water

Water scarcity for agriculture is a reality. In many regions, water canal systems are overexploited as well as aquifers. Simultaneously, there is an increasing water demand for industry and growing cities (Fischer et al. 2014).

Most of digestate liquid streams could be a source of water for irrigation purposes, but clean water is needed for irrigation due to limits on nutrients and pollutants. Moreover, traditional spraying irrigation techniques are not indicated for digestates due to the high ammoniacal nitrogen content. From digestates, irrigation water can be produced with reverse osmosis or condensation from evaporation processes, which were both previously discussed (Sect. 5.1.1).

In many cases, UC-AD are localized far from agriculture. Depending on transporting distances, in situ or industrial digestate water reuse can be a better option. Among the few large scale experiments in literature, Chiumenti et al. (2013b) observed 1.7 t/h of UF + RO permeate for 3.6 ton/h of digestates, which represented 48% of the digestate mass. The final permeate water had 1300 mg/L of DM and less than 100 mg/L of COD (single RO step). Adam et al. (2018) obtained 10–12% of final permeate after NF followed by two consecutive RO. The permeates had about 2 mg/L of suspended solids and 27–32 mgO_2/L of COD. As a quality reference, the Food and Agriculture Organization of the United Nations (FAO) recommends less than 50 mg/L of suspended solids to avoid clogging of drip irrigation systems (Ayers et al. 1985). For unrestricted use of irrigation water, the FAO recommends water salinity lower than 0.7 dS/m (Ayers et al. 1985), which is respected by the permeate water from Chiumenti et al. but not from Adam et al. For "class A" reuse water (all food crops), the new European Legislation proposal on water reuse specifies limits of 10 mg/L of BOD_5 and total suspended solids, among a few other parameters (European Comission 2018).

Fortunately, water is not sufficiently scarce to have an important added value. If it was the case, dilution of AD inputs would also tend to be avoided thus less water would be recovered from digestates. Much likely, the feasibility of the reuse water processes relies on the sales of concentration fractions and/or cost reduction compared to other options.

5.3.2 Animal feed products

In most of the countries, regulation framework would not allow the commercialization of waste-derived products for animal feed, especially outside the agricultural sphere such as digestates from mixed-source feedstock. The following possibilities are strongly hypothetical, depending on legislation evolution along with research programs to confirm their innocuity.

5.3.2.1 Harvested biomass for animal feeding Protein-rich microalgae and macrophyte (discussed in Sect. 5.1.2.1) are often indicated as potential source for primary animal feed. They are often reported as high-grade nutritional food for livestock and aquaculture (Uggetti et al. 2014; Pascual 2016). No information on marketing prices could be found as no industrial production of such biomasses from digestate is available. As a reference, high protein animal feed (50–70% protein) can be found in global international wholesale websites ranging from 400 up to 1300 USD/t.

Little attention seems to be dedicated to the treatment of digestate with mushrooms composting. According to Stoknes et al. (2013), common edible *Agaricus bisporus* is conventionally produced in a mixtures of compost, straw, horse manure, chicken manure and gypsum. They were able to substitute manure from the mixture with a dewatered digestate from municipal source-separated food waste, without affecting mushroom yield. To achieve thermogenesis in early process stages, they had to add only 30 g/kg (dry basis) of chicken manure with as much as 470 g/kg of digestate.

5.3.2.2 Earthworms from vermicomposting for animal feeding Al Seadi et al. (2013) indicated that earthworms from digestate vermicomposting (Sect. 5.1.2.5) can be used to feed chickens but no further information was provided. Indeed, earthworms are known from decades for its protein content (60–70%) and its application for organic waste treatment and animal feed (Edwards 1985).

5.3.2.3 Feed additives for livestock Biochar is already used as a feed supplement for livestock. Positive effects include rapid decrease on diarrhea incidence, improvement of feed intake, reduction of allergies, among other benefits. However, no specific information is available on the biomass origin of these biochar (Gerlach and Schmidt 2012; Schmidt 2012).

Humic substances from leonardite are widely commercialized as feed additive (HUMINTECH 2015a). In the academy, Montoneri et al. (2013) tested as feed additives different "soluble biobased products" extracted from several streams of an UC-AD treating biowaste (including digestate) (as discussed in Sect. 5.1.2.7). The products performed similarly to fossil humic substances for the reduction of ammonia production. The tests were performed under simulated cecal fermentation and in vitro intestinal fermentation.

5.3.3 Biopesticides

The evolution of disease-causing organisms towards plant resistance genes and defense agents is one of the greatest hazard to the sustainability of modern agriculture and food safety (Fischer et al. 2014). Along with that, concerns to improve environmental safety, food quality and human health tends to boost the demand for eco-friendly alternatives to the conventional chemical pesticides.

Bacillus thuringiensis, referred usually as "Bt", are the most usual bacteria do produce biopesticides for insect control. Bt-based products represent about 75% of the global biopesticide use (Olson 2015). They are currently commercialized by various biotech companies under different names and configurations (Rosas-Garcia 2009). By 2013, the biopesticide market represented a 3 billion USD industry but mere 5% of the pesticides market. Biopesticide are expected to grow to above 4.5 billion USD by 2023. Around 2050, the use of biopesticides is expected to overpass the use of synthetic pesticides (Olson 2015).

Support media is an important limitation for the cost-effectiveness to produce Bt-biopesticides. They were estimated to contribute up to 25% of the total production cost (Brar et al. 2007). Digestates can be used as nutrient source and growth support for producing Bt-products. Certainly one of the pioneers of this possibility were Cerda et al. (2019), as part of the DECISIVE project (H2020-EU.3.5.4, ID 689229). They have proofed the concept by successfully producing biopesticides through solid-state fermentation with a biowaste digestate from a UC-AD plant. The conditions, though, remain distant from full-scale implementation.

It is important to mentation that the regulatory framework is favorable towards biopesticides in the USA and in the EU (Villaverde et al. 2014; U.S. EPA 2019).

5.3.4 Adsorbents: biochar

For almost every activated carbon (AC) application, digestate biochars can be and has been assessed as an alternative. It implies in a long list of potential biochar industrial applicability (Schmidt 2012). As previously stated, digestate biochars present a relatively high specific surface (up to 470 m^2/g BET surface), high pore volume (0.06 to 0.55 cm^3/g) (Inyang et al. 2012; Stefaniuk and Oleszczuk 2015; Opatokun et al. 2016; Wongrod et al. 2018; Jiang et al. 2018; Liu et al. 2020) and polar functional groups conferring to it adsorbent properties (Jiang et al. 2018).

As AC surface is usually above 1000 m^2/g (Tadda et al. 2016), biochar performance could be expected to be poorer than AC, if the biochar is not activated before or after pyrolysis through physical and chemical treatments (Sizmur et al. 2017). However, in comparison to carbonaceous materials such as wood, digestate biochar tend to present much higher N and O content and much more polar functional groups on its surface. For this reason, depending on the adsorbate, biochar can present better adsorption results than AC despite its smaller surface. Moreover, some studies indicate the advantage of using digestate instead of raw substrate for pyrolysis. Yao et al. (2011) compared the adsorption of phosphates with biochar from sugar beet railing and its digestate to a commercial AC. Among the three, the digestate biochar had the best phosphate removal efficiency (73% compared to almost 0 for the rest). Moreover, production of biochar from the digestate was significantly higher (45.5% compared to 36.3%), with similar bio-oil production (12.5 and 10.9%) and a greater specific surface.

According to Schmidt (2012), the high prices of biochar previously discussed are due to its valuable application in animal farming. The author states that around 90% of the biochar produced in Europe (mostly from wood biomass) is used either as silage agent (no official reference), in animal litter (adsorption of ammonia), animal feed additive or effluent treatment (composting).

5.3.5 Engineered materials

5.3.5.1 Bioplastics After hydrolysis and extraction of complex soluble OM from a UC-AD digestate (as discussed in Sect. 5.1.2.7), Franzoso et al. (2016) investigated the production of poly(vinyl alcohol-co-ethylene). With up to 10% digestate soluble OM, the resulting blend presented lower melt viscosity and similar or better mechanical properties. To produce bioplastics from UC-AD digesates, this option seems more feasible than the chain-elongation pathway after fermentation. This is due to the same reason of the unlike production of bioethanol: lack of residual biodegradable OM (intrinsic to digestates) and low residual fiber content (if low fibrous inputs).

5.3.5.2 Civil construction Sewage sludge (dried or as ashes) has been investigated for integration with civil construction for several decades (Tay 1987). Sludge incineration ashes can be integrated in mainly two manners to civil construction materials: (i) as a constituent of sintered materials such as bricks, tiles and pavers; (ii) as part of the Portland cement manufacturing either as an additive to cement composition or included as clinker material in furnaces to optimize cement mineral composition. The main limitation is the content of P that can be include undesirable cement characteristics (Donatello and Cheeseman 2013). No specific studies could be found on UC-AD digestates.

5.3.6 *Biosurfactants*

Biosurfactants are traditionally defined as amphiphilic organic compounds that can be naturally produced by plants and microorganisms (Vijayakuma and Saravanan 2015). Due to higher biodegradability and less toxicity, they are an emerging alternative to chemical surfactants. Indeed, most of the surfactants in the market are produced by the petrochemical industry. In 2011, global biosurfactants market worthen around 1.7 billion USD (Reis et al. 2013). Surfactants have a wide range of industrial applications including food industry, pharmaceutical, cosmetics, textile and pollution remediation. Biosurfactants are usually classified as glycolipids and lipopeptides and produced through various aerobic microorganisms consuming mainly carbohydrates and lipids. Their production cost is about 3 to 10 times higher than that of chemical surfactants (Reis et al. 2013).

Cerda et al. (2019) explored the production of sophorolipids (a glycolipid) from a biowaste digestate from a UC-AD by using *Starmella bombicola* (a yeast) as inoculum under aerated conditions. However, two major bottlenecks were identified: (i) optimal fermentation needed a pH around 3.5 but digestate had an important buffering capacity for acidification and (ii) the yeast used in the study requires initial sugar concentration above 100 g/L, which is far from digestate composition as they are consumed during AD.

Apparently neglected by biosurfactant reviews, a more practical approach has been extensively investigated: the application of humic-like substances (HLS) extracted from organic residues as biosurfactants (as discussed in Sect. 5.1.2.7). Extracted from UC-AD digestate, composts, sewage sludge or OFMSW, different uses for HLS-biosurfactants were demonstrated, including: heavy oil removal (Baxter et al. 2014), soil remediation from aromatic hydrocarbons (Conte et al. 2005; Montoneri et al. 2009, 2014), textile dyeing (Montoneri et al. 2009; Savarino et al. 2009), emulsions (Vargas et al. 2014), among other (Montoneri et al. 2011). Many of these publications seems to trace back to researchers from the University of Turin (Italy) and more precisely to the BiochemEnergy project (Montoneri et al. 2011). Indeed, these research teams have been investigating this subject from over a decade (Quagliotto et al. 2006). The BiochemEnergy project estimated a product value ranging from 1 to 100 USD/kg which is high compared to an operational cost estimated from 0.10 to 0.50 USD/kg.

Fossil-based humic substances are industrially used due to its surface-active properties, for example, as drilling fluid (HUMINTECH 2015b). However, no cases of commercial application of HLS extracted from digestates were found. Industrial biosurfactants are expected to change water surface tension from 72 down to 35 mN/m (Akbari et al. 2018). The best performing HLS-biosurfactants from organic residues reduced surface tension to as low as 30–36 mN/m (Quagliotto et al. 2006; Savarino et al. 2009).

With proven applicability, favorable economics and increasing environmental awareness, HLS from UC-AD digestates as biosurfactants are a promising value-added product.

5.3.7 *Flame retardants: struvite or ammonium sulfate*

The currently conventional wood treatment for fire prevention relies on chemicals that contain halogenated compounds, which are harmful to the human health and the environment (Guo et al. 2019).

Wastewater recovered struvite (discussed in Sect. 5.1.1.6) was demonstrated to be an alternative eco-friendly flame retardant to textile and wood (Yetilmezsoy et al. 2018; Guo et al. 2019). Guo et al. (2019) indicated that the main mechanism of struvite wood fire protection was the enhancement of char formation due to two mechanisms: (i) heat absorption with the releasing of a non-flammable gas (preventing temperature increase) and (ii) the amorphous $MgHPO_4$ resulting from struvite thermal decomposition promotes a gas barrier and increase wood structure preventing wood devolatilization (Guo et al. 2019). In both studies, however, the performance was not compared to traditional commercial products.

Ammonium sulfate (discussed in Sect. 5.1.1.5) is another common digestate derived product. In the 1970s it gained interest for its use as flame retardant for wood (George and Susott 1971) and later for organic mulch protection in fire-risk regions such as California (Hickman and Perry 1996). No recent publication is available on this subject.

5.3.8 *Ammonium nitrate for the manufacturing of explosives*

Ammonium nitrate can be obtained from digestates after NH_3 stripping and scrubbing with nitric acid instead of the usual sulfuric acid (producing ammonium sulfate) (Sect. 5.1.1.5). Ammonium nitrate is currently the most important constituent for the manufacturing of modern explosives. However, no study could be identified to assess the compatibility and feasibility of producing prilled, porous and pure ammonium nitrate from digestates while aiming the manufacturing of industrial explosives (Meyer et al. 2007).

5.3.9 *Functional landfill cover layer*

If there is no better option for the destination of a digestate or a partial stream, they can be used as engineered cover layers to promote nutrient removal from leachates in landfills. For example, Peng et al. (2018) demonstrated this concept at laboratory scale with an OFMSW digestate. They succeeded on removing leachate nitrates, which was associated mainly to denitrification, but also adsorption. This can be a useful destination to environmental services providers operating both landfills and UC-AD plants.

5.3.10 *Conclusion of industrial valorization possibilities*

Several options other than agriculture and energy could be identified to valorize UC-AD digestates. From these options, effectively, it seems that only biochar can be considered as a full-scale developed product with industrial applications. However, no case of UC-AD digestates biochar marketing could be identified. After that, another promising strategy is the use of humic-like substances as biosurfactants, which is still under development or demonstration. There are indeed several opportunities for industrial synergy if the AD plant is localized nearby an industrial area, but most of them still require extensive research and development.

6 Conclusion

Technically, there are several possibilities to reach value-added products from UC-AD digestates. Agriculture is the first destination to be considered due to (i) a more advanced technological state-of-the-art, (ii) the positive effects of AD on digestate fertilizing value and (iii) for closing nutrient cycles and promoting carbon sequestration in soil. Aiming agriculture, several processes are already available either to concentrate desirable characteristics of digestates, enhance OM stability or to produce pure and reformulated fertilizers. However, most of the processes still have major drawbacks and great marge for optimization. Globally, reflecting the AD industrial sector, most of the research literature is oriented to WWTP (sludge) digestates or agricultural digestates. Many specific issues of UC-AD digestates from other organic residues are poorly explored. Adding to that, OM valorization seems much less developed and explored than nutrient recovery. The only full-scale OM transformation techniques seem to be composting (fully commercial) or thermal conversion (few installations, mostly demonstrators). Despite the full development of the agricultural destination for digestate byproducts, there is a wide range of urgent subjects for R&D, including process optimization, environmental safety/performance of final products (e.g., pathogens, emerging pollutants, nutrient leaching, atmospheric emissions and life cycle assessments), assessment and enhancement of product performance regarding crop needs (e.g., nutrient balance, nutrient accessibility, OM stability) and market research (e.g. market size and final consumer needs).

Especially for UC-AD designers and operators, when the agricultural destination is not possible, thermal conversion processes are a technically advanced option to valorize the solids through the production of biofuels and/or biochar. In the near future, they could be used to start a whole biorefinery system. Similarly, biomass harvesting processes such as microalgae are rapidly upscaling, enabling to valorize the nutrients on the digestate liquid phase while capturing atmospheric CO_2 and producing renewable biomass for biorefinery. In both cases, the main underlying mechanisms seem to be clearly understood, but R&D programs are still necessary to overcome major bottlenecks and provide more industrial pilot demonstration and validation.

Several other products were successfully obtained from UC-AD digestates such as biopesticides, biosurfactants and composite materials. Many of these approaches seem promising, but they seem limited to a few research groups working under bench to pilot scale. They need to be further investigated and upscaled from process to product application.

If UC-AD digestates could be effectively valorized into value-added products, would it be possible to drive AD inputs and/or parameters for enhancing digestate value (reverse engineering)? What balance to be found with revenues from gate fees and/or optimization for biogas production? Few researchers seem to address these questions.

As regulations and public acceptation are constantly evolving, perhaps one of the greatest recalcitrant barriers can be the price competition with traditional fossil/ore-based products. Many of them such as crude oil, coal, N and P fertilizers are internationally traded as commodities under extremely volatile prices. Adding to that, traditional industrial products have been optimized for application performance and cost reduction. With increasing environmental awareness, a consumer-driven market change towards upcycled and eco-friendly products is fundamental and might enhance digestate intrinsic value, thus UC-AD economic feasibility and environmental performance.

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References

Ackerman JN, Zvomuya F, Cicek N, Flaten D (2013) Evaluation of manure-derived struvite as a phosphorus source for canola. Can J Plant Sci 93:419–424. https://doi.org/10.4141/cjps2012-207

Adam G, Mottet A, Lemaigre S et al (2018) Fractionation of anaerobic digestates by dynamic nanofiltration and reverse osmosis: an industrial pilot case evaluation for nutrient recovery. J Environ Chem Eng 6:6723–6732. https://doi.org/10.1016/j.jece.2018.10.033

Ağdağ ON, Sponza DT (2005) Co-digestion of industrial sludge with municipal solid wastes in anaerobic simulated landfilling reactors. Process Biochem 40:1871–1879. https://doi.org/10.1016/j.procbio.2004.06.057

Agência Nacional do Petróleo Gás Natural e Biocombustíveis (ANP) (2017) Resolução N^{o} 685 de 29/06/2017

Akbari S, Abdurahman NH, Yunus RM et al (2018) Biosurfactants—a new frontier for social and environmental safety: a mini review. Biotechnol Res Innov 2:81–90. https://doi.org/10.1016/J.BIORI.2018.09.001

Akhiar A, Battimelli A, Torrijos M, Carrere H (2017) Comprehensive characterization of the liquid fraction of digestates from full-scale anaerobic co-digestion. Waste Manag 59:118–128. https://doi.org/10.1016/j.wasman.2016.11.005

Al Seadi T, Lukehurst C, Al Saedi T et al (2012) Quality management of digestate from biogas plants used as fertiliser. IEA Bioenergy Task 37:40

Al Seadi T, Drosg B, Fuchs W et al (2013) Biogas digestate quality and utilization. In: Wellinger A, Murphy J, Baxter D (eds) The biogas handbook. Elsevier, Amsterdam, pp 267–301

Alaerts GJ, Mahbubar R, Kelderman P (1996) Performance analysis of a full-scale duckweed-covered sewage lagoon. Water Res 30:843–852. https://doi.org/10.1016/0043-1354(95)00234-0

Alburquerque JA, de la Fuente C, Ferrer-Costa A et al (2012) Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass Bioenergy 40:181–189. https://doi.org/10.1016/j.biombioe.2012.02.018

Aleta P et al (2018) The effect of organic matter on the removal of phosphorus through precipitation as struvite and calcium phosphate in synthetic dairy wastewater. Membr Water Treat 9:163–172. https://doi.org/10.12989/mwt.2018.9.3.163

Almeida C, Marisa R, Dror I et al (2019) Assessing fate and bioavailability of trace elements in soils after digestate application

Al-Wabel MI, Hussain Q, Usman ARA et al (2018) Impact of biochar properties on soil conditions and agricultural sustainability: a review. Land Degrad Dev 29:2124–2161. https://doi.org/10.1002/ldr.2829

Amlinger F, Blytt LD (2013) How to comply with the EU animal by-products regulations at composting and anaerobic digestion plants. European Compost Network ECN e.V, Bochum

André L, Pauss A, Ribeiro T (2018) Solid anaerobic digestion: state-of-art, scientific and technological hurdles. Bioresour

Technol 247:1027–1037. https://doi.org/10.1016/j.biortech.2017.09.003

Antón R, Cuevas J, Carreras N et al (2017) Effects of treated urban waste digestates on hydroponically grown tomato (*Solanum lycopersicon* L.), pp 3–4

Appels L, Baeyens J, Degrève J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Prog Energy Combust Sci 34:755–781. https://doi.org/10.1016/j.pecs.2008.06.002

Arlabosse P, Blanc M, Tran D (2010) Projet DIVA—Tâche 4.1.1—evaluation technique et environnementale du séchage des digestats—Livrable L4.1.1.1

Atiyeh RM, Lee S, Edwards CA et al (2002) The influence of humic acids derived from earthworm-processed organic wastes on plant growth. Bioresour Technol 84:7–14. https://doi.org/10.1016/S0960-8524(02)00017-2

Ayers RS, Westcot DW et al (1985) Chapter 1. Water quality evaluation. In: Water quality for agriculture. Food and Agriculture Organization of the United Nations Rome

Bachmann S, Uptmoor R, Eichler-Lobermann B (2016) Phosphorus distribution and availability in untreated and mechanically separated biogas digestates. Sci Agric 73:9–17. https://doi.org/10.1590/0103-9016-2015-0069

Balat M, Balat M, Kırtay E, Balat H (2009) Main routes for the thermo-conversion of biomass into fuels and chemicals. Part 2: gasification systems. Energy Convers Manag 50:3158–3168. https://doi.org/10.1016/j.enconman.2009.08.013

Banks CJ, Chesshire M, Heaven S, Arnold R (2011) Anaerobic digestion of source-segregated domestic food waste: performance assessment by mass and energy balance. Bioresour Technol 102:612–620. https://doi.org/10.1016/j.biortech.2010.08.005

Bashiri R, Farhadian M, Asadollahi MA, Jeihanipour A (2016) Anaerobic digested sludge: a new supplementary nutrient source for ethanol production. Int J Environ Sci Technol 13:763–772. https://doi.org/10.1007/s13762-015-0925-8

Batstone DJ, Puyol D, Flores-Alsina X, Rodríguez J (2015) Mathematical modelling of anaerobic digestion processes: applications and future needs. Rev Environ Sci Biotechnol 14:595–613. https://doi.org/10.1007/s11157-015-9376-4

Battista F, Bolzonella D (2019) Exploitation of solar energy for ammonium sulfate recovery from anaerobic digestate of different origin. Waste Biomass Valorization. https://doi.org/10.1007/s12649-019-00597-x

Bauer A, Mayr H, Hopfner-Sixt K, Amon T (2009) Detailed monitoring of two biogas plants and mechanical solid-liquid separation of fermentation residues. J Biotechnol 142:56–63. https://doi.org/10.1016/j.jbiotec.2009.01.016

Baxter MD, Acosta E, Montoneri E, Tabasso S (2014) Waste biomass-extracted surfactants for heavy oil removal. Ind Eng Chem Res 53:3612–3621. https://doi.org/10.1021/ie402239p

Benoît P, Brugère H, Casellas M et al (2014) Caractéristiques physico-chimiques et biologiques des Mafor

Bloomfield C, McGrath SP (1982) A comparison of the extractabilities of Zn, Cu, Ni and Cr from sewage sludges prepared by treating raw sewage with the metal salts before or after anaerobic digestion. Environ Pollut Ser B Chem Phys 3:193–198

Bolzonella D, Fatone F, Gottardo M, Frison N (2018) Nutrients recovery from anaerobic digestate of agro-waste: techno-economic assessment of full scale applications. J Environ Manag 216:111–119. https://doi.org/10.1016/J.JENVMAN.2017.08.026

Bonetta S, Bonetta S, Ferretti E et al (2014) Agricultural reuse of the digestate from anaerobic co-digestion of organic waste: microbiological contamination, metal hazards and fertilizing performance. Water Air Soil Pollut 225:1–11. https://doi.org/10.1007/s11270-014-2046-2

Braak E, Auby S, Piveteau S et al (2015) Phosphorus recycling potential assessment by a biological test applied to wastewater sludge. Environ Technol. https://doi.org/10.1080/09593330.2015.1116612

Brändli RC, Bucheli TD, Kupper T et al (2007) Fate of PCBs, PAHs and their source characteristic ratios during composting and digestion of source-separated organic waste in full-scale plants. Environ Pollut 148:520–528. https://doi.org/10.1016/j.envpol.2006.11.021

Brar SK, Verma M, Tyagi RD et al (2007) Techno-economic analysis of bacillus thuringiensis biopesticides production from wastewater and wastewater sludge. In: Moving forward wastewater biosolids sustainability: technical, managerial and public synergy from the International Water Association, 24-27, Jun 2007, Moncton, NB, Canada, pp 731–738

Brémond U, Bertrandias A, Loisel D et al (2020) Assessment of fungal and thermo-alkaline post-treatments of solid digestate in a recirculation scheme to increase flexibility in feedstocks supply management of biogas plants. Renew Energy 149:641–651. https://doi.org/10.1016/j.renene.2019.12.062

Brunetti G, Farrag K, Plaza C, Senesi N (2012) Advanced techniques for characterization of organic matter from anaerobically digested grapemarc distillery effluents and amended soils. Environ Monit Assess 184:2079–2089. https://doi.org/10.1007/s10661-011-2101-z

Cabeza R, Steingrobe B, Römer W, Claassen N (2011) Effectiveness of recycled P products as P fertilizers, as evaluated in pot experiments. Nutr Cycl Agroecosyst 91:173–184. https://doi.org/10.1007/s10705-011-9454-0

Calvo P, Nelson L, Kloepper JW (2014) Agricultural uses of plant biostimulants. Plant Soil 383:3–41. https://doi.org/10.1007/s11104-014-2131-8

Capdevielle A, Sýkorová E, Béline F, Daumer M-L (2015) Effects of organic matter on crystallization of struvite in biologically treated swine wastewater. Environ Technol. https://doi.org/10.1080/09593330.2015.1088580

Capson-Tojo G, Rouez M, Crest M et al (2016) Food waste valorization via anaerobic processes: a review. Rev Environ Sci Biotechnol 15:499–547

Carballa M, Omil F, Lema JM (2009) Influence of different pretreatments on anaerobically digested sludge characteristics: suitability for final disposal. Water Air Soil Pollut 199:311–321. https://doi.org/10.1007/s11270-008-9880-z

Case SDC, Oelofse M, Hou Y et al (2017) Farmer perceptions and use of organic waste products as fertilisers—a survey study of potential benefits and barriers. Agric Syst 151:84–95. https://doi.org/10.1016/J.AGSY.2016.11.012

Cavalli D, Cabassi G, Borrelli L et al (2016) Nitrogen fertilizer replacement value of undigested liquid cattle manure and digestates. Eur J Agron 73:34–41. https://doi.org/10.1016/j.eja.2015.10.007

Cerda A, Mejias L, Rodríguez P et al (2019) Valorisation of digestate from biowaste through solid-state fermentation to obtain value added bioproducts: a first approach. Bioresour Technol 271:409–416. https://doi.org/10.1016/J.BIORTECH.2018.09.131

Chen R, Li R, Deitz L et al (2012) Freshwater algal cultivation with animal waste for nutrient removal and biomass production. Biomass Bioenergy 39:128–138. https://doi.org/10.1016/j.biombioe.2011.12.045

Chiumenti A (2015) Complete nitrification-denitrification of swine manure in a full-scale, non-conventional composting system. Waste Manag 46:577–587. https://doi.org/10.1016/j.wasman.2015.07.035

Chiumenti A, da Borso F, Chiumenti R et al (2013a) Treatment of digestate from a co-digestion biogas plant by means of vacuum evaporation: tests for process optimization and environmental sustainability. Waste Manag 33:1339–1344. https://doi.org/10.1016/j.wasman.2013.02.023

Chiumenti A, Da Borso F, Teri F et al (2013b) Full-scale membrane filtration system for the treatment of digestate from a co-digestion plant. Appl Eng Agric 29:985–990. https://doi.org/10.13031/aea.29.10117

Christel W, Bruun S, Magid J, Jensen LS (2014) Phosphorus availability from the solid fraction of pig slurry is altered by composting or thermal treatment. Bioresour Technol 169:543–551. https://doi.org/10.1016/j.biortech.2014.07.030

Conte P, Agretto A, Spaccini R, Piccolo A (2005) Soil remediation: humic acids as natural surfactants in the washings of highly contaminated soils. Environ Pollut 135:515–522. https://doi.org/10.1016/j.envpol.2004.10.006

Coppens J, Grunert O, Van Den Hende S et al (2016) The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J Appl Phycol 28:2367–2377. https://doi.org/10.1007/s10811-015-0775-2

Dabert P (2015) Final report—DIVA project—caracterisation des digestats et de leurs filieres de valorisation agronomique. Projet ANR-10-BIOE-007

Dahlin J, Herbes C, Nelles M (2015) Biogas digestate marketing: qualitative insights into the supply side. Resour Conserv Recycl 104:152–161. https://doi.org/10.1016/j.resconrec.2015.08.013

De Moor S, Velghe F, Wierinck I et al (2013) Feasibility of grass co-digestion in an agricultural digester, influence on process parameters and residue composition. Bioresour Technol 150:187–194. https://doi.org/10.1016/j.biortech.2013.10.011

Delfosse P, Luxem P, Glaude E et al (2011) Séchage et valorization des digestats en agriculture: bénéfices économiques et environnementaux

Demirbas A (2006) Biogas production from the organic fraction of municipal solid waste. Energy Sources Part Recovery Util Environ Eff 28:1127–1134. https://doi.org/10.1080/009083190910479

Deremince B, Königsberger S (2017) Statistical report of the European Biogas Association 2017. European Biogas Association, Brussels

Desbois D, Legris B (2007) Prix et coûts de production de six grandes cultures: blé, maïs, colza, tournesol, betterave et pomme de terre. L'agriculture Nouv Défis Insee Réf

Desmidt E, Ghyselbrecht K, Zhang Y et al (2015) Global phosphorus scarcity and full-scale p-recovery techniques: a review. Crit Rev Environ Sci Technol 45:336–384. https://doi.org/10.1080/10643389.2013.866531

Directive Council (1975) Council directive 75/442/EEC of 15 July 1975 on waste. Off J Eur Communities 194:39–41

Donatello S, Cheeseman CR (2013) Recycling and recovery routes for incinerated sewage sludge ash (ISSA): a review. Waste Manag 33:2328–2340. https://doi.org/10.1016/j.wasman.2013.05.024

Drosg B, Fuchs W, Seadi TA et al (2015) Nutrient recovery by biogas digestate processing. IEA Bioenergy, Dublin

Edwards CA (1985) Production of feed protein from animal waste by earthworms. Philos Trans R Soc B Biol Sci 310:299–307. https://doi.org/10.1098/rstb.1985.0120

Ehmann A, Lewandowski I (2013) Biomass ashes: characteristics and potential for use as fertilizer. In: 15th Ramiran international conference. Versailles, France, pp 5–8

Epstein E (2011) Industrial composting: environmental engineering and facilities management. CRC Press, Boca Raton

Erkelens M, Ward AJ, Ball AS, Lewis DM (2014) Microalgae digestate effluent as a growth medium for *Tetraselmis* sp. in the production of biofuels. Bioresour Technol 167:81–86. https://doi.org/10.1016/j.biortech.2014.05.126

European Biogas Association (2013) EBA opinion on the digestate drying at biogas plants, pp 22–25

European Biogas Association (2015) Digestate factsheet: the value of organic fertilisers for Europe's economy, society and environment, pp 1–4

European Comission (2011) Tackling the challenges in commodity markets and on raw materials

European Comission (2018) ANNEXES to the proposal for a regulation of the European parliament and of the council on minimum requirements for water reuse. Brussels, 28/05/2017

Evans TD (2007) Recovering ammonium and struvite fertilisers from digested sludge dewatering liquors. In: Proceedings of IWA specialist conference: moving forward–wastewater biosolids sustainability

Eyheraguibel B, Silvestre J, Morard P (2008) Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. Bioresour Technol 99:4206–4212. https://doi.org/10.1016/j.biortech.2007.08.082

Fagbohungbe MO, Herbert BMJ, Hurst L et al (2016) The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion. Waste Manag. https://doi.org/10.1016/j.wasman.2016.11.028

Fascella G, Montoneri E, Ginepro M, Francavilla M (2015) Effect of urban biowaste derived soluble substances on growth, photosynthesis and ornamental value of Euphorbia x lomi. Sci Hortic 197:90–98. https://doi.org/10.1016/j.scienta.2015.10.042

Fascella G, Montoneri E, Francavilla M (2018) Biowaste versus fossil sourced auxiliaries for plant cultivation: the Lantana case study. J Clean Prod 185:322–330. https://doi.org/10.1016/j.jclepro.2018.02.242

Fattah KP, Fattah K-P, Zhang Y, ZhangY Mavinic DS, Mavinic DS, Koch FA, Koch FA (2010) Use of carbon dioxide stripping for struvite crystallization to save caustic dosage: performance at pilotscale operation. Can J Civ Eng 37:1271–1275. https://doi.org/10.1139/L10-055

Fischer RA, Byerlee D, Edmeades G (2014) Crop yields and global food security. ACIAR, Canberra

Franzoso F, Vaca-Garcia C, Rouilly A et al (2016) Extruded versus solvent cast blends of poly(vinyl alcohol-*co*-ethylene) and biopolymers isolated from municipal biowaste. J Appl Polym Sci. https://doi.org/10.1002/app.43009

Frischmann P (2012) Enhancement and treatment of digestates from anaerobic digestion

Fuchs W, Drosg B (2013) Assessment of the state of the art of technologies for the processing of digestate residue from anaerobic digesters. Water Sci Technol 67:1984–1993. https://doi.org/10.2166/wst.2013.075

Fuldauer LI, Parker BM, Yaman R, Borrion A (2018) Managing anaerobic digestate from food waste in the urban environment: evaluating the feasibility from an interdisciplinary perspective. J Clean Prod 185:929–940. https://doi.org/10.1016/j.jclepro.2018.03.045

Funke A, Reebs F, Kruse A (2013) Experimental comparison of hydrothermal and vapothermal carbonization. Fuel Process Technol 115:261–269. https://doi.org/10.1016/J.FUPROC.2013.04.020

Gasum (2016) Daily total of 500 kg of nitrogen recovered from biogas plant reject water of sewage sludge origin. https://www.gasum.com/en/About-gasum/for-the-media/News/2016/Daily-total-of-500-kg-of-nitrogen-recovered-from-biogas-plant-reject-water-of-sewage-sludge-origin/. Accessed 22 Jan 2019

George CW, Susott RA (1971) Effects of ammonium phosphate and sulfate on the pyrolysis and combustion of cellulose. Intermountain Forest & Range Experiment Station, Forest Service, U.S. Dept. of Agriculture, Ogden, Utah

Gerlach A, Schmidt H-P (2012) The use of biochar in cattle farming. Ithaka J 2012:281–285

Gong H, Yan Z, Liang KQ et al (2013) Concentrating process of liquid digestate by disk tube-reverse osmosis system. Desalination 326:30–36. https://doi.org/10.1016/j.desal.2013.07.010

Govasmark E, Stäb J, Holen B et al (2011) Chemical and microbiological hazards associated with recycling of anaerobic digested residue intended for agricultural use. Waste Manag 31:2577–2583. https://doi.org/10.1016/j.wasman.2011.07.025

Guercini S, Castelli G, Rumor C (2014) Vacuum evaporation treatment of digestate: full exploitation of cogeneration heat to process the whole digestate production. Water Sci Technol 70:479–485

Guilayn F, Braak E, Piveteau S, Daumer M-L (2017) Sequencing biological acidification of waste-activated sludge aiming to optimize phosphorus dissolution and recovery. Environ Technol 38:1399–1407. https://doi.org/10.1080/09593330.2016.1230653

Guilayn F, Jimenez J, Martel J-L et al (2019a) First fertilizing-value typology of digestates: a decision-making tool for regulation. Waste Manag 86:67–79. https://doi.org/10.1016/j.wasman.2019.01.032

Guilayn F, Jimenez J, Rouez M et al (2019b) Digestate mechanical separation: efficiency profiles based on anaerobic digestion feedstock and equipment choice. Bioresour Technol 274:180–189. https://doi.org/10.1016/j.biortech.2018.11.090

Guilayn F, Benbrahim M, Rouez M et al (2020) Humic-like substances extracted from different digestates: first trials of lettuce biostimulation in hydroponic culture. Waste Manag 104:239–245. https://doi.org/10.1016/j.wasman.2020.01.025

Guo H, Luković M, Mendoza M et al (2019) Bioinspired struvite mineralization for fire resistant wood. ACS Appl Mater Interfaces Acsami. https://doi.org/10.1021/acsami.8b19967

Gusiatin ZM, Kurkowski R, Brym S, Wiśniewski D (2016) Properties of biochars from conventional and alternative feedstocks and their suitability for metal immobilization in industrial soil. Environ Sci Pollut Res 23:21249–21261. https://doi.org/10.1007/s11356-016-7335-4

Hagemann N, Spokas K, Schmidt HP et al (2018) Activated carbon, biochar and charcoal: linkages and synergies across pyrogenic carbon's ABCs. Water Switz 10:1–19. https://doi.org/10.3390/w10020182

He X, Zhang T, Ren H et al (2016) Phosphorus recovery from biogas slurry by ultrasound/H2O2 digestion coupled with HFO/biochar adsorption process. Waste Manag. https://doi.org/10.1016/j.wasman.2016.08.032

Hickman GW, Perry E (1996) Using ammonium sulfate fertilizer as an organic mulch fire retardant. J Arboric 22:279–280

Hidalgo D, Corona F, Martín-Marroquín JM et al (2015) Resource recovery from anaerobic digestate: struvite crystallisation versus ammonia stripping. Desalination Water Treat 3994:1–7. https://doi.org/10.1080/19443994.2014.1001794

Hjorth M, Nielsen AM, Nyord T et al (2009) Nutrient value, odour emission and energy production of manure as influenced by anaerobic digestion and separation. Agron Sustain Dev 29:329–338

Hjorth M, Christensen KV, Christensen ML, Sommer SG (2010) Solid-liquid separation of animal slurry in theory and practice. A review. Sustain Dev 30:153–180

HUMINTECH (2015a) Humic acids—animal breeding. https://www.humintech.com/livestock-breeding/applications/animal-breeding.html. Accessed 7 Apr 2019

HUMINTECH (2015b) Industry applications practice—humic acids, drilling fluids. https://www.humintech.com/industry/applications/drilling-fluids.html. Accessed 7 May 2019

Inyang M, Gao B, Yao Y et al (2012) Removal of heavy metals from aqueous solution by biochars derived from anaerobically digested biomass. Bioresour Technol 110:50–56. https://doi.org/10.1016/j.biortech.2012.01.072

Jamaludin Z, Rollings-Scattergood S, Lutes K, Vaneeckhaute C (2018) Evaluation of sustainable scrubbing agents for ammonia recovery from anaerobic digestate. Bioresour Technol 270:596–602. https://doi.org/10.1016/j.biortech.2018.09.007

Jiang B, Lin Y, Mbog JC (2018) Biochar derived from swine manure digestate and applied on the removals of heavy metals and antibiotics. Bioresour Technol 270:603–611. https://doi.org/10.1016/J.BIORTECH.2018.08.022

Jirka S, Tomlinson T (2014) 2013 State of the biochar industry a survey of commercial activity in the biochar field

Kataki S, Baruah DC (2018) Prospects and issues of phosphorus recovery as struvite from waste streams

Kataki S, West H, Clarke M, Baruah DC (2016) Phosphorus recovery as struvite from farm, municipal and industrial waste: feedstock suitability, methods and pre-treatments. Waste Manag 49:437–454. https://doi.org/10.1016/j.wasman.2016.01.003

Kiely G, Tayfur G, Dolan C, Tanji K (1997) Physical and mathematical modelling of anaerobic digestion of organic wastes. Water Res 31:534–540. https://doi.org/10.1016/S0043-1354(96)00175-3

King C, Bardos P, Nortcliff S et al (2013) Final report: market expectations and requirements for digestate

Kizito S, Wu S, Kipkemoi Kirui W et al (2015) Evaluation of slow pyrolyzed wood and rice husks biochar for adsorption of ammonium nitrogen from piggery manure anaerobic digestate slurry. Sci Total Environ 505:102–112. https://doi.org/10.1016/j.scitotenv.2014.09.096

Kjerstadius H, La Cour Jansen J, De Vrieze J et al (2013) Hygienization of sludge through anaerobic digestion at 35, 55 and 60 C. Water Sci Technol 68:2234–2239. https://doi.org/10.2166/wst.2013.486

Koszel M, Lorencowicz E (2015) Agricultural use of biogas digestate as a replacement fertilizers. Agric Agric Sci Procedia 7:119–124. https://doi.org/10.1016/j.aaspro.2015.12.004

Kothari R, Pandey AK, Kumar S et al (2014) Different aspects of dry anaerobic digestion for bio-energy: an overview. Renew Sustain Energy Rev 39:174–195. https://doi.org/10.1016/j.rser.2014.07.011

Koutra E, Grammatikopoulos G, Kornaros M (2017) Selection of microalgae intended for valorization of digestate from agro-waste mixtures. Waste Manag. https://doi.org/10.1016/j.wasman.2017.12.030

Kratzeisen M, Starcevic N, Martinov M et al (2010) Applicability of biogas digestate as solid fuel. Fuel 89:2544–2548. https://doi.org/10.1016/j.fuel.2010.02.008

Krishnasamy K, Nair J, Bauml B (2012) Hydroponic system for the treatment of anaerobic liquid. Water Sci Technol 65:1164–1171. https://doi.org/10.2166/wst.2012.031

Krishnasamy K, Nair J, Bell R (2014) Evaluation of anaerobic digestate as a substrate for vermicomposting. Int J Environ Waste Manag 14:149–164

Labatut RA, Angenent LT, Scott NR (2011) Biochemical methane potential and biodegradability of complex organic substrates. Bioresour Technol 102:2255–2264. https://doi.org/10.1016/j.biortech.2010.10.035

Lallanilla M (2013) What causes fertilizer explosions? In: Live science. https://www.livescience.com/28841-fertilizer-explosions-ammonium-nitrate.html. Accessed 7 Apr 2019

Lauterböck B, Ortner M, Haider R, Fuchs W (2012) Counteracting ammonia inhibition in anaerobic digestion by removal with a hollow fiber membrane contactor. Water Res 46:4861–4869. https://doi.org/10.1016/j.watres.2012.05.022

Lebuf V, Accoe F, Vaneeckhaute C et al (2012) Nutrient recovery from digestates: techniques and end-products. In: Venice 2012: fourth international symposium on energy from biomass and waste, p 18

Lebuf V, Accoe F, Van Elsacker S et al (2013) Techniques for nutrient recovery from digestate: inventory, p 26

Lehmann L, Bloem E, Schick J et al (2015) The influence of anaerobic digestion on the concentration of antibiotics, heavy metals and on phosphorous-solubility of digestates. In: 16th Ramiran international conference. Hamburg, Germany

Leng RA (1999) Duckweed: a tiny aquatic plant with enormous potential for agriculture and environment

Levasseur P, Toudic A, Bonhomme S, Lorinquer E (2017) Gestion et treatement des digestats issues de methanisation

Li Y, Park SY, Zhu J (2011) Solid-state anaerobic digestion for methane production from organic waste. Renew Sustain Energy Rev 15:821–826. https://doi.org/10.1016/j.rser.2010.07.042

Li Y, Liu H, Su D, Yan F (2016) Characterization and thermophilic anaerobic digestion of organic fraction of municipal solid waste. Waste Biomass Valorization 7:325–330. https://doi.org/10.1007/s12649-015-9444-6

Liebetrau J, Denysenko V, Gromke JD (2017) IEA bioenergy task 37: country report Germany

Lin H, Gan J, Rajendran A et al (2015) Phosphorus removal and recovery from digestate after biogas production. In: Biernat K (ed) Biofuels—status and perspective. InTech

Liu J, Huang S, Chen K et al (2020) Preparation of biochar from food waste digestate: pyrolysis behavior and product properties. Bioresour Technol 302:122841. https://doi.org/10.1016/j.biortech.2020.122841

Logan M, Visvanathan C (2019) Management strategies for anaerobic digestate of organic fraction of municipal solid waste: current status and future prospects. Waste Manag Res 37:27–39. https://doi.org/10.1177/0734242X18816793

Lyons G, Genc Y (2016) Commercial humates in agriculture: real substance or smoke and mirrors? Agronomy 6:50

Mailler R, Gasperi J, Chebbo G, Rocher V (2014) Priority and emerging pollutants in sewage sludge and fate during sludge treatment. Waste Manag 34:1217–1226. https://doi.org/10.1016/j.wasman.2014.03.028

Mailler R, Gasperi J, Patureau D et al (2017) Fate of emerging and priority micropollutants during the sewage sludge treatment: case study of Paris conurbation. Part 1: contamination of the different types of sewage sludge. Waste Manag 59:379–393. https://doi.org/10.1016/j.wasman.2016.11.010

Major J (2010) Guidelines on practical aspects of biochar application to field soil in various soil management systems. Int Biochar Initiat 8:1–23

Makádi M, Tomócsik A, Orosz V (2012) Digestate: a new nutrient source—review. In: Kumar DS (ed) Biogas. InTech, pp 296–310

Marazzi F, Sambusiti C, Monlau F et al (2017) A novel option for reducing the optical density of liquid digestate to achieve a more productive microalgal culturing. Algal Res 24:19–28. https://doi.org/10.1016/J.ALGAL.2017.03.014

Marcato CE, Pinelli E, Pouech P et al (2008) Particle size and metal distributions in anaerobically digested pig slurry. Bioresour Technol 99:2340–2348. https://doi.org/10.1016/j.biortech.2007.05.013

Marcato CE, Pinelli E, Cecchi M et al (2009) Bioavailability of Cu and Zn in raw and anaerobically digested pig slurry. Ecotoxicol Environ Saf 72:1538–1544. https://doi.org/10.1016/j.ecoenv.2008.12.010

Marcilhac C, Sialve B, Pourcher A-M et al (2014) Digestate color and light intensity affect nutrient removal and competition phenomena in a microalgal-bacterial ecosystem. Water Res 64:278–287. https://doi.org/10.1016/J.WATRES.2014.07.012

Marcilhac C, Sialve B, Pourcher A-M et al (2015) Control of nitrogen behaviour by phosphate concentration during microalgal-bacterial cultivation using digestate. Bioresour Technol 175:224–230. https://doi.org/10.1016/J.BIORTECH.2014.10.022

Mariani L (2015) Levantamento de unidades de produção de biogás no Brasil para fins energéticos ou Mecanismo de Desenvolvimento Limpo. CIBiogas

Massaccesi L, Sordi A, Micale C et al (2013) Chemical characterisation of percolate and digestate during the hybrid solid anaerobic digestion batch process. Process Biochem 48:1361–1367. https://doi.org/10.1016/j.procbio.2013.06.026

Mehta CM, Batstone DJ (2013) Nutrient solubilization and its availability following anaerobic digestion. Water Sci Technol 67:756–763. https://doi.org/10.2166/wst.2012.622

Menardo S, Balsari P, Dinuccio E, Gioelli F (2011a) Thermal pre-treatment of solid fraction from mechanically-separated raw and digested slurry to increase methane yield. Bioresour Technol 102:2026–2032. https://doi.org/10.1016/j.biortech.2010.09.067

Menardo S, Gioelli F, Balsari P (2011b) The methane yield of digestate: effect of organic loading rate, hydraulic retention time, and plant feeding. Bioresour Technol 102:2348–2351. https://doi.org/10.1016/j.biortech.2010.10.094

Meyer R, Khler J, Homburg A (2007) Explosives. Wiley, Weinheim

Minasny B, Malone BP, McBratney AB et al (2017) Soil carbon 4 per mille. Geoderma 292:59–86. https://doi.org/10.1016/j.geoderma.2017.01.002

Ministry of Land Infrastructure Transport and Tourism (2013) Efforts for use of methane gas in wastewater treatment of Japan. Japan

Ministry of New and Renewable Energy (2014) Annual report 2013–2014

Möller K, Müller T (2012) Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng Life Sci 12:242–257. https://doi.org/10.1002/elsc.201100085

Möller K, Stinner W, Deuker A, Leithold G (2008) Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. Nutr Cycl Agroecosyst 82:209–232. https://doi.org/10.1007/s10705-008-9196-9

Möller K, Schulz R, Müller T (2010) Substrate inputs, nutrient flows and nitrogen loss of two centralized biogas plants in southern Germany. Nutr Cycl Agroecosyst 87:307–325. https://doi.org/10.1007/s10705-009-9340-1

Monballiu A, Desmidt E, Ghyselbrecht K, Meesschaert B (2019) The inhibitory effect of inorganic carbon on phosphate recovery from UASB effluent as calcium phosphate. Water Sci Technol. https://doi.org/10.2166/wst.2019.026

Monfet E, Aubry G, Ramirez AA (2018) Nutrient removal and recovery from digestate: a review of the technology. Biofuels 9:247–262. https://doi.org/10.1080/17597269.2017.1336348

Monlau F, Sambusiti C, Ficara E et al (2015) New opportunities for agricultural digestate valorization: current situation and perspectives. Energy Environ Sci. https://doi.org/10.1039/C5EE01633A

Montoneri E (2017) Municipal waste treatment, technological scale up and commercial exploitation: the case of bio-waste lignin to soluble lignin-like polymers. Food Waste Reduct Valoris Sustain Assess Policy Anal. https://doi.org/10.1007/978-3-319-50088-1_6

Montoneri E, Boffa V, Savarino P et al (2009) Use of biosurfactants from urban wastes compost in textile dyeing and soil remediation. Waste Manag 29:383–389

Montoneri E, Mainero D, Boffa V et al (2011) Biochemenergy: a project to turn an urban wastes treatment plant into biorefinery for the production of energy, chemicals and consumer's products with friendly environmental impact. Int J Glob Environ Issues 11:170. https://doi.org/10.1504/IJGENVI.2011.043528

Montoneri C, Montoneri E, Tomasso L, Piva A (2013) Compost derived substances decrease feed protein N mineralization in swine cecal fermentation. J Agric Sci Tor 5:31–44. https://doi.org/10.5539/jas.v5n3p31

Montoneri E, Tomasso L, Colajanni N et al (2014) Urban wastes to remediate industrial sites: a case of polycyclic aromatic hydrocarbons contamination and a new process. Int J Env Sci Technol 2:251–262. https://doi.org/10.1007/s13762-013-0211-6

Morard P, Eyheraguibel B, Morard M, Silvestre J (2011) Direct effects of humic-like substance on growth, water, and mineral nutrition of various species. J Plant Nutr 34:46–59. https://doi.org/10.1080/01904167.2011.531358

Morero B, Vicentin R, Campanella EA (2017) Assessment of biogas production in Argentina from co-digestion of sludge and municipal solid waste. Waste Manag 61:195–205. https://doi.org/10.1016/j.wasman.2016.11.033

Mulbry W, Westhead EK, Pizarro C, Sikora L (2005) Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresour Technol 96:451–458. https://doi.org/10.1016/J.BIORTECH.2004.05.026

Mumme J, Eckervogt L, Pielert J et al (2011) Hydrothermal carbonization of anaerobically digested maize silage. Bioresour Technol 102:9255–9260. https://doi.org/10.1016/j.biortech.2011.06.099

Muscolo A, Sidari M, Nardi S (2013) Humic substance: relationship between structure and activity. Deeper information suggests univocal findings. J Geochem Explor 129:57–63. https://doi.org/10.1016/j.gexplo.2012.10.012

Nagy D, Balogh P, Gabnai Z et al (2018) Economic analysis of pellet production in co-digestion biogas plants. Energies 11:1135. https://doi.org/10.3390/en11051135

Neumann J, Meyer J, Ouadi M et al (2016) The conversion of anaerobic digestion waste into biofuels via a novel thermo-catalytic reforming process. Waste Manag 47:141–148. https://doi.org/10.1016/J.WASMAN.2015.07.001

Nguyen D, Gadhamshetty V, Nitayavardhana S, Khanal SK (2015) Automatic process control in anaerobic digestion technology: a critical review. Bioresour Technol 193:513–522. https://doi.org/10.1016/j.biortech.2015.06.080

Nkoa R (2014) Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agron Sustain Dev 34:473–492. https://doi.org/10.1007/s13593-013-0196-z

Olga P, Fabrizio G, Elio D, Paolo B (2014) Improved pig slurry mechanical separation using chitosan and biochar. Biosyst Eng 127:115–124. https://doi.org/10.1016/j.biosystemseng.2014.08.009

Oliveira V, Labrincha J, Dias-Ferreira C (2018) Extraction of phosphorus and struvite production from the anaerobically digested organic fraction of municipal solid waste. J Environ Chem Eng 6:2837–2845. https://doi.org/10.1016/J.JECE.2018.04.034

Olson S (2015) An analysis of the biopesticide market now and where it is going. Outlooks Pest Manag 26:203–206. https://doi.org/10.1564/v26_oct_04

Opatokun SA, Strezov V, Kan T (2014) Product based evaluation of pyrolysis of food waste and its digestate. Energy 92:349–354

Opatokun SA, Kan T, Al Shoaibi A et al (2016) Characterization of food waste and its digestate as feedstock for thermochemical processing. Energy Fuels 30:1589–1597. https://doi.org/10.1021/acs.energyfuels.5b02183

Opatokun SA, Yousef LF, Strezov V (2017) Agronomic assessment of pyrolysed food waste digestate for sandy soil management. J Environ Manag 187:24–30. https://doi.org/10.1016/j.jenvman.2016.11.030

Osborn PD (1985) Handbook of energy data and calculations: including directory of products and services. Elsevier Science, London

Owamah HI, Dahunsi SO, Oranusi US, Alfa MI (2014) Fertilizer and sanitary quality of digestate biofertilizer from the co-digestion of food waste and human excreta. Waste Manag 34:747–752. https://doi.org/10.1016/j.wasman.2014.01.017

Palumbo G, Schiavon M, Nardi S et al (2018) Biostimulant potential of humic acids extracted from an amendment obtained via combination of olive mill wastewaters (OMW) and a pre-treated organic material derived from municipal solid waste (MSW). Front Plant Sci 9:1–14. https://doi.org/10.3389/fpls.2018.01028

Pantelopoulos A, Magid J, Jensen LS (2016) Thermal drying of the solid fraction from biogas digestate: effects of acidification, temperature and ventilation on nitrogen content. Waste Manag 48:218–226. https://doi.org/10.1016/j.wasman.2015.10.008

PAQUES (2019) PHOSPAQ^{TM}. https://en.paques.nl/products/other/phospaq. Accessed 8 Apr 2019

Park S, Kim M (2016) Effect of ammonia on anaerobic degradation of amino acids. KSCE J Civ Eng 20:129–136. https://doi.org/10.1007/s12205-015-0240-4

Pascual A (2016) LIFE LEMNA—duckweed technology for improving nutrient management and resource efficiency in pig production systems. http://www.life-lemna.eu/. Accessed 21 Jan 2019

Pedrazzi S, Allesina G, Belló T et al (2015) Digestate as bio-fuel in domestic furnaces. Fuel Process Technol 130:172–178. https://doi.org/10.1016/j.fuproc.2014.10.006

Peng W, Pivato A, Lavagnolo MC, Raga R (2018) Digestate application in landfill bioreactors to remove nitrogen of old landfill leachate. Waste Manag 74:335–346. https://doi.org/10.1016/j.wasman.2018.01.010

Peng W, Lü F, Hao L et al (2020) Digestate management for high-solid anaerobic digestion of organic wastes: a review. Bioresour Technol 297:122485. https://doi.org/10.1016/j.biortech.2019.122485

Piveteau S, Picard S, Dabert P, Daumer ML (2017) Dissolution of particulate phosphorus in pig slurry through biological acidification: a critical step for maximum phosphorus recovery as struvite. Water Res 124:693–701. https://doi.org/10.1016/j.watres.2017.08.017

Pognani M, D'Imporzano G, Scaglia B, Adani F (2009) Substituting energy crops with organic fraction of municipal solid waste for biogas production at farm level: a full-scale plant study. Process Biochem 44:817–821. https://doi.org/10.1016/j.procbio.2009.03.014

Polak J, Sułkowski WW, Bartoszek M, Papież W (2005) Spectroscopic studies of the progress of humification processes in humic acid extracted from sewage sludge. J Mol Struct 744:983–989. https://doi.org/10.1016/j.molstruc.2004.12.054

Poulsen HD (1998) Zinc and copper as feed additives, growth factors or unwanted environmental factors. J Anim Feed Sci 7:135–142

Powers WJ, Van Horn HH, Wilkie AC et al (1999) Effects of anaerobic digestion and additives to effluent or cattle feed on odor and odorant concentrations. J Anim Sci 77:1412–1421

Prevot AB, Avetta P, Berto S et al (2015) Soluble bio-based substances isolated from urban wastes. https://doi.org/10.1007/978-3-319-14744-4

P-REX (2017) Main P-REX deliverables—technical factsheets. https://doi.org/10.5281/zenodo.242550

Prot T, Nguyen VH, Wilfert P et al (2019) Magnetic separation and characterization of vivianite from digested sewage sludge. Sep Purif Technol 224:564–579. https://doi.org/10.1016/j.seppur.2019.05.057

Quagliotto P, Montoneri E, Tambone F et al (2006) Chemicals from wastes: compost-derived humic acid-like matter as surfactant. Environ Sci Technol 40:1686–1692

Quintern M, Morley M (2017) One solution fits all—how households, schools, prisons, municipalities and industries successfully use vermicomposting for organic waste upcycling. In: Proceedings Sardinia 2017—sixteenth international waste management and landfill symposium. CISA, S. Margherita di Pula, Cagliari, Italy

Rajpal A, Bhargava R, Chopra AK, Kumar T (2014) Vermistabilization and nutrient enhancement of anaerobic digestate through earthworm species Perionyx excavatus and Perionyx sansibaricus. J Mater Cycles Waste Manag 16:219–226. https://doi.org/10.1007/s10163-013-0167-0

Raymond B, Federici BA (2017) In defence of Bacillus thuringiensis, the safest and most successful microbial insecticide available to humanity—a response to EFSA.

FEMS Microbiol Ecol 93:1–8. https://doi.org/10.1093/femsec/fix084

Reis RS, Pacheco GJ, Pereira AG, Freire DMG (2013) Biosurfactants: production and applications. In: Biodegradation—life of science. InTech, p 64

Reza MT, Coronella C, Holtman KM et al (2016) Hydrothermal carbonization of autoclaved municipal solid waste pulp and anaerobically treated pulp digestate. ACS Sustain Chem Eng 4:3649–3658. https://doi.org/10.1021/acssuschemeng.6b00160

Risberg K, Cederlund H, Pell M et al (2017) Comparative characterization of digestate versus pig slurry and cow manure—chemical composition and effects on soil microbial activity. Waste Manag 61:529–538. https://doi.org/10.1016/j.wasman.2016.12.016

Robson TC, Braungardt CB, Rieuwerts J, Worsfold P (2014) Cadmium contamination of agricultural soils and crops resulting from sphalerite weathering. Environ Pollut 184:283–289. https://doi.org/10.1016/j.envpol.2013.09.001

Romero Güiza MS, Mata Alvarez J, Chimenos Rivera JM, Astals Garcia S (2016) Nutrient recovery technologies for anaerobic digestion systems: an overview. Rev Investig Optim Nuevos Procesos En Ing 29:7–26. https://doi.org/10.18273/revion.v29n1-2016001

Rosas-Garcia N (2009) Biopesticide production from bacillus thuringiensis: an environmentally friendly alternative. Recent Pat Biotechnol 3:28–36. https://doi.org/10.2174/187220809787172632

Salati S, Papa G, Adani F (2011) Perspective on the use of humic acids from biomass as natural surfactants for industrial applications. Biotechnol Adv 29:913–922. https://doi.org/10.1016/j.biotechadv.2011.07.012

Sambusiti C, Monlau F, Ficara E et al (2015) Comparison of various post-treatments for recovering methane from agricultural digestate. Fuel Process Technol 137:359–365. https://doi.org/10.1016/j.fuproc.2015.04.028

Sambusiti C, Monlau F, Barakat A (2016) Bioethanol fermentation as alternative valorization route of agricultural digestate according to a biorefinery approach. Bioresour Technol 212:289–295. https://doi.org/10.1016/j.biortech.2016.04.056

Savarino P, Montoneri E, Bottigliengo S et al (2009) Biosurfactants from urban wastes as auxiliaries for textile dyeing. Ind Eng Chem Res 48:3738–3748

Sawatdeenarunat C, Nguyen D, Surendra KC et al (2016) Anaerobic biorefinery: current status, challenges, and opportunities. Bioresour Technol 215:304–313. https://doi.org/10.1016/j.biortech.2016.03.074

Scarlat N, Dallemand J-F, Fahl F (2018) Biogas: developments and perspectives in Europe. Renew Energy 129:457–472. https://doi.org/10.1016/J.RENENE.2018.03.006

Schievano A, D'Imporzano G, Salati S, Adani F (2011) On-field study of anaerobic digestion full-scale plants (Part I): an on-field methodology to determine mass, carbon and nutrients balance. Bioresour Technol 102:7737–7744. https://doi.org/10.1016/j.biortech.2011.06.006

Schmidt H-P (2012) 55 uses of biochar. Ithaka J 1:286–289

Serna-Maza A, Heaven S, Banks CJ (2015) Biogas stripping of ammonia from fresh digestate from a food waste digester. Bioresour Technol 190:66–75. https://doi.org/10.1016/j.biortech.2015.04.041

Seruga P, Krzywonos M, Paluszak Z et al (2020) Pathogen reduction potential in anaerobic digestion of organic fraction of municipal solid waste and food waste. Molecules 25:275. https://doi.org/10.3390/molecules25020275

Shalaby EA (2014) A review of selected non-edible biomass sources as feedstock for biodiesel production. In: Biernat K (ed) Biofuels—status and perspective. IntechOpen

Sheets JP, Yang L, Ge X et al (2015) Beyond land application: emerging technologies for the treatment and reuse of anaerobically digested agricultural and food waste. Waste Manag 44:94–115. https://doi.org/10.1016/j.wasman.2015.07.037

Sigurnjak I, Vaneeckhaute C, Michels E et al (2017) Fertilizer performance of liquid fraction of digestate as synthetic nitrogen substitute in silage maize cultivation for three consecutive years. Sci Total Environ 599–600:1885–1894. https://doi.org/10.1016/J.SCITOTENV.2017.05.120

Silva E, Brás I (2016) Potential valorization as fertilizers of Humic Substances extracted from landfill leachate. In: International congress on water, waste and energy management

Sizmur T, Fresno T, Akgül G et al (2017) Biochar modification to enhance sorption of inorganics from water. Bioresour Technol 246:34–47. https://doi.org/10.1016/J.BIORTECH.2017.07.082

Smith SR (2009) A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. Environ Int 35:142–156. https://doi.org/10.1016/J.ENVINT.2008.06.009

Smith KA, Jeffrey WA, Metcalfe JP et al (2010) Nutrient value of digestate from farm-based biogas plants. In: 14th Ramiran international conference. Lisboa, Portugal, pp 2–5

Someus E (2015) REFERTIL project closing summary

Stasinakis AS (2012) Review on the fate of emerging contaminants during sludge anaerobic digestion. Bioresour Technol 121:432–440. https://doi.org/10.1016/j.biortech.2012.06.074

Stefaniuk M, Oleszczuk P (2015) Characterization of biochars produced from residues from biogas production. J Anal Appl Pyrolysis 115:157–165. https://doi.org/10.1016/J.JAAP.2015.07.011

Stefaniuk M, Bartmiński P, Różyło K et al (2015) Ecotoxicological assessment of residues from different biogas production plants used as fertilizer for soil. J Hazard Mater 298:195–202. https://doi.org/10.1016/j.jhazmat.2015.05.026

Stoknes K, Beyer DM, Norgaard E (2013) Anaerobically digested food waste in compost for *Agaricus bisporus* and *Agaricus subrufescens* and its effect on mushroom productivity. J Sci Food Agric 93:2188–2200. https://doi.org/10.1002/jsfa.6026

Stoknes K, Scholwin F, Krzesiński W et al (2016) Efficiency of a novel "Food to waste to food" system including anaerobic digestion of food waste and cultivation of vegetables on digestate in a bubble-insulated greenhouse. Waste Manag 56:466–476. https://doi.org/10.1016/j.wasman.2016.06.027

Straka F, Jenicek P, Zabranska J et al (2007) Anaerobic fermentation of biomass and wastes with respect to sulfur and nitrogen contents in treated materials. In: Proceedings Sardinia 2007. S. Margherita di Pula, Cagliari, Italy

SUEZ (2017a) SUEZ aide Montpellier à mieux valoriser ses déchets. https://www.suez.com/fr/Notre-offre/Succes-commerciaux/Nos-references/Methanisation-des-dechets-Ametyst-Montpeliier. Accessed 7 Apr 2019

SUEZ (2017b) PhosphogreenTM: recycler le phosphore des eaux usées pour produire et revendre de l'engrais. Guide Nouv Tech Eau Ind Nuis 4052:143

Sun Q, Li H, Yan J et al (2015) Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation. Renew Sustain Energy Rev 51:521–532. https://doi.org/10.1016/J.RSER.2015.06.029

Szogi AA, Vanotti MB (2015) Phosphorus recovery prior to land application of biosolids using the "Quick wash" process developed by USDA. In: 16th Ramiran international conference. Hamburg, Germany

Tadda MA, Ahsan A, Shitu A et al (2016) A review on activated carbon: process, application and prospects. J Adv Civ Eng Pract Res 2:7–13

Taghizadeh-Toosi A, Clough TJ, Sherlock RR, Condron LM (2012) Biochar adsorbed ammonia is bioavailable. Plant Soil 350:57–69. https://doi.org/10.1007/s11104-011-0870-3

Takemura K, Endo R, Shibuya T, Kitaya Y (2019) Application of biogas digestate as a nutrient solution for the hydroponic culture of chrysanthemum morifolium ramat with rock-wool substrate. Waste Biomass Valorization. https://doi.org/10.1007/s12649-018-00576-8

Talboys PJ, Heppell J, Roose T et al (2016) Struvite: a slow-release fertiliser for sustainable phosphorus management? Plant Soil 401:109–123. https://doi.org/10.1007/s11104-015-2747-3

Tambone F, Genevini P, D'Imporzano G, Adani F (2009) Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. Bioresour Technol 100:3140–3142. https://doi.org/10.1016/j.biortech.2009.02.012

Tambone F, Scaglia B, D'Imporzano G et al (2010) Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81:577–583. https://doi.org/10.1016/j.chemosphere.2010.08.034

Tambone F, Terruzzi L, Scaglia B, Adani F (2015) Composting of the solid fraction of digestate derived from pig slurry: biological processes and compost properties. Waste Manag 35:55–61. https://doi.org/10.1016/j.wasman.2014.10.014

Tampio E (2016) Utilization of food waste via anaerobic digestion: from feedstock to biogas and fertilizers. Tampere University of Technology (thesis)

Tan Z, Lin CSK, Ji X, Rainey TJ (2017) Returning biochar to fields: a review. Appl Soil Ecol 116:1–11. https://doi.org/10.1016/j.apsoil.2017.03.017

Tao W, Fattah KP, Huchzermeier MP (2016) Struvite recovery from anaerobically digested dairy manure: a review of application potential and hindrances. J Environ Manag 169:46–57. https://doi.org/10.1016/J.JENVMAN.2015.12.006

Tay J (1987) Bricks manufactured from sludge. J Environ Eng 113:278–284. https://doi.org/10.1061/(ASCE)0733-9372(1987)113:2(278)

Teglia C, Tremier A, Martel J-L (2010) Characterization of solid digestates: part 1, review of existing indicators to assess solid digestates agricultural use. Waste Biomass Valorization 2:43–58. https://doi.org/10.1007/s12649-010-9051-5

Teglia C, Tremier A, Martel J-L (2011) Characterization of solid digestates: part 2, assessment of the quality and suitability for composting of six digested products. Waste Biomass Valorization 2:113–126. https://doi.org/10.1007/s12649-010-9059-x

Tesfamichael AA, Stoknes K (2017) Substitution of peat with vermicompost from food waste digestate and green waste compost. Acta Hortic 1168:399–406. https://doi.org/10.17660/ActaHortic.2017.1168.51

The Commission of the European Communities (2011) Comission Regulation (EU) No 142/2011 of 25 February 2011. Off J Eur Union

Tian Z, Zhang Y, Yu B, Yang M (2016) Changes of resistome, mobilome and potential hosts of antibiotic resistance genes during the transformation of anaerobic digestion from mesophilic to thermophilic. Water Res 98:261–269. https://doi.org/10.1016/j.watres.2016.04.031

Tigini V, Franchino M, Bona F, Varese GC (2016) Is digestate safe? A study on its ecotoxicity and environmental risk on a pig manure. Sci Total Environ 551:127–132

Tremier A, Buffet J, Daumoin M (2014) Projet DIVA—Tâches 4.1.2—comportement des digestats en compostage 1–20

Tur Cardona J, Speelman S, Verpecht A, Buysse J (2015) Farmers' reasons to accept bio-based fertilizers—a choice experiment in Flanders. In: 16th Ramiran international conference. Hamburg, Germany

Uggetti E, Sialve B, Trably E, Steyer JP (2014) Integrating microalgae production with anaerobic digestion: a biorefinery approach. Biofuels Bioprod Biorefining 8:516–529. https://doi.org/10.1002/bbb.1469

U.S. Department of Agriculture (USDA), U.S. Environmental Protection Agency (EPA), U.S. Department of Energy (DOE) (2014) Biogas Opportunities Roadmap, Washington, DC

U.S. Environmental Protection Agency (EPA) (2012) Technology assessment report—aqueous sludge gasification technologies (version 1.5). Greenhouse Gas Technology Center, Southern Research Institute

U.S. Environmental Protection Agency (EPA) (2019b) Biopesticides. https://www.epa.gov/pesticides/biopesticides. Accessed 21 Jan 2019

Uysal A, Yilmazel YD, Demirer GN (2010) The determination of fertilizer quality of the formed struvite from effluent of a sewage sludge anaerobic digester. J Hazard Mater 181:248–254. https://doi.org/10.1016/j.jhazmat.2010.05.004

Vaccari FP, Baronti S, Lugato E et al (2011) Biochar as a strategy to sequester carbon and increase yield in durum wheat. Eur J Agron 34:231–238. https://doi.org/10.1016/j.eja.2011.01.006

VALDIPRO (2015) Digestat séché—séchage de la fraction solide ou du digestat brut. Rennes

Van Bruwaene R, Kirchmann R, Impens R (1984) Cadmium contamination in agriculture and zootechnology. Experientia 40:43–52

Vaneeckhaute C, Meers E, Michels E et al (2013a) Ecological and economic benefits of the application of bio-based mineral fertilizers in modern agriculture. Biomass Bioenergy 49:239–248. https://doi.org/10.1016/j.biombioe.2012.12.036

Vaneeckhaute C, Meers E, Michels E et al (2013b) Closing the nutrient cycle by using bio-digestion waste derivatives as synthetic fertilizer substitutes: a field experiment. Biomass Bioenergy 55:175–189. https://doi.org/10.1016/j.biombioe.2013.01.032

Vaneeckhaute C, Zeleke AT, Tack FMG, Meers E (2016) Comparative evaluation of pre-treatment methods to enhance phosphorus release from digestate. Waste Biomass Valor. https://doi.org/10.1007/s12649-016-9647-5

Vaneeckhaute C, Lebuf V, Michels E et al (2017) Nutrient recovery from digestate: systematic technology review and product classification. Waste Biomass Valorization 8:21–40. https://doi.org/10.1007/s12649-016-9642-x

Vanotti MB, Dube PJ, Szogi AA, García-González MC (2017) Recovery of ammonia and phosphate minerals from swine wastewater using gas-permeable membranes. Water Res 112:137–146. https://doi.org/10.1016/j.watres.2017.01.045

Vargas AKN, Bianco Prevot A, Montoneri E et al (2014) Use of biowaste-derived biosurfactants in production of emulsions for industrial use. Ind Eng Chem Res 53:8621–8629. https://doi.org/10.1021/ie4037609

Vijayakuma S, Saravanan V (2015) Biosurfactants-types, sources and applications. Res J Microbiol 10:181–192. https://doi.org/10.3923/jm.2015.181.192

Villaverde JJ, Sevilla-Morán B, Sandín-España P et al (2014) Biopesticides in the framework of the European Pesticide Regulation (EC) No. 1107/2009. Pest Manag Sci 70:2–5. https://doi.org/10.1002/ps.3663

Vondra M, Máša V, Touš M, Konečná E (2018) Vacuum evaporation of a liquid digestate from anaerobic digestion: a techno-economic assessment. Chem Eng Trans 70:769–774. https://doi.org/10.3303/CET1870129

Wahal S, Viamajala S, Hansen CL (2010) Chemical speciation in the effluent of an anaerobic digester treating dairy waste: implications for nutrient recovery and reuse. Trans ASABE 53:1727–1732. https://doi.org/10.13031/2013.34883

Wiśniewski D, Gołaszewski J, Białowiec A (2015) The pyrolysis and gasification of digestate from agricultural biogas plant. Arch Environ Prot 41:70–75. https://doi.org/10.1515/aep-2015-0032

Wongrod S, Simon S, van Hullebusch ED et al (2018) Changes of sewage sludge digestate-derived biochar properties after chemical treatments and influence on As(III and V) and Cd(II) sorption. Int Biodeterior Biodegrad 135:96–102. https://doi.org/10.1016/J.IBIOD.2018.10.001

Wu S, He H, Inthapanya X et al (2017) Role of biochar on composting of organic wastes and remediation of contaminated soils—a review. Environ Sci Pollut Res 24:16560–16577. https://doi.org/10.1007/s11356-017-9168-1

Xia A, Murphy JD (2016) Microalgal cultivation in treating liquid digestate from biogas systems. Trends Biotechnol 34:264–275. https://doi.org/10.1016/j.tibtech.2015.12.010

Yao Y, Gao B, Inyang M et al (2011) Biochar derived from anaerobically digested sugar beet tailings: characterization and phosphate removal potential. Bioresour Technol 102:6273–6278. https://doi.org/10.1016/J.BIORTECH.2011.03.006

Yetilmezsoy K, Ilhan F, Kocak E, Akbin HM (2017) Feasibility of struvite recovery process for fertilizer industry: a study of financial and economic analysis. J Clean Prod 152:88–102. https://doi.org/10.1016/j.jclepro.2017.03.106

Yetilmezsoy K, Kocak E, Akbin HM, Özçimen D (2018) Utilization of struvite recovered from high-strength ammonium-containing simulated wastewater as slow-release fertilizer and fire-retardant barrier. Environ Technol 3330:1–18. https://doi.org/10.1080/09593330.2018.1491642

Yokoyama S, Matsumura Y (2015) The present status and future scope of bioenergy in Japan. Nihon Energi GakkaishiJournal Jpn Inst Energy 94:1079–1086. https://doi.org/10.3775/jie.94.1079

Youngquist CP, Mitchell SM, Cogger CG (2016) Fate of antibiotics and antibiotic resistance during digestion and composting: a review. J Environ Qual 45:537. https://doi.org/10.2134/jeq2015.05.0256

Yuste R (2016) Biofuels production in conventional oil refineries through bio-oil co-processing (REPSOL). In: Joint workshop of ENMIX, FASTCARD, CASCATBEL, BIOGO. Stuttgart, Germany

Zandvoort MH, van Hullebusch ED, Fermoso FG, Lens PNL (2006) Trace metals in anaerobic granular sludge reactors: bioavailability and dosing strategies. Eng Life Sci 6:293–301

Zeng Y, Guardia A, Ziebal C et al (2014) Nitrogen dynamic and microbiological evolution during aerobic treatment of digested sludge. Waste Biomass Valorization 5:441–450. https://doi.org/10.1007/s12649-013-9275-2

Zeng Y, De Guardia A, Dabert P (2015) Improving composting as a post-treatment of anaerobic digestate. Bioresour Technol 201:293–303. https://doi.org/10.1016/j.biortech.2015.11.013

Zhang Y, Banks CJ (2013) Impact of different particle size distributions on anaerobic digestion of the organic fraction of municipal solid waste. Waste Manag 33:297–307. https://doi.org/10.1016/j.wasman.2012.09.024

Zhang CM, Mao ZG, Wang X et al (2010) Effective ethanol production by reutilizing waste distillage anaerobic digestion effluent in an integrated fermentation process coupled with both ethanol and methane fermentations. Bioprocess Biosyst Eng 33:1067–1075. https://doi.org/10.1007/s00449-010-0432-8

Zirkler D, Peters A, Kaupenjohann M (2014) Elemental composition of biogas residues: variability and alteration during anaerobic digestion. Biomass Bioenergy 67:89–98. https://doi.org/10.1016/j.biombioe.2014.04.021

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The impact of biogas digestate typology on nutrient recovery for plant growth: Accessibility indicators for first fertilization prediction

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ABSTRACT

In recent years, anaerobic digestion of organic waste (OW) is rapidly appearing as a winning waste management strategy by producing energy and anaerobic digestates that can be used as fertilizers in agricultural soils. In this context, the management of the OW treatment process to maximize agro-system sustainability satisfying the crop nutrient demands represents the main goal. To investigate these traits, two protocols to assess the plant availability of digestate nitrogen (N) and phosphorus (P) were evaluated. With this aim, the N and P availability was determined on 8 digestates and 2 types of digestate-based compost from different OW via sequential chemical extractions (SCE). In addition, the digestates were tested in soil incubations and in plant pot tests with Italian ryegrass and compared with chemical fertilizer and a non-amended control soil. The N extracted from digestates via SCE was related to soil N mineralization and plant N recovery. The C: N ratio had negative impact on mineralized N and its recovery in shoots ($Shoots_N = -0.0085.(C/N)+0.172$, $r^2 = 0.67$), whereas water extractable mineral N was positevely related to the root N apparent recovery fraction (N-ARF) with ($Roots_N = 5E^{-5}.N_{solublemin}+0.0138$, $r^2 = 0.53$). The shoot P-ARF was positively correlated with the inorganic water extractable fraction of P ($Shoots_P = 0.1153.H_2O\text{-}P_i - 0.2777.H_2O\text{-}P_o + 0.0249$, $r^2 = 0.71$) whereas the root P-ARF was positively correlated with the less accessible fractions ($Roots_P =$ (b) $0.0955.NaHCO_3\text{-}P_o+0.0955.NaOH\text{-}P_o\text{-}0.0584NaHCO_3\text{-}P_i+0.0128$, $r^2 = 0.8641$). Feedstock digestate typology impacted the N and P recovery results leading to a better description of the typology properties and a first nutrients ARF prediction.

1. Introduction

An increasing interest exists in improving the soil quality following the utilization of organic wastes (OW) within the environmentally friendly bio-refinery approach (Alburquerque et al., 2012; Gissén et al., 2014). In this respect, anaerobic digestion is a major building block in the circular bioeconomy. Furthermore, both energy (ie. biomethane) and organic fertilizers (ie. digestate) replacing the chemical fertilizers provided to crops can be recovered from anaerobic digestion of OW. From an agronomic and economic point of view, digestate use as a fertilizer can be considered not only as a supplement for traditional organic fertilizers (i.e. slurry) but also as an alternative fertilizer, which under certain soil conditions, is more effective than using NPK fertilizer (Barłóg et al., 2019). Indeed, anaerobic digestion is reported as a suitable treatment to easily recover P and N fertilizers from digestate (Mazzini et al., 2020). This is due to organic matter and free organic nutrients biodegraded into mineral forms. However, agricultural reuse of treated OW as soil fertilizers is limited by environmental constraints related to the quality of organic matter, nutrient availability and safety issues. Furthermore, the chemical composition of digestate depends on the nature of feedstock and digestion process (Alburquerque et al., 2012, Guilayn et al., 2019, Barłóg et al., 2019). Indeed, a statistical classification of 91 digestates based on their agronomic quality (i.e. C, N, P, total solids, organic matter) was applied on a large range of raw digestates by Guilayn et al. (2019) and revealed 6 groups of different digestate typology. By analyzing the obtained digestates groups, two criteria impacted the classification: the TS concentration of the digestates associated with dry or wet anaerobic digestion process and the feedstock type, as following: liquid fibrous feedstock (crop residues silage, cattle slurry), liquid sewage sludge, liquid pig slurry, slurries co-digested with silage and green wastes (dry anaerobic digestion), municipal solid wastes (dry anaerobic digestion) and fibrous feedstock (dry anaerobic digestion of cattle manure and green waste).

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In this framework, mineral N can be readily available for crops but can be temporarily immobilized by microbial biomass during OW mineralization in soil (Lashermes et al., 2010). Therefore, it is important to know the OW mineralization N kinetics to optimize the N supply synchronisation with plant requirement in agricultural systems.

In this context, to better control and manage the quality of digestates, a better knowledge of the accessibility and availability of nutrients and organic matter would improve the fertilizing potential of these products. According to Möller and Müller (2012), an accurate characterization of digestate nutrient content and organic matter (OM) composition, combined with experiments to assess the N mineralization and N immobilization processes after field spreading, would be essential for a better characterization of the driving factors driving N turnover in the soil.

Total content of nutrients in OW harldy predicts their fertilizer potential. Ahmad et al. (2018) reported more than 80% of applied P is rapidly immobilized being unavailable for plant following adsorption/precipitation processes or conversion into organic form. To gain a better insight on this issue, many authors proposed to characterize the available P via P fractionation. He et al. (2010) reported that bioavailability of applied P depends on the presence of specific P forms and that labile P includes the sum of inorganic and organic P from H_2O and $NaHCO_3$ extracted fractions. In this regard, Grigatti et al. (2015, 2017, 2019), performed a dedicated SCE on digestates and composts in order to measure the phosphorous potentially available for plants. The authors found good correlations between the water- and the sodium bicarbonate-extractable P from composts with short- and medium-term plant P uptake.

Lashermes et al. (2010) proposed an OW classification based on their chemical characteristics to predict N availability. However, only 2% of the 273 OW studied came from similar typology of digestates coming from fibrous feedtstock anaerobic digestion with low N availability (i.e. digestates was classified in a group with initial N concentration under 65 g kg^{-1} and C: N ratio around 15). However, according to Guilayn et al. (2019), the N content from digestates varies from 20 g kg^{-1} to 175 g kg^{-1} and C: N ratio varies from 2 to more than 30. It would be interesting to complete the Lashermes study with different typologies of digestate to classify their fertilizer potential. To do that, the first step would be to evaluate the impact of digestate typology on plant growth. Recently, a methodology proposed for organic matter characterization has been applied to a large range of OW in order to predict both bio-avalability and complexity/biodegradability of the organic matter (Jimenez et al., 2015). This technique has been successfully used for OM biodegradation kinetics modelling (anaerobic digestion, compost, soil) (Jimenez et al., 2017) and for organic N dynamics in anaerobic digestion (Bareha et al., 2018, 2019). The basic idea was to find a consistent characterization method in order to describe the bioprocess kinetics and to model the whole treatment chain in terms of organic carbon fate, e.g. anaerobic digestion, compost and organic matter fate in soil. The methodology used is based on SCE to simulate organic matter bioaccessibility for microorganisms combined with three-dimensional fluorescence spectroscopy to analyze the organic matter complexity. The challenge is now to transfer this technique to the characterization of nitrogen accessibility in order to predict its fate after land-spreading and its availability for plant growth.

Apart from the previously mentioned studies, there is no literature information on the development of indicators for both N and P plant availability from digestates. This is the reason this study aimed to propose indicators based on chemical extractions and characterization for N and P.

In this light, the objectives of this work were to determine the plant-available N and P fractions based on characterization of the accessibility study in order to: (i) assess the impact of typology of OW on the nutrient availability, and (ii) use these indicators to predict plant's nutrients recovery. This study, focussed on a wide range of anaerobic digestates from many types of OW. The results could be used for rapid diagnostic of the (N, P) fertilization potential of a digestate.

2. Materials and methods

2.1. Organic waste

In order to ensure a representative survey, the anaerobic digestates used in this work were selected on the basis of the results reported by Guilayn et al. (2019). The typology developed by Guilayn et al. (2019) was used to select the digestate samples out of a large panel of digestates. For this study, 6 main groups were selected from a statistical study applied to the agronomic characteristics: sewage sludge, municipal waste, cow manure (dry-anaerobic digestion), pig manure (liquid anaerobic digestion), centralised (co-digestion), and crop residues. Table 1 presents the samples used with the type of OW used as feedstock and the conditions of the anaerobic digestion process. Post-treated digestates through phase separation (solid or liquid) and composting were also added. Indeed, phase separation is the most classical digestate post-treatment (Alburquerque et al., 2012), leading to two products of different quality (i.e. solid phase as soil amendment and liquid phase as fertilizer). Besides, two digestate-based types of compost (FFMSW_2 and Sludge_2) were used since composting is widely adopted treatment for the solid phase of urban OW digestate to meet the European fertilizing regulation parameters (EU Fertilisers Regulation COM, 2016). The organic waste samples were freeze-dried and ground (ø 1 mm), in order to reduce the particle size effect in the soil incubations and plant growth experiments.

2.2. Analytical measurements

Freeze-dried ground samples were further ball milled and analysed for the main physico-chemical parameters. The moisture was determined at 105 ± 2 °C until constant weight (24–48 h), the volatile solid (VS) was determined on the total solids (TS) at 550 °C for 4 h. The total carbon (TC) and total nitrogen (TN) were determined via an elemental analyser (FlashSmart, Thermo Fisher Scientific). The total nutrient and trace elements were determined by ICP (Inductively Coupled Plasma-OES, Spectro Arcos, Ametek) on ≈250 mg of samples after microwave assisted digestion with 65% HNO_3 + 37% HCl. All the analyses were done in duplicates.

Based on Jimenez et al. (2015), the sequential chemical extractions of organic matter were applied on the freeze-dried and grounded samples (0.5 g). An orbital shaker was used for the extractions steps as following described:30 mL of 0.01 M $CaCl_2$ (twice, 1 h, 30 °C, 300 rpm) ; 0.01 M (NaOH + NaCl) (4 times, 15 min, 30 °C, 300 rpm); 0.1 M HCl (once, 1 h, 30 °C, 300 rpm), 0.1 M NaOH (4 times, 1 h, 30 °C, 300 rpm) and 72% H_2SO_4 (twice, 3 h, 30 °C, 300 rpm). The fractions names are respectively: Soluble extracted from Particulate Organic Matter (SPOM), Readily Extractible Organic Matter (REOM), Slowly Extractible Organic Matter (SEOM) and Poorly Extractible Organic Matter (PEOM). The not extracted fraction is called Non Extractible Organic Matter (NEOM). Each extraction was done in two replicates, centrifuged and the supernatant collected and filtered. The recovered pellets were submitted to the subsequent extraction. Ammonium and total nitrogen were measured in the supernatants using HachLange® kits based on colorimetry methods (LCK 303, 2–47 mg N L^{-1}, and LCK 238, 5–40 mg L^{-1} respectively).

The organic products were submitted to P fractionation via SCE according to the method of Dou et al. (2000). Freeze-dried and ball-

Table 1
Definition and origins of digestate samples.

Sample Name	Type	Scale	Anaerobic Digestion conditions	Digester Hydraulic retention time	Post-treatment	Substrate	Reference
Agri_1	Digestate	Operating scale (farmer)	Dry batch mesophilic	60 days	–	Cow manure	This study
Agri_2	Digestate	Operating scale (farmer)	Liquid continuous mesophilic	60 days	–	Pig manure co-digested with energetic crop and vegetable residues	
Agri_3	Digestate	Lab scale	Dry batch mesophilic	100 days	–	wheat straw silage	
Sludge_1	Solid phase from digestate	Operating scale (private agency)	Liquid continuous mesophilic	20 days	Press filter	wastewater treatment sludge	
Sludge_2	Compost of solid digestate	Operating scale (private agency)	Liquid continuous mesophilic	20 days	Press filter and composting	digestate of wastewater treatment sludge (1/3) and green wastes (2/3)	
FFMSW_1	Digestate	Operating scale (private agency)	Dry continous thermophilic	20 days	–	Fermentable fraction from municipal solid waste (FFMW)	
FFMSW_2	Compost of digestate	Operating scale (private agency)	Dry continuous thermophilic 20	20 days	Composting	96%FFMSW, 50% green waste, 14% agro-industrial wastes	
BW_1	Digestate	Operating scale (governmental)	Liquid continous mesophilic	60 days	–	Biowastes (60%) from supermarkets co-digested with agro-industrial wastes (28%) and crop residues (12%)	
Centr_1	Solid phase of digestate	Operating scale (governmental)	Liquid continous mesophilic	45 days	screw press	Oil (20%), crop residues (10%), agro_industrial wastes (55%), sewage sludge (10%), biowaste (5%)	
Centr_2	Liquid phase of digestate	Operating scale (governmental)	Liquid continous mesophilic	45 days	screw press		
D1	Digestate	Operating scale	Liquid continous thermophilic	na	–	Wastewater treatment sludge	Grigatti et al. (2019)
D2	Digestate	Operating scale	Liquid continous mesophilic	na	–	Wine sludge	
BD	Digestate	Operating scale	Liquid continous thermophilic	na	–	Bovine slurry and energy crops	

milled products were extracted for 24 h with deionized water (H_2O) in an end-over-end shaker, and then centrifuged. The supernatants were passed through a Whatman #42 filter, and the recovered pellets were extracted with 0.5 M $NaHCO_3$ (pH 8.5) for 24 h. The same procedure was repeated with 0.1 N NaOH and 1 N HCl. The residual pellets were treated with a mixture of 96% H_2SO_4 and 30% H_2O_2 via hot acid digestion at 360 °C. Inorganic P (P_i) in the extracts was determined via the molybdenum blue method of Murphy and Riley (1962). The P recovered in each fraction [water extractable P (H_2O-P), bicarbonate extractable P ($NaHCO_3$-P), alkali extractable P (NaOH-P), acid extractable P (HCl-P), Residual-P] was calculated as follows:

$$P_{i\,fraction\ x}(\%) = \frac{Pi_{fraction\ x}}{P_{tot\ OW}} \times 100 \qquad (1)$$

where $P_{i;fraction\ x}$ is the inorganic P determined in each fraction (H_2O, $NaHCO_3$, NaOH, HCl, Residual), and $P_{tot\ OW}$ is the total-P determined in the different organic waste via ICP after microwave-assisted acid digestion.

The total recovery was calculated as the sum of all fractions (H_2O-P + $NaHCO_3$-P + NaOH-P + HCl-P + Residual-P) by using the Equation2:

$$Tot\ P_{recovery}(\%) = \frac{\sum_{H2O}^{Residual} P_{i;o\ fraction\ x}}{P_{tot\ OW}} \times 100 \qquad (2)$$

where $\sum_{H2O}^{Residual} P_{i;o\ fraction\ x}$ represents the sum of the single P recovery values (P_i) in each fraction (H_2O-P + $NaHCO_3$-P + NaOH-P + HCl-P + Residual-P), and $P_{tot\ OW}$ is the total P determined in the different products via ICP after microwave-assisted acid digestion. Organic P was calculated as the difference between P_{tot} and P_i in the first four fractions.

According to He et al. (2010), H_2O-P, NaHCO3-P, NaOH-P and HCl-P are respectively water-soluble, bioavailable, potential bioavailable (Fe/Al bound) and Ca-bound P.

2.3. X-ray diffraction

Samples submitted to X-ray diffraction analysis were carefully ground and placed in a flat stage sample holder. Data were collected by means of a PANalytical X'Pert PRO powder diffractometer equipped with a fast X'Celerator detector. Cu Kα radiation was used (40 mA, 40 kV). The 2θ range was from 5° to 60° with a step size of 0.1° and time/step of 100 s. Data were processed for phase identification using a HighScore Plus software package (PANalytical).

2.4. Soil incubation

2.4.1. Soil

The soil used for the incubation and pot trial was collected from the top layer in a field in the Po Valley (Bologna, Italy). This soil showed the following characteristics: pH (H_2O 1:2.5), 7.90; particle-size distribution; 184 mg kg^{-1}, sand, 425 mg kg^{-1}, silt, 391 mg kg^{-1} clay; total $CaCO_3$, 85 g kg^{-1}; total organic carbon (TOC), 10.2 g kg^{-1} ; total Kjeldahl nitrogen (TKN), 1.60 g kg^{-1}; C: N, 8.3; exchangeable K, 330 mg kg^{-1} as K_2O, CEC 27.2 meq. 100 g^{-1}. The total (extractable in aqua regia + HF) Al, Fe and P were 35661, 22,224 and 808 mg kg^{-1} respectively. The NH_4-oxalate (pH 3) extractable Al and Fe were 764 and 2158 mg kg^{-1} respectively, while the Na dithionite-citrate extractable Al and Fe were 281 and 2462 mg kg^{-1} respectively.

2.4.2. Incubation tests

Soil incubation was conducted in 500 mL plastic vessels with perforated plastic cap, on 250 g of soil (TS basis). The soil was rewetted at 60% of the water holding capacity (WHC) and pre-incubated for 4 weeks at 25 °C in the dark. Water content was kept at 60% of WHC by weighing the vessels every 2–3 days and by adjusting with water drops if needed. After this period, the organic products (i.e. digestates) were added to the soil at 170 kg N ha^{-1}, defined as the maximum N load per year authorized by the European Nitrates Directive (1991) and accurately mixed by hand. Chemical references used as positive control ($Ctrl^{+}$) were added by distributing N (as NH_4NO_3) at the same concentration of 170 kg N ha^{-1}, and in addition P and K were added at 117 and 147 kg ha^{-1} (as KH_2PO_4). On the basis of N loading the P added to the soil with the different organic product was on average 20.45 ± 2.5 mg P kg $soil^{-1}$. Calculation of the amount of digestate mass added is described by the Equations (3)–(5) considering 0.3 m of soil depth (d) and a bulk density of 1.33 g/cm^3 for 1 ha of soil.

$$\mathrm{dosis}\left(\frac{\mathrm{mgN}}{\mathrm{kgsoil}}\right)=\frac{dosis\left(\frac{mgN}{ha}\right)}{area_{1ha}\times d}=\frac{170\times 10^{6}}{10^{4}\times 0.3\times 1.3\times 10^{3}}=44\ \mathrm{mgN}\ \mathrm{kg}^{-1} \tag{3}$$

$$\mathrm{dosis}\left(\frac{\mathrm{gN}}{\mathrm{kgsoil}}\right)=\frac{\mathrm{m_{digestate}}\times \mathrm{N_{digestate}}\times \mathrm{TS_{digestate}}}{\mathrm{m_{soil}}} \tag{4}$$

From the Eq. (4), digestate amount is calculated as:

$$\mathrm{m_{digestate}}=\frac{\mathrm{dosis}\times \mathrm{m_{soil}}}{\mathrm{N_{digestate}}\times \mathrm{TS_{digestate}}} \tag{5}$$

$m_{digestate}$ is the digestate mass (kg)
$N_{digestate}$ is the N concentration of the digestate (gN kgTS-1)
$TS_{digestate}$ is the Total solids content of the digestate (% of fresh matter)
m_{soil} is the considered soil mass in the test (kg)

Three replicates for each treatment were assessed in a completely randomized block design, in addition to an unfertilized treatment control ($Ctrl^{-}$). Two parallel soil sampling series were done to follow the P and N evolution during incubation. Olsen-P, was determined according to Watanabe and Olsen (1965) at days: 0, 14, 28, 56, 84. The Olsen-P data were used to calculate the relative percentage extractable (RPE) P, used to normalise the P extractability for each treatment relative to Olsen-P obtained for $Ctrl^{+}$ (Grigatti et al., 2019). The mineral N course in soil was assessed by extracting soil samples (1 g TS basis) at days 0, 14, 28, 56 and 84 with 1 M KCl for 30 min on an end-over-and end shaker. The solution was filtered over a Whatman #42 filter and ammonium and nitrates were determined using Hach Lange® kits (LCK 304, 0.015–2 mg N L^{-1} and LCK 339, 0.23–13.5 mg N L^{-1} respectively).

2.5. Plant pot trials

Based on Grigatti et al. (2014) and Grigatti et al. (2015), the digestate samples were added to the ground soil at 170 mg N kg^{-1} and thoroughly mixed by hand in 2 L plastic pots (ø 140 mm × h 150 mm). These were filled with 1 L of inert material (agricultural light expanded clay), and 1 kg of each different treated soil in 3 replicates. Pots were seeded with 0.8 g of seeds of Italian ryegrass (*Lolium multiflorum* subsp. Italicum), cv. Sprint, covered with a thin layer of sand to prevent drying, watered and placed in a growth chamber at 16–18 h day-night photoperiod at 13–23 °C (±3 °C) day-night temperature, the light was ensured by 6 Philips Master Tld 58 W-840 tubes. Besides the organic products, an unfertilized control ($Ctrl^{-}$), and a chemical reference (at the same rate used for soil incubation) was added ($Ctrl^{+}$). The same treatments and dosis performed in soil incubation test were applied. The use of fast growing species as ryegrass in controlled conditions (moisture; temperature; light) leads to a multiple harvest approach giving the opportunity to the best description of apparent N and P utilization kinetics in the time frame of a growing season (Gunnarsson et al., 2010; Schiemenz and Eichler-Löbermann, 2010; Tampio et al., 2016).

After emergence, plants were regularly watered with tap water to keep soil at 60% Water Holding Capacity (WHC). At each harvest, ryegrass plants were cut 2 cm above ground and collected 3 times: at 28, 56 and 84 days after sowing. The plant biomass was then dried in a forced air oven at 60 °C for 3 days and weighed, to determine dry weight (DW) per pot. Dry biomass was also ball-milled for subsequent analysis. At the last harvest, the roots were divided from the soil with a combined water-sieving separation, and the root biomass was then dried as above, weighed and milled. On plant tissue (shoots and roots), the total P content was determined by means of ICP after (HNO_3, H_2O_2,) microwave assisted digestion. The total N content was determined by elementar analysis as the OW samples. Apparent plant N and P utilization efficiency was calculated on the basis of the Apparent Recovery Fraction (ARF) approach (Gunnarsson et al., 2010), according to the Eq. (6):

$$\mathrm{ARF\_X}(\%)=\sum_{\mathbf{i}=1}^{3}\frac{\mathbf{Xuptaketreatment_{ti}}-\mathbf{iXuptakectrl}-_{\mathbf{ti}}}{\mathbf{Xadded}} \tag{6}$$

in which X uptake treatment (t_n) is the total nitrogen or phosphorus uptake (mg pot^{-1}) of a fertilizer treatment at time t (i = cut 1–3.); X uptake $ctrl^{-}_{ti}$ is the total nitrogen or phosphorus uptake (mg pot^{-1}) of the unfertilized control at time t (i = cut 1–3); X added is the total nitrogen or phosphorus added to the pot (mg pot^{-1}).

Chemical nutrient equivalent coefficient k_{eq} is defined as the equivalent chemical nutrient dosis (i.e. positive control) percentage needed to reach similar crop yield and is calculated according the Eq. (7).

$$\mathrm{k_{eq}X}=\frac{\mathrm{ARF\_X}}{\mathrm{ARF_{Ctrl+}}} \tag{7}$$

2.6. Statistical analysis

All the data from the plant growth experiment were analyzed by means of Kruskal-Wallis non parametric test. Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) were also performed on all the data using FactomineR package from the R software. Partial Least Square Regression (PLSR) was performed for variables prediction using the SIMCA® software. For PCA analysis, data from Grigatti et al. (2019) were included as far as similar experiments were done on P speciation and plant pot tests. The three samples were D1, D2 and BD, which are respectively digestate from thermophilic wastewater sludge digestion, digestate from mesophilic winery sludge treatment and digestate from mesophilic bovine slurry and energy crops treatment. Table 1 presents these samples, the conditions of the anaerobic digestion process and the associated feedstocks.

3. Results and discussion

3.1. Digestates characterization

The main physico-chemical characteristics of the studied digestates are reported in the Table 2. A high variability of the parameters analysed was apparent. Indeed, organic matter ranged between 40 and and 90% (TS basis), the TKN varied between 15 and 45 g kg^{-1} while P ranged between 4 and 20 g kg^{-1}. The most N-rich samples (44–48 g kg^{-1}) were the liquid phase of the centralised digestate (Centr_2), the pig manure digestate (Agri_2) and the sludge digestates (Sludge_1, D1 and D2). The poorest samples were the digestates of FFMSW and its compost (between 15 and 17 g N kg^{-1}). The richest P samples were the sludge digestate (Sludge_1) and its compost (Sludge_2), along with the solid phase of the centralised digestate (Centr_1) and digestate D2 (sludge from winery processing) with a P content ranging between 18 and 20 g kg^{-1}. The FFMSW_1 digestate and its compost FFMSW_2 besides the silage straw digestate (Agri_3) were the poorest P samples (4 g P kg^{-1}). PCA and HCA analyses were performed on the samples characteristics for a whole sight of the digestates profiles thus allowing the comparison with the typology of Guilayn et al. (2019). The results are presented in the Supplementary Material (Fig. A.1). The PCA was applied on nine parameters (i.e from TS to K in Table 2). The first two components recovered $\approx$ 80% variance. The first component (PC1) was mainly related to the TS, negatively related to OM and K.

PC1 clustered dried (i.e. FFMSW_1, composts Sludge_2 and FFMSW_2) and liquid AD digestates (i.e. all the others). The second component (PC2) was formed by P, which was negatively related to the C: N ratio related to fibrous composition. The sludge digestate samples were associated with P concentration variable whereas Agri_3 (wheat straw digestate) was mainly associated to C: N ratio variable. Furthermore, Sludge_1 and 2 were the samples containing the highest metals concentrations (Fe, Al, Pb, Cu, Mn, Mo, and Cd).

These results were in agreement with the raw digestate typology found by Guilayn et al. (2019). Indeed, Agri_1 and Agri_3 characteristics were in agreement with the characteristics associated to the fibrous feedstock digestate group after dried digestion (i.e. cattle manure and crop residues). Agri_2 characteristics were consistent with those of the pig slurry digestate group from the typology. Sludge_1 characteristics were consistent with those of the sludge digestate group and FFMSW_1 characteristics fit with the municipal solid wastes digestate group characteristics. BW_1 characteristics were mainly close to the biowaste and municipal solids waste digestate composition after dry digestion. However, NH_4 concentration and NH_4: TN ratio had similar values than the manure co-digestion (i.e. NH_4: TN ratio > 70%). This is probably due to the high ratio of agro-industrial waste in the feedstock used in addition of biowastes. Only digestate from the wet digestion of fibrous substrate was not taken into account (silage) in this study. Composts Sludge_2 and FFMSW_2 and Centr_1 and 2 obtained after phase separation were not compared with the typology as they were not raw digestates.

Attention should be paid to the results for TKN and N-NH_4^+ concentration obtained by the raw sample analysis and to the freeze-dried sample extraction analysis. Regarding some digestates, highly significant positive differences were obtained between raw and freeze-dried samples analyses (from 40% for Centr_2 to 61% for Agri_2 of TKN loss). This result was due to ammonia volatilization during freeze-drying operation. The loss of ammonia could have an impact on soil incubation and plant N recovery results. Considering the 8 others digestates, no significant differences appeared and a linear relation was obtained between raw sample analysis and freeze-dried analysis (i.e. TKN_raw = 0.9443.TKN_freeze-dried, R^2 = 0.8832). Furthermore, the N typology of Agri_2 became similar as Agri_1 and 3 (fibrous feedstock involving manures). NH_4: TN ratio evolved from 76% to 39%. This ratio decreased also for Centr_2 from 46% to 9% after freeze-drying. Consequently, Agri_2 and Centr_2 results were used in statistical tests by using the freeze-dried analysis. N-ARF recovery will be discussed accordingly. However, concerning liquid digestates with high ammonia content as pig slurry digestates, the best option would be to perform soil incubation and plant pot trials with fresh matter as Rigby and Smith (2013), de la Fuente et al. (2013). A higher mass of soil would be required to maintain the soil WHC with the liquid digestate moisture, according Eq. (5).

This first characterization approach showed that the digestates selected were representative of a large range of digestates (i.e. municipal waste, sludge, manure, crop residue, bio-wastes, centralised).

Table 2
Physico-chemical characteristics of the investigated digestates.

Parameters	*Units*	Agri_1	Agri_2	Agri_3	Sludge_1	Sludge_2	FFMSW_1	FFMSW_2	BW_1	Centr_1	Centr_2	D1	D2	BD
TS	%	17.1%	4.4%	14%	22.4%	59.1%	19.7%	53.3%	24.9%	26.4%	6%	3.94%	3.09%	5.05%
VS	%TS	70.2%	70.1%	87.0%	51.7%	48.5%	48.7%	41.8%	79.0%	82.2%	60.0%	58.2%	59.4%	68.3%
TOC	g kg^{-1}	382.90	404.71	454.83	283.35	258.01	279.30	232.55	438.64	438.17	320.53	344.2	397.8	515.0
COD	g kg^{-1}	1104.00	1400.56	1235.50	679.00	726.00	712.09	536.00	1291.00	1245.00	1497.58	nd*	nd	Nd
TKN**	g kg^{-1}	25.85	115.00	nd	40.36	26.40	22.20	13.61	29.79	23.33	75.67	nd	nd	nd
TKN***	g kg^{-1}	27.98	**44.53**	17.72	43.06	26.61	17.78	15.39	30.89	28.62	**45.47**	47.8	44.9	43.0
C/N		13.68	3.52	25.67	6.58	9.70	15.71	15.11	14.20	15.31	7.05	7.2	8.9	12.0
NH_4^+-N**	g kg^{-1}	6.28	87.85	2.99	10.80	0.92	10.5	0.04	27.68	6.81	34.74	nd	nd	nd
P	g kg^{-1}	5.36	10.37	4.00	20.44	16.15	4.21	4.23	10.79	6.93	17.98	7.1	18.4	6.2
K	g kg^{-1}	21.22	27.41	12.98	2.15	4.90	11.49	8.22	7.25	4.86	16.10	nd*		
S	g kg^{-1}	3.59	6.23	1.63	6.23	5.33	2.22	5.00	2.16	6.27	7.58			
Al	g kg^{-1}	2.31	3.23	0.65	6.66	12.56	8.68	2.73	8.46	2.68	9.33			
Ca	g kg^{-1}	18.53	23.08	13.44	69.08	41.25	37.05	29.72	42.31	17.56	27.08	10	37	11
Fe	g kg^{-1}	1.58	2.81	0.94	42.60	37.48	5.79	5.06	7.89	11.54	12.07	1.9	8.6	1.0
Ca/P		0.29	0.27	0.24	2.08	2.32	1.38	1.19	0.73	1.67	0.67	1.4	2.0	1.7
Mg	g kg^{-1}	5.49	6.28	1.56	3.52	3.72	3.24	4.29	3.47	1.98	5.13	2.8	11.6	5.2
References	This study											Grigatti et al. (2019)		

[*] nd : Not determined.

[**] Measured on raw samples.

[***] Measured on from freeze-dried samples.

3.1.1. X-ray powder diffraction

The X-ray powder diffraction has been widely use for the study of the crystalline P species in anaerobic digestate and compost (Li et al., 2019; Grigatti et al., 2017, 2019). In this light the XRD profiles from the tested products can give a valuable insight to their P extractability. The XRD patterns from the digestates are presented in Fig. 1. The XRD patterns of samples Agri_1, Agri_2 and Agri_3 were similar showing the presence of crystalline inorganic phases, namely quartz (PDF no. 01-087-2096), calcite (PDF no. 01-085-1108) and sylvine (KCl, PDF no. PDF no.41-1476).

However, these three samples differed significantly in their amorphous phase content, which was definitively higher in Agri_3, followed by Agri_1, whereas sample Agri_2 was mostly constituted of crystalline material. Furthermore, Agri_2 presents also struvite peaks (PDF no. 15-0762), that could be detected on account of the higher crystallinity of this sample. Amongst the analyzed samples, Sludge_1 was the most crystalline one, with no significant presence of amorphous material. The Sludge_1 pattern showed the presence of calcite and quartz as main constituting phases, together with a low amount of vivianite ($Fe_3(PO_4)_2(H_2O)_8$, PDF no. 01-075-1186). Li et al. (2018) found also that quartz was one of the major phases for sewage sludge. Sludge_2 was somewhat similar to Sludge_1, but less crystalline so that only calcite and quartz were detected (in this case quartz is more abundant than calcite). FFMSW_1 and FFMSW_2 were very similar both regarding the amount of amorphous phase which is present together with the fine crystalline fraction of the materials. Regarding the qualitative detection of crystalline phases, it was possible to identify quartz (the most abundant), calcite, together with low amounts of kaolinite ($Al_2(Si2O_5)(OH)_4$, PDF no.01-080-0885) and sylvite. BW_1, Centr_1 and Centr_2 patterns all displayed the presence of quartz, calcite and struvite as crystalline components, but significantly differed for the amount of amorphous material that was relatively low in Centr_2, and higher and similar in BW_1 and Centr_1. The relationship between the XRD outcomes and the P extractability are dicussed in the following section.

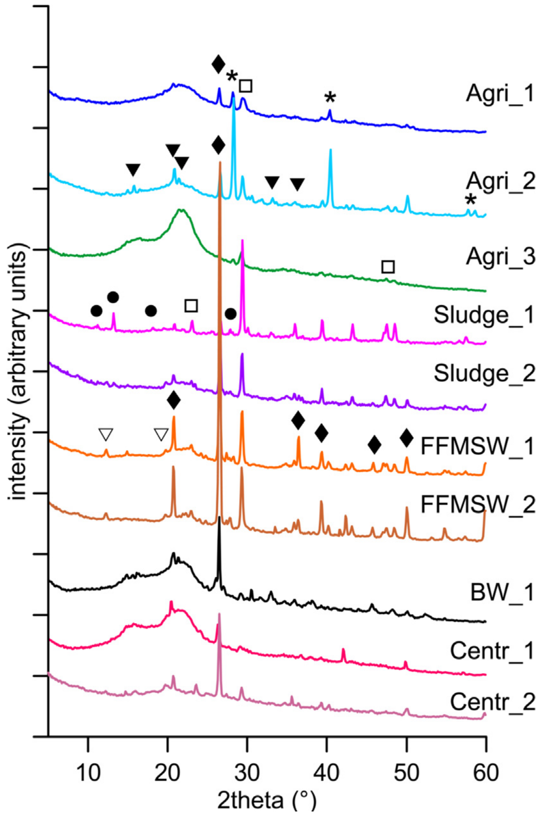


Fig. 1. X-ray diffraction of the digestates. The main peaks of different crystalline phases are identified by symbols: ▼ struvite; ◆ quartz; ☐ calcite; ● vivanite; * sylvine; ▽ kaolinite.

3.1.2. Sequential chemical extractions of nitrogen and phosphorous

The N fractionation protocol used in this work was developed by Jimenez et al. (2015), and has been validated to describe OM and N evolution during biological degradation processes (Jimenez et al., 2017; Bareha et al., 2018). The P fractionation has been adapted from Hedley et al. (1982), using methodology by Grigatti et al. (2015, 2017, 2019). These authors showed the link between labile-P from organic waste samples and the plant P-uptake. The N and P speciations have been assessed applying both fractionation protocols to the selected digestates (Fig. 2 a and b respectively). The results showed that the digestates performed different N and P speciations. This was related to the digestate typology and the nature of the feedstock for anaerobic digestion, according to Guilayn et al. (2019). The observed variability in N and P speciation showed the significant effect of the feedstock on the tests discussed in Section 3.2. In the study of N accessibility (Fig. 2a), mineral nitrogen was assessed in the water extractable fraction SPOM. The non-extractable organic nitrogen varied between 25 and 48% except for Agri_3 with only 5% N in the NEOM fraction.

HCA was applied on the N speciation data (not shown). Four groups were found, ordered by N accessibility basis as follows: (i) Group 1: High NH_4-N SPOM samples: Centr_1 and BW_1. This result is consistent since these samples had poor TS; (ii) Group 2: High organic SPOM and REOM: Agri_2 and Sludge_1. SPOM and REOM are mainly composed of accessible and readily extractable proteins; (iii) Group 3: High organic SEOM: Agri_3. SEOM is mainly composed of complex proteins and humic-like substances; (iv) Group 4: High PEOM organic N content samples: Agri_1, FFMSW_1, Centr_2, FFMSW_2 and Sludge_2. PEOM extraction targets holocellulose-like compounds found in fibrous digestates as Agri_1, FFMSW_1 and 2. Phase separation concentrates OM and fibers in the solid phase (Guilayn et al., 2019) as Centr_2. Finally, the compost Sludge_2 was also clustered in this group because of its co-composting with green wastes.

P was extracted in each fraction as reported in Fig. 2b. Phosphorus fractionation showed that inorganic P content was higher than organic P in all the fractions, as reported in the studies of Grigatti et al. (2015, 2017, 2019) on digestates and composts, and as observed by He et al. (2010) on poultry litter and dried wastewater sludge. Mazzini et al. (2020) showed that 78 to 93% of TP was inorganic following the SCE from six types of animal slurries digestates. The authors showed relevant NaOH (5–43% of organic P) and HCl (2–25% of organic P) extractable organic P, relating this to inorganic P microbial immobilization during anaerobic digestion for microorganism growth. Indeed, soluble inorganic P would be transformed into organic P compounds such as phosphates monoesters or DNA. This biological organic-P may be rapidly mineralized once in soil (He et al., 2010). In this context, NaOH and H_2O were the most organic-P rich fractions from the samples investigated in this work. Furthermore, Agri_1, 2 and 3, and FFMSW_1 mainly showed NaOH extractable organic P. Agri_3 contained the highest organic P content ($\approx$40%), and 1.5 to 4- folds higer than other samples. In water Agri_2 and 3 showed 60% of organic P, higher than BD (49%).

The results obtained by He et al. (2010) on poultry litter showed that a large part of organic P was in the NaOH fraction and was mainly related to phytate-like. Mazzini et al. (2020) showed similar results on crop residues digestates. The authors showed also that organic P were mainly extracted in NaOH fraction of 6 agrowastes digestates as shown by Agri_1 and 3. Organic P was

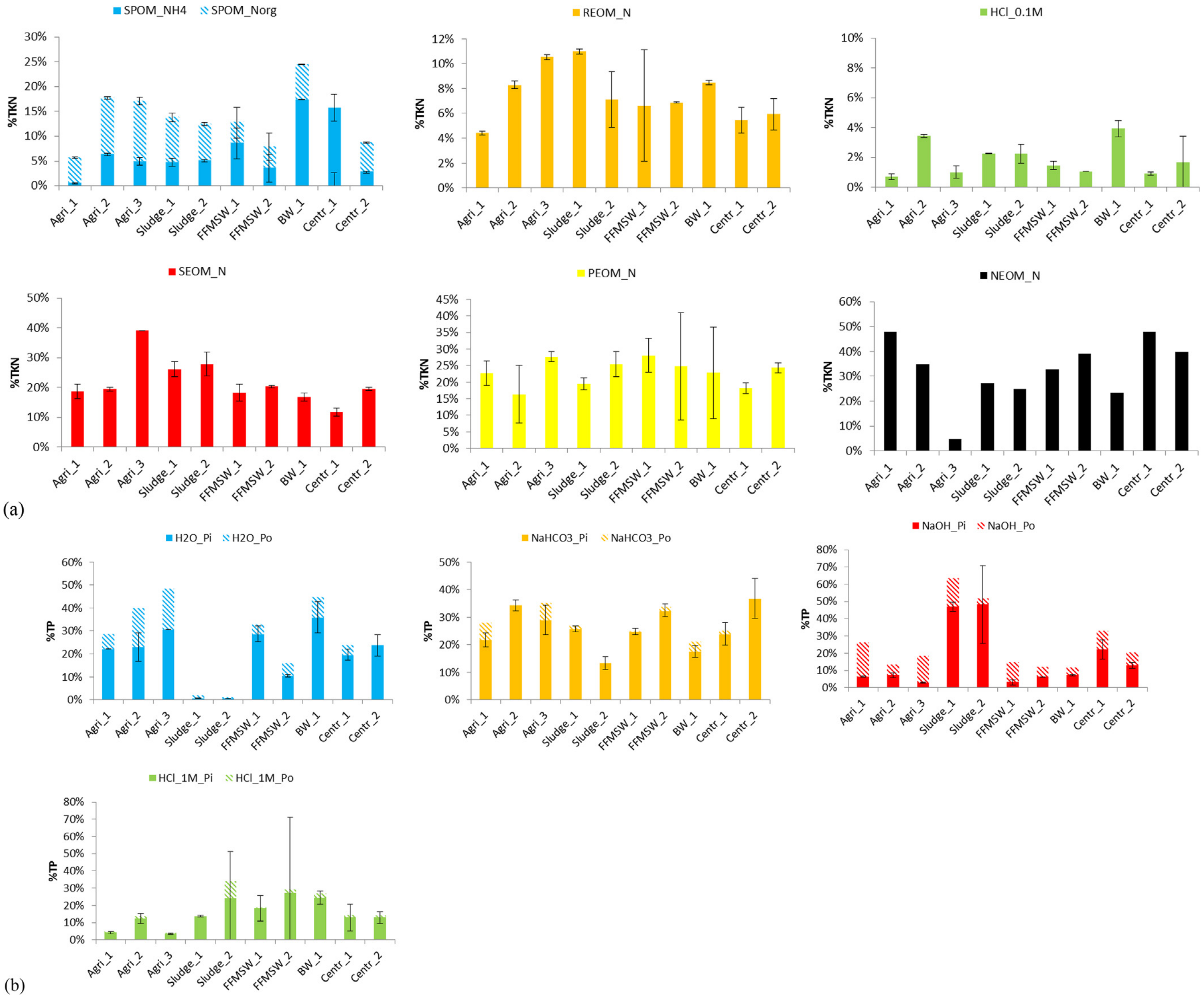


Fig. 2. SCE fractions of TKN (a) and TP (b) obtained for all the digestates, according chemical accessibility. Error bars, SE (n = 2).

observed in HCl fraction from Sludge_1 and 2 in this study. He et al. (2010), observed also organic P in HCl fractions related to non-hydrolysable fractions in the dried wastewater sludge. The $NaHCO_3$ and the HCl fractions showed to be the most inorganic P rich fractions from the samples tested in this work thus fitting to available P and Ca-bounded P. The labile-P fraction (H_2O + $NaHCO_3$) was found at the highest level in the agricultural residue digestates (Agri_1, 2 and 3). Similar observations on bovine manure and energy crops digestate (BD) were made by Grigatti et al. (2019). Then the biowaste and agro-food industries digestates (BW_1, Centr_1 and 2) had also high labile-P. The FFMSW samples showed intermediate P accessibility. The sludge samples were characterized by a high NaOH extractable P, related to metal-bounded P. Indeed, this result was consistent with both Al and Fe content (≈7 and ≈37 g kg^{-1}). Li et al. (2018), showed mainly NaOH extractable Al, while Fe was NaOH soluble being also extracted by HCl in sewage sludge, thus showing Fe-bounded P was partially occluded. Amongst the others samples, compost Sludge_2 showed important poorly available HCl-P, being very similar to FFMSW_2, these results were consistent with the P fractionation of composts showed by Grigatti et al. (2015, 2017, 2019).

Finally, HCA showed six groups (clustering not shown). Groups were ordered according to the chemical availability level (i.e. Labile-P > NaOH-P > HCl-P) as follow: (i) Very high P accessibility related to high H_2O-P fractions: digestates from liquid feedstock (no manure), biowastes and winery (BW_1, D1 and D2). These samples were characterized by amorphous P and crystalline P (quartz and struvite); (ii) High P accessibility associated with organic H_2O-P and $NaHCO_3$-P: digestates from liquid manure (BD and Agri_2). These samples were characterized by crystalline P (struvite); (iii) Moderate P accessibility (intermediary P speciation): digestates from organic fraction from municipal wastes digestates and centralised digestates (FFMSW_1, FFMSW_2, Centr_1 and Centr_2). These samples were characterized by an amorphous phase and mainly quartz and calcite as crystalline phases; (iv) Poor-P accessibility associated with organic $NaHCO_3$-P and NaOH-P, associated with digestates from fibrous substrates as cow manure with crops residues and wheat straw (Agri_1 and Agri_3). These samples were characterized by a high amorphous phase; (v) Very poor P accessibility associated with inorganic NaOH-P and low fraction of available P: digestate from solid phase of sludge (Sludge_1). This sample was characterized by a crystalline P phase (mainly quartz and calcite); (vi) The poorest accessible P associated with inorganic HCl-P was compost of sludge digestate (Sludge_2). This sample was characterized by less crystalline P than Sludge_1 with calcite and quartz.

For some groups, the feedstock type seemed to have an impact on the P accessibility of digestates (solid phase of sludge, liquid manure, liquid feedstocks, fibrous feedstock). Another observation was that the amorphous and crystalline characteristics also seemed to be associated with some groups: fibrous digestates were mainly composed of amorphous phase and contained organic P in $NaHCO_3$ and NaOH. The labile P fractions had in common amorphous P and struvite as crystalline P. The most crystalline P-rich fractions were found in the solid phase of sludge digestates before and after composting, containing low available P and high sparingly soluble HCl-P.

The eight tested digestates represented a wide range of inherent characteristics and accessibility patterns for both N and P. In this framework, the soil and plant test results can give a deeper insight to these issues as discussed in the following section.

3.2. Soil incubation and plant pot trials

Table 3 shows the cumulative amount of biomass harvested in ryegrass plant pot tests. Any significant differences between treatments for tissue and root (Kruskal-Wallis test p = 0.89 and 0.23 respectively). Nevertheless, it appears that poor biomass was obtained in Agri_2 treatment (pig manure digestate), and in FFMSW_2 treatment (FFMSW digestate compost), close to the negative control. The best results were obtained in Centr_1 and BW_1 (solid phase of a centralised digestate and biowaste digestate). The total plant biomass (shoot + root) was the highest (g pot^{-1}) in Centr_1 (4.11) ≥ Sludge_2 (3.75) ≥ BW_1 (3.72) ≥ FFMSW_1 (3.70) ≥ Ctrl$^+$ (3.65) ≥ Sludge_1 (3.48) ≥ Agri_3 (3.38) ≥ Centr_2 (3.23) ≥ Agri__1 (3.18) ≥ Agri_2 (3.02) ≥ FFMSW_2 (2.93) ≥ Ctrl$^-$ (2.90).

3.2.1. Nitrogen fate

The N mineralization data from soil incubation (Fig. 3) showed the net cumulated mineral N (i.e. NH_4 + NO_3 mass) during soil incubation (Fig. 3a) and the net cumulated mineralization rate of organic N (Fig. 3b).

Concerning the available mineral N (N-min), BW_1, Centr_2, Sludge_1, Sludge_2, Agri_2 and FFMSW_1 achieved the highest cumulated values after 84 days of incubation. On the contrary, Centr_1, FFMSW_2, Agri_3 and Agri_1 performed poorly cumulated N-min. These latter were more fibrous samples, with high C: N ratios.

The cumulated organic N mineralization rate was calculated (Fig. 3b). Two groups of treatments appeared and were classified in the same order than the cumulated N-min: (i) positive mineral-

Table 3

Dry biomass (DW), total P uptake and N uptake in ryegrass shoots during three successive harvests (day 28–84) and roots at the final harvest (day 84).

	Treatment	Days after sowing						
		Shoots					Roots	Total
		28	56	84	*Mean*	0–84	84	84
	DW (g pot^{-1})							
	Ctrl-	0.87	0.53	0.25	*0.55*	1.65	1.25	2.90
	Ctrl+	1.00	0.70	0.39	*0.70*	2.09	1.56	3.65
1	Agri_1	0.88	0.50	0.29	*0.56*	1.67	1.51	3.18
2	Agri_2	0.86	0.58	0.35	*0.60*	1.79	1.23	3.02
3	Agri_3	0.84	0.44	0.27	*0.52*	1.56	1.82	3.38
4	Sludge_1	0.94	0.58	0.42	*0.65*	1.94	1.55	3.49
5	Sludge_2	0.94	0.57	0.37	*0.63*	1.88	1.87	3.75
6	FFMSW_1	0.85	0.58	0.36	*0.60*	1.79	1.91	3.70
7	FFMSW_2	0.88	0.47	0.26	*0.54*	1.61	1.32	2.93
8	BW_1	0.99	0.53	0.41	*0.64*	1.92	1.79	3.72
9	Centr_1	0.89	0.54	0.37	*0.60*	1.81	2.30	4.11
10	Centr_2	0.96	0.58	0.41	*0.65*	1.95	1.28	3.23
	P uptake (mg pot^{-1})							
	Ctrl-	1.93	1.60	1.02	*1.52*	4.55	2.25	6.80
	Ctrl+	2.17	1.48	1.43	*1.70*	5.09	2.24	7.33
1	Agri_1	2.41	1.14	1.61	*1.72*	5.16	2.87	8.03
2	Agri_2	1.81	1.15	1.76	*1.58*	4.73	2.19	6.92
3	Agri_3	1.87	1.07	1.76	*1.57*	4.70	2.86	7.56
4	Sludge_1	2.28	1.23	2.70	*2.07*	6.21	2.96	9.17
5	Sludge_2	2.29	1.53	2.32	*2.05*	6.14	3.18	9.32
6	FFMSW_1	2.05	1.76	2.41	*2.07*	6.21	2.62	8.84
7	FFMSW_2	2.65	1.13	1.35	*1.71*	5.13	2.22	7.35
8	BW_1	2.76	1.40	2.38	*2.18*	6.54	2.39	8.93
9	Centr_1	2.77	1.36	2.26	*2.13*	6.39	2.58	8.97
10	Centr_2	2.67	1.53	2.31	*2.17*	6.51	2.25	8.76
	N (mg pot^{-1})							
	Ctrl-	25.01	9.32	6.52	*13.62*	40.85	12.44	53.28
	Ctrl+	45.70	16.69	9.23	*23.87*	71.62	14.89	86.51
1	Agri_1	25.29	7.35	8.21	*13.62*	40.85	14.76	55.61
2	Agri_2	29.41	9.03	8.98	*15.81*	47.42	13.31	60.73
3	Agri_3	21.84	6.48	6.80	*11.70*	35.11	14.71	49.82
4	Sludge_1	36.91	10.51	12.39	*19.94*	59.81	15.13	74.93
5	Sludge_2	35.32	9.72	10.52	*18.52*	55.55	17.49	73.05
6	FFMSW_1	28.77	10.34	9.67	*16.26*	48.78	15.35	64.13
7	FFMSW_2	29.80	8.97	7.41	*15.39*	46.17	15.45	61.62
8	BW_1	32.03	9.97	11.88	*17.96*	53.88	16.51	70.39
9	Centr_1	30.74	9.28	10.00	*16.67*	50.02	18.45	68.47
10	Centr_2	34.63	10.39	12.16	*19.06*	57.18	14.04	71.22

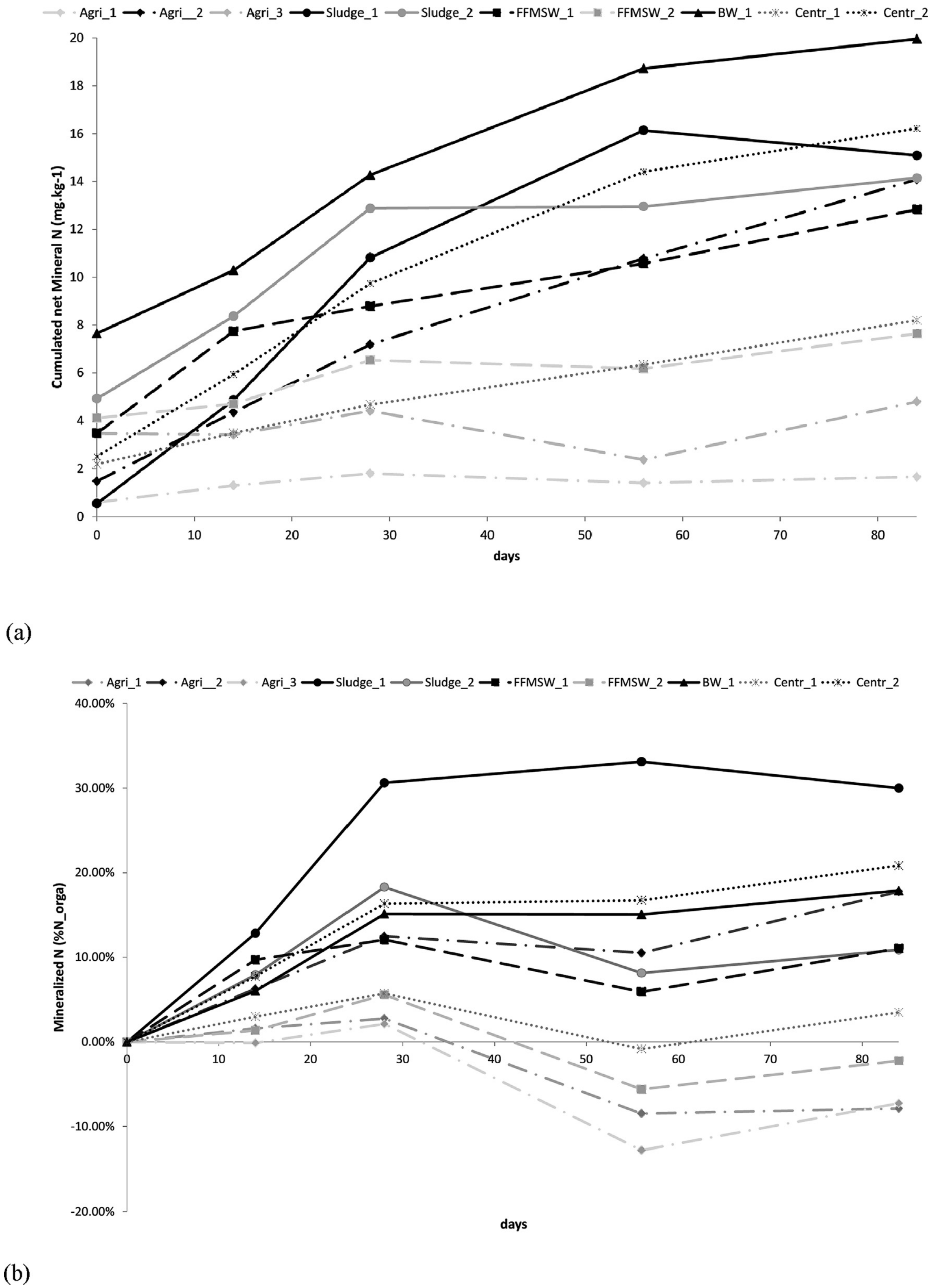


Fig. 3. Cumulated mineral nitrogen concentration evolution (a) and cumulated mineralized organic nitrogen percentage (b) during soil incubation. Net values. *Black: high mineralized N group; Grey: low mineralized N group (circle + full line: sludge digestates, square + large dashes: FFMSW digestates, diamond + dashes and dots line: Agrowastes digestates, triangle: biowaste digestate, cross + dotted line: centralised digestates).*

ization rate associated with the Sludge_1, Centr_2, BW_1, Agri_2, Sludge_2 and FFMSW_1 treatments and (ii) negative mineralization rate associated with Centr_1, FFMSW_2, Agri_1 and Agri_3. Sludge_1 showed the highest N mineralization rate (30%) suggesting that this digestate was rich in hydrolysable proteins. Organic N from the liquid digestates Centr_2 and Agri_2 showed respectively 21% and 18% of mineralized organic N, following Sludge_1 (30%). This result is consistent with Guilayn et al. (2019) who reported that the highest organic N came from sludge digestate and pig-slurry digestate group. Sludge_2 and FFMSW_1 performed lower N mineralization rates (11%). Similar results were observed in liquid or solid digestates by Rigby and Smith (2013). They showed that the solid phase from municipal solid wastes digestate (C: N ratio of 11) had negative N mineralization rate whatever the soil composition used (sandy, silty or clay). On the contrary, Rigby and Smith (2013) showed that the liquid digestates from slurry (C: N ratio of 4), had 16% to 40% N mineralization while food waste digestate (C: N ratio of 14) reached 30% N mineralization.

The negative mineralization rates group was associated to an immobilization of mineral nitrogen by the soil microorganisms during their growth (De la Fuente et al., 2013). This was related to the increased microbial activity following the OW addition to the soil. Indeed, nitrogen is the main limiting nutrient for plant growth, especially for a fast growing, highly demanding species such as Italian ryegrass (Grigatti et al., 2011). These results are in agreement with Cavalli et al. (2017) which reported immobilization for high C: N ratio anaerobic digestates. The authors showed the low C: N ratio (5 to 7) associated to the raw digestate and its liquid phase (cattle slurry/mais silage) induced net N mineralization ($\approx$ 30%). On the contrary, the cellulose- and volatile fatty acids-rich solid phase (C: N ratio, 20) induced lower net N mineralization (9–16%). Indeed, negative correlations were found between the N mineralization rate and C: N ratio (r= −0.73, p = 0.029) as observed by Morvan et al. (2006) on animal manure wastes.

Considering the impact of process treatment of digestates, composting appeared to negatively affect N mineralization. N mineralization rate from Sludge_2 (11%) was lower than Sludge_1 (30%) and FFMSW_1 (11%) had highest mineralized values than FFMSW_2 (-2%). Phase separation impacted also organic N mineralization as shown by Cavalli et al. (2017). Solid phase (Centr_1) reached negative values of minerlization whereas Centr_2 reached 21% of mineralized N.

Plant growth experiments were affected by fertilizer treatment for N-ARF (Kruskal-Wallis, p = 0.026) only for shoot plant tissues. Cumulated N recovery by plant tissues was plotted in the Fig. 4. The chemical treatment showed the greatest shoot N recovery (70%) with a total of 76% of N-ARF. N uptake recovery was mainly observed in shoots (64% to 87% of the total recovery). Root N-ARF was measured between 7% and 36% of the total N-ARF, except for Agri_1 and Agri_3 treatments where negative to zero values were obtained.

Sludge_1 and Sludge_2 achieved the best N-ARF amongst the digestates treatments (16.5 and 15.1%). These results were consistent with the Sludge_1 treatment yielding the highest N-min available for plant growth. Centr_2 (13.7%), BW_1 (13.2%) and Centr_1 (11.6%) treatments showed intermediate total N recovery. The FFMSW_1, FFMSW_2 treatments followed with 8.2% and 6.4% respectively. Finally, a last group formed by Agri_1 and Agri_3 was observed with low N-ARF of 1.8% and 2.6% respectively. In soil incubation tests, Agri_1 and 3 showed N immobilization. Accordingly to these outcomes, the plant N-ARF was negative or close to 0 and close to the unfertilized soil treatment.

Considering the digestate treatments impact, composting (i.e. FFMSW_2 and Sludge_ 2) lowered the fertilizing N value of its associated digestate (i.e. FFMSW_1 and Sludge_1 respectively). Phase separation impacted also the N fertilizing potential as shown by Tambone et al. (2017). Centr_2 treatment induced higher N mineralization rates and N-ARF than Centr_1 treatment. According to N speciation and characterization, the solid phase Centr_1 contained more fibers than the liquid phase Centr_2.

PCA and HCA were performed to find correlations between the N availability data, digestate characteristics, mineralized N percentages and N-ARF from shoots and roots as reported in Fig. A.2a (supplementary material). No significant correlation was observed between the total N content, the mineralization rate and the N-ARF. However, a strong correlation was obtained between the C: N ratio and N-ARF from shoots-N (r = −0.82, p = 0.004) as in the mineralization N tests without plants. This was described by a linear equation (Eq. (8)) based on the data of this study and validated by data from digestates and composts studied in Grigatti et al. (2015). Similarly, Decoopman et al. (2017) found a correlation between C: N ratio of agricultural digestates and plant N-ARF measured in field experiments on cereals.

$$\mathrm{Shoots_N} = -0.0085 \times \frac{\mathrm{C}}{\mathrm{N}} + 0.1782, \mathrm{r}^2 = 0.6718 \qquad (8)$$

The fiber component of digestates had a negative impact on mineralised N and N recovery by plant tissues. Similarly, the fiber fraction PEOM of digestates was negatively correlated with cumulated mineralized N (−0.58, p = 0.08) and shoots-N although to a lesser extent (r = −0.58, p = 0.44). Moreover, cumulative mineralized N and shoots-N ARF were positively correlated (r = 0.72, p = 0.02) which was consistent with the availability of N for plant growth. Concerning roots, there was a positive correlation between roots N-ARF and nitrates content (r = 0.72, p = 0.017), SPOM_NH$_4$ (r = 0.61, p = 0.06) whereas SPOM_org and roots_N ARF were negatively correlated (r = −0.59, p = 0.07). A linear equation (Eq. (9)) was found between Roots N-ARF and water extracted mineral N (i.e. $N_{soluble\_min}$ =NO_3^-+ SPOM_NH$_4$).

$$\mathrm{Roots_N} = 5\mathrm{E}^{-5} \times \mathrm{N_{solublemin}} + 0.0138, \mathrm{r}^2 = 0.5313 \qquad (9)$$

According to Gunnarsson et al. (2010), plants respond to N availability with a different root/shoot nutrient allocations and plant growth rate could be influenced by the ammonia/nitrates ratio in soil solution due to the different N sources. Authors observed an increase in root biomass as a result of the availability of a high amount of ammonia.

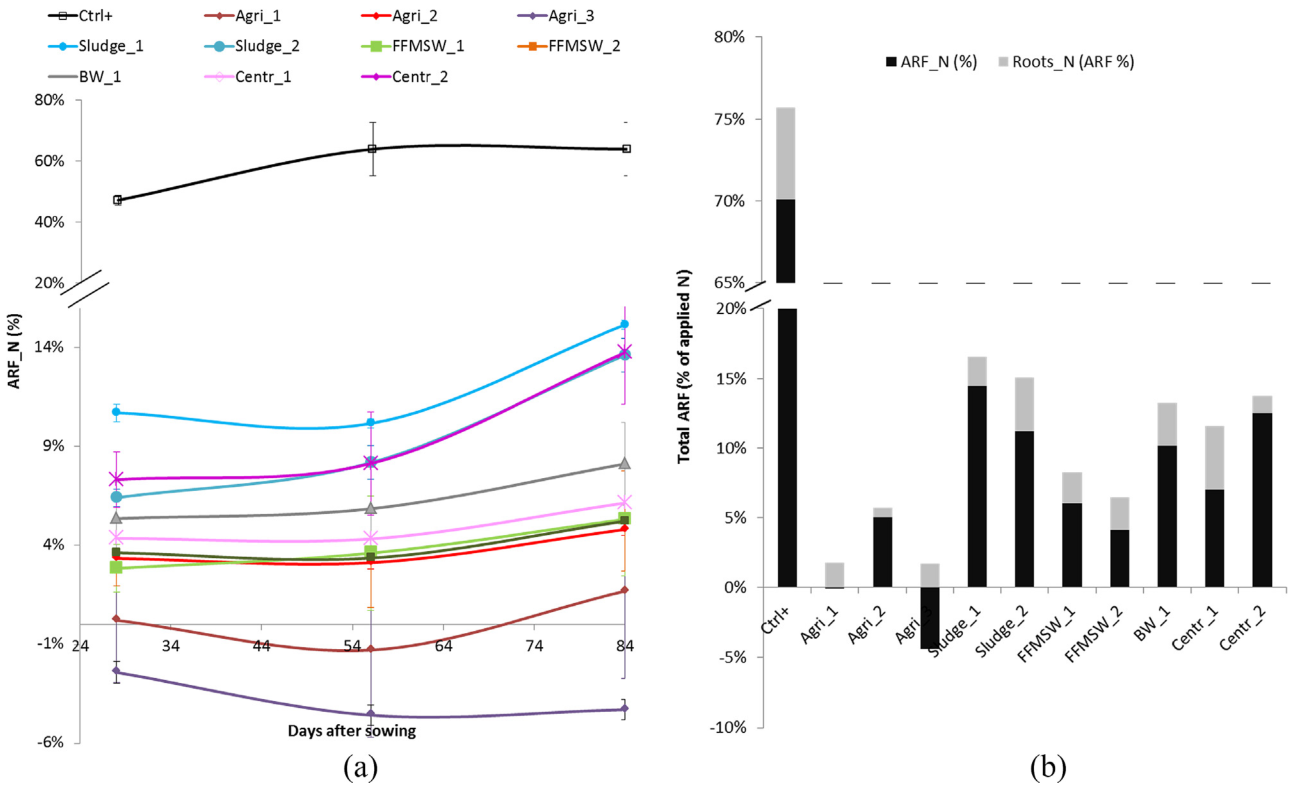
HCA revealed four clusters (Supplementary material Fig. A.2.a) as follows: (i) Group 1: Agri 3 associated with fibrous digestate with high C: N (>25), high SEOM_N and PEOM_N and low shoots-N ARF. This type of digestate is not suitable as N fertilizer; (ii) Group 2: FFMSW_2 and Agri_1 15 < C/N < 13, intermediary protein-like and fibrous substrate digestates with high organic SPOM_N and PEOM_N for Agri_1 as compost FFMSW_2. They recovered very low roots N-ARF and intermediate level of shoots N-ARF; (iii) Group 3: Centr_1, FFMSW_1 and BW_1 municipal solids and biowastes digestates, with C: N ratios of 15 in average, high ammonium and nitrate levels in the labile fraction and high levels of total N recovery; (iv) Group 4: The protein-like digestates (Sludge_1, Centr_2, Agri_2) which have a poor C: N (<10), poor PEOM_N, high SPOM_Norg content and the highest total N recovery. Sludge_2 was classified in the Group 4 because of its lower C: N ratio (9.7) and its high N mineralization level.

C: N ratio is associated to the fibrous level of an OW and seemed to be discriminant enough to predict N-ARF and organic N mineralization rate. Digestates with C: N ratios $\geq$ 15 were not suitable as N fertilisers whereas digestates with lower C: N ratios had a high potential of N fertilizer.

Accessibility fractionation of N allowed a consistent digestate classification. Furthermore, interesting correlation between roots-ARF N and water extractable nitrates and ammonia were found.

3.2.2. Phosphorous fate

The Olsen-P course during the soil incubation is shown by Fig. 5a. The Olsen-P evolution in soil depends on several phenomena: (i) the physico-chemical conditions of the soil can trap some weakly bound P, (ii) the organic part of P can be mineralized and (iii) P can be taken up by soil microorganisms for their growth.



(a) (b)

Fig. 4. Nitrogen apparent recovery fraction (ARF) of ryegrass shoots (a) and in both shoots and roots (b) in three harvests during pot trial. Error bars, SE (n = 3).

The control soil had the lowest Olsen-P content throughout the incubation (12–14 mg kg soil^{-1}), whereas the compared treatments exhibited different Olsen-P courses in time. All the tested samples showed an available P depletion during the first two weeks. The chemical control (Ctrl^{+}) performed the fastest depletion. Indeed, the chemical P treatment (phosphate salt) can be rapidly fixed with Ca components in calcareous soil (Alburquerque et al., 2012). In this context, the presence of organic matter and the different P forms from digestates can have positive interactions with soil, performing lower fixation and higher efficiency in comparison to chemical P sources (Alburquerque et al., 2012).

In some treatments, an Olsen-P increase appeared between 14 and 28 days (Agri_2, Centr_1, BW_1 and FFMSW_1) probably due to organic P mineralization before another decrease until 56 days. This latter decrease led to a lower quantity of available P for plant growth. The RPE obtained was between 50 and 84%. The RPE of the Centr_2 treatment was the highest with 84% vs. the chemical P treatment, followed by Sludge_2 (74%). FFMSW_1, Agri_2 and Agri_3 were the samples where the P fixation was the highest (RPE of 60% in average). The other digestates were intermediate between the two groups. FFMSW_1, Agri_2 and Agri_3 contained high fractions of water P.

The pot tests showed the treatments affected the plant P tissue (p-value = 0.021). In this context the control soil performed the worst at the first cut (1.93 mg pot^{-1}), showing very poor performance during the plant pot test reaching 4.55 mg pot^{-1} on average (Fig. 5b and c). The shoot-P ARF is shown in Fig. 4a and b. P-ARF from Agri_2 and Agri_3 was negative from the beginning. This result was consistent with the soil incubation observations. This can be linked with high fixation of P in soil as suggested by Ahmad et al. (2018). In the second cut (day 56), the cumulative shoots P-ARF decreased in all the treatments. Only FFMSW_1 and Centr_2 showed opposite outcomes. Then, between the second and the third cut (day 56; 84), shoot-P ARF further increased. In the end the whole P-ARF (%) was: Centr_1 (5.78)≈FFMSW(5.31)≥ BW_1 (4.35)>Centr_2(3.78)>Sludge_1 (2.72)≥Agri_1 (2.44)≥ Sludge_2 (2)>FFMSW_2 (1.6)>Agri_2 (0.6)≈Agri_3 (0.5%).

FFMSW_2, Agri_2 and 3 showed lower shoots P-ARF than the chemical treatment (1.81%). That means they were not suitable for P fertilization on calcareous soil.

The root-P content was unaffected by the treatment. However, the root P-ARF (%) was: Agri_1 (2.46) > Agri_3 (2.04) > FFMSW_1 (1.18) = Sludge_1 (1.17) = Sludge_2 (1.16) > Centr_1 (1.04) > BW_1 (0.3) > Centr_2 (0.00) > FFMSW_2 (-0.08) > Agri_2 (-0.21). Shoots-P and roots-P recovery potential were different depending on the treatment, except for Agri_2 and FFMSW_2 which had the lowest ARF P recovery for both.

Briat et al. (2020) reported that availability of soil P (and K) for plants highly depends on the N availability. They reported that P concentrations on shoots were correlated with N concentrations in shoots. Indeed, a significant correlation was observed in this study between total N-ARF and total P-ARF (r = 0.8152, p = 0.0022) with a linear relationship (ARF_N/ARF_P = 7.7535, r^2 = 0.6626) thus proving N and P recovery by plants were linked.

PCA and HCA analysis were performed on both shoots and roots P uptake and P speciation for the 8 digestates studied beside the three digestates from Grigatti et al. (2019), as shown by Fig. A.2b in Supplementary Material. Six groups were found according HCA analysis. The results showed that the P-ARF was classified not only according to the digestate's nature but also according to their P accessibility. The groups found were: (i) group 1 : Sludge_2, compost of solid phase of sludge digestate, HCl rich with an intermediate level of P recovered in shoots and roots; (ii) group 2 : Sludge_1, solid phase of sludge digestate (inorganic $NaHCO_3$ and NaOH fractions rich), with an intermediate level of P recovered in shoots and roots; (iii) groups 3–4 : Centr_1, FFMSW_1, FFMSW_2, Centr_2, Agri_2 (intermediate to low total P-ARF); (iv) groups 5–7 : BW_1,

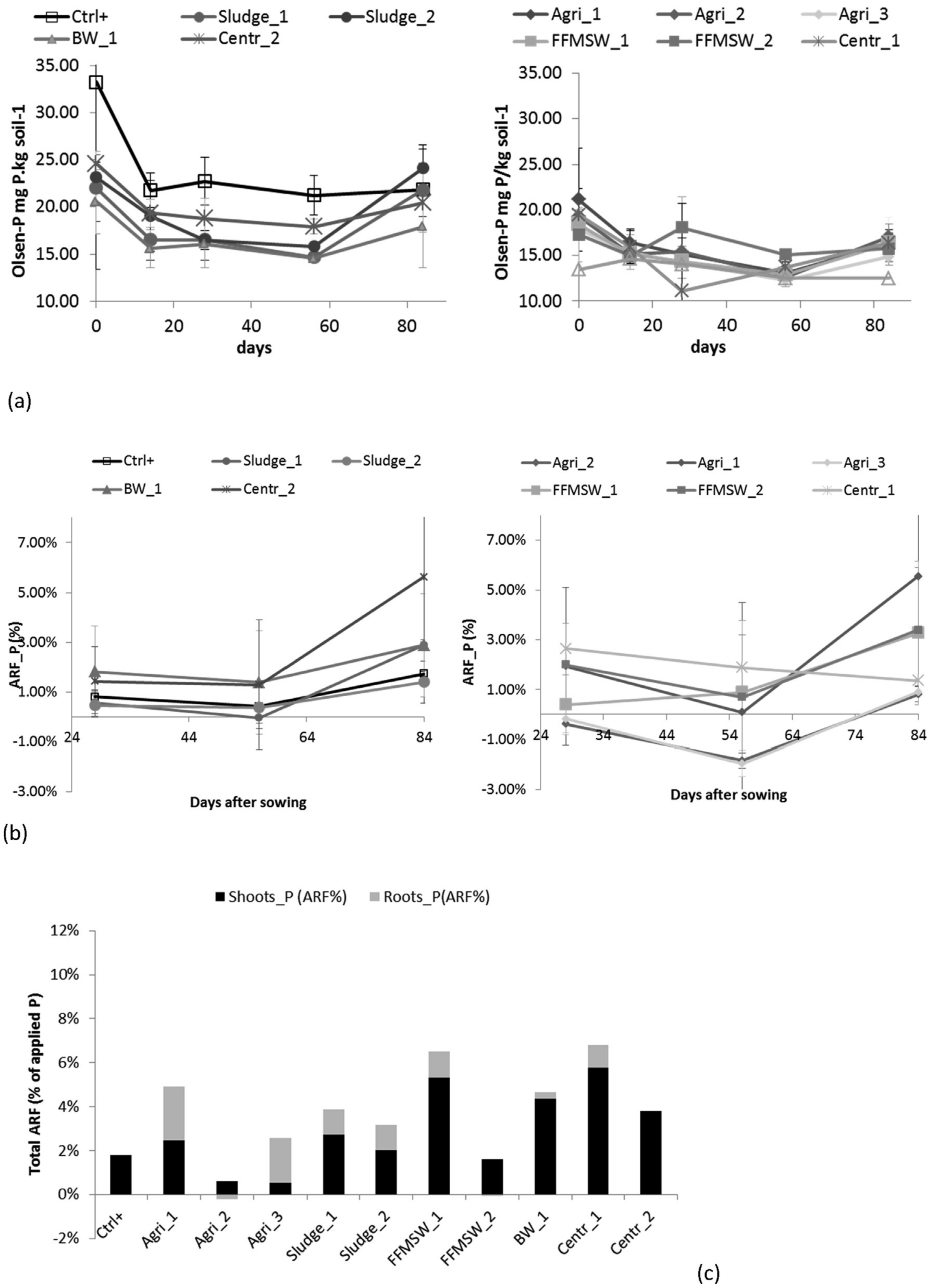


Fig. 5. Olsen-P evolutions during the soil incubation of the tested digestates (a), cumulative phosphorous apparent recovery fraction (ARF) of ryegrass shoots (b) and in both shoots and roots (c) in three harvests during pot trial. Error bars, SE (n = 3).

D1, D2 and BD (high total P-ARF and highest inorganic H_2O-P); (v) group 6 : Agri_1, Agri_3, fibrous digestates. Agri_1 has high P total recovery in both shoots and roots as BD, but Agri_3 shows a high recovery only in roots. It seems that the fibrous characteristic enhances P recovery above all in in plants roots through the organic fraction of NaOH-P.

The groups obtained were similar to digestate typology groups, except for Agri_2 and BD which were not in a same group. When doing statistical treatments on the shoots results and P speciation for the 8 digestates used in addition to the 3 digestates used by Grigatti et al. (2019), some correlations were found. Water-P fraction was positively correlated with shoots (r = 0.49, p = 0.09). This result was also observed by Grigatti et al. (2017) for composts. As reported by Ahmad et al., 2018, available P consists in the exchangeable and soluble P corresponding to water and Na-bounded P fractions (H_2O + $NaHCO_3$) whereas occluded P consists into P bound with metals (Fe, Al) and Ca. However, a simple linear model did not fit between inorganic H_2O-P fraction (H_2O-P_i) and shoots P-ARF. PLS model was used showing that two main significant variables were needed to predict shoots P-ARF (Eq. (10))·H_2O-P_i fraction impacted positively shoots P-ARF, as expected, whereas organic H_2O-P fraction (H_2O-P_o) had a negative impact. Therefore, available form of P for shoots growth was associated to H_2O-P_i fraction, as expected·H_2O-P_o fraction is accessible but has to be mineralized to be available.

$$Shoots_P = 0.1153 \times H_2OP_i - 0.2777 \times H_2OP_o + 0.0249, r^2 = 0.71 \tag{10}$$

No correlations were found between ARF results and TP or phosphates concentrations (r = –0.12, p = 0.74, r = –0.26, p = 0.46 respectively) meaning that the TP concentration is not enough to predict P uptake by plants.

Organic $NaHCO_3$-P fraction ($NaHCO_3$-P_o) was positively correlated with roots (r = 0.62, p = 0.025 with D1, D2 and BD). Organic NaOH-P fraction (NaOH-P_o) was also positively correlated with roots (r = 0.79, p = 0.006). A PLS regression model was found between roots P-ARF and inorganic and organic P content in $NaHCO_3$-P and NaOH-P fractions (Eq. (11)).

$$\begin{aligned} Roots_P = {} & 0.0955 \times NaHCO_3P_o + 0.0955 \times NaOHP_o \\ & -0.0584 \times NaHCO_3P_i + 0.0128, r^2 = 0.8641 \end{aligned} \tag{11}$$

Negative impact of inorganic labile $NaHCO_3$ was found whereas organic P forms impacted positively the roots P-ARF. As previously mentioned, phytate and others phosphates esters can be extracted in the NaOH-P fraction (He et al., 2010). Roots can exude phosphatases able to hydrolyse phytate and organic P forms able to be absorbed by roots (Lambers et al., 2006; Gerke, 2015).

Interestingly, roots and shoots P recoveries were correlated with different P fractions. Finally, chemical nutrient equivalents were calculated for all the treatments. The obtained values were above 100% except for Agri_2 (35%) and Agri_1 if only shoots were considered. That means that all the studied digestates (except from pig slurry) can substitute chemical P needs.

Similarly to N results, phase separation impacted the P fertilizing potential. The solid phase of centralised digestate Centr_1 was more suitable as P fertilizer than Centr_2. Composting impacted also the fertilizing value of the municipal waste digestates by lowering the P fertilizer potential relative to its respective digestate. This trend was also showed by Sludge_1 (digestate) versus Sludge_2 (composted digestate). Knowledge of the availability of P and its effects makes possible to anticipate fertilisation according to the soil composition (Alburquerque et al., 2012) and not only based on overall P analysis. Indeed, this was the case of sludge digestates Sludge_1 and 2 which had the highest P contents (same level of Centr_1) but their treatments obtained moderate P-ARF. From P results, first guidelines can be given according to the digestate feedstock typology for the studied calcareous soil and ryegrass. Pig manure digestate (Agri_2) seemed not suitable for P fertilization. Fibrous digestates rich on wheat straw Agri_1 and 3 had very low P fertilizing potential whereas FFMSW_1 presented higher potential as the solid phase Centr_1. The compost FFMSW_2 had also low P fertilizing potential and the liquid phase Centr_2 was intermediate. Sludge 1 and 2 had intermediary results and biowaste digestate BW_1 showed high N and P fertilizing potential.

4. Conclusions

This study focused on different typologies of digestates classified according to their process and to their feedstock. In this context, both P and N speciations showed a wide accessibility range according to feedstocks charateristics. The chemical accessibility indicators described the nutrient availability for plants and allowed the digestates classification on N and P fertilizing potential basis. The N and P speciation impacted the results from incubations with bare soil as well as the apparent coefficients of the use of N and P by the plant for its growth. Depending on the tissue collected (shoot or root), the speciation variables having a significant impact were different for P and N, for the type of calcacerous soil used. First models were found to predict P recovery in shoots and roots using P speciation. Furthermore, C: N ratio value was significant and could be used for shoots N-ARF prediction wheras mineral water extracted N could be used for roots N-ARF prediction. Thus, a more detailed knowledge of the digestates would allow more adequate control of fertilization. Moreover, composting and phase separation impacted the nutrient recovery and can be used as an actuator to propose different organic fertilizers type. In terms of perspectives, field trials on contrasted soils qualities for crops with contrasting nutrient needs should be carried out in order to offer a guide to fertilization by type of digestate. Finally, N and P speciation studied could be used in dynamic models to improve soil and plant model predictions for digestate use in agriculture.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2020.07.052.

References

Ahmad, M., Ahmad, M., El-Naggar, A.-H., Usman, A.D., Abduljabbar, A., Vithanage, M., Elfaki, J., Al-Faraj, A., Al-Wabel, M.I., 2018. Aging effects of organic and inorganic fertilizers on phosphorus fractionation in a calcareous sandy loam soil. Pedosphere 28 (6), 873–883.

Alburquerque, J.A., de la Fuente, C., Campoy, M., Carrasco, L., Nájera, I., Baixauli, C., Caravaca, F., Roldán, A., Cegarra, J., Bernal, M.P., 2012. Agricultural use of digestate for horticultural crop production and improvement of soil properties. Eur. J. Agron. 43, 119–128.

Bareha, Y., Girault, R., Jimenez, J., Trémier, A., 2018. Characterization and prediction of organic nitrogen biodegradability during anaerobic digestion: a bioaccessibility approach. Bioresour. Technol. 263, 425–436.

Bareha, Y., Girault, R., Guezel, S., Chaker, J., Trémier, A., 2019. Modeling the fate of organic nitrogen during anaerobic digestion: development of a bioaccessibility based ADM1. Water Res. 154, 298–315.

Barłóg, P., Hlisnikovský, L., Kunzová, E., 2019. Yield, content and nutrient uptake by winter wheat and spring barley in response to applications of digestate, cattle slurry and NPK mineral fertilizers. Arch. Agron. Soil Sci.

Briat, J.-F., Gojon, A., Plassard, C., Rouached, H., Lemaire, G., 2020. Reappraisal of the central role of soil nutrient availability in nutrient management in light of recent advances in plant nutrition at crop and molecular levels. Eur. J. Agron. 116.

Cavalli, D., Corti, M., Baronchelli, D., Bechini, L., Marino Gallina, P., 2017. CO2 emissions and mineral nitrogen dynamics following application to soil of undigested liquid cattle manure and digestates. Geoderma 308, 26–35.

Decoopman, B., Houot, S., Germain, M., Hanocq, D., Airiaud, A., Lejare, L., Lerouc, C., 2017. Valeur azote des digestats de methanisation. Rencontres COMIFER-GEMAS.

Dou, Z., Toth, J.D., Galligan, D.T., Ramberg Jr, C.F., Ferguson, J.D., 2000. Laboratory procedures for characterizing manure phosphorus. J. Environ. Qual. 29, 508–514.

EU, 1991. European Commission Directive of the Council of December 12, 1991 Concerning the Protection of Waters Against Pollution Caused by Nitrates from Agricultural Sources (91/676/EEC) European Commission, Brussels, 1991, 1–8.

EU Fertilisers Regulation COM, 2016. 157 final 2016/00084 (COD)Proposal for a Regulation on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009

de la Fuente, C., Alburquerque, J.C., Bernal, M., 2013. Soil C and N mineralisation and agricultural value of the products of an anaerobic digestion system. Biol. Fertil. Soils 49, 313–322.

Gerke, J., 2015. Phytate (Inositol Hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants. A review. Plants 4, 253–266.

Gissén, C., Prade, T., Kreuger, E., Nges, I.A., Rosenqvist, H., Svensson, S.-E., Lantz, M., Mattsson, J.E., Börjesson, P., Björnsson, L., 2014. Comparing energy crops for biogas production – yields, energy input and costs in cultivation using digestate and mineral fertilization. Biomass Bioenergy 4, 199–2010.

Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C., 2015. Phosphorous in digestate-based compost: chemical speciation and plant-availability. Waste BiomassValor 6, 481–493.

Grigatti, M., Boanini, E., Mancarella, S., Simoni, A., Centemero, M., Veeken, A.H., 2017. Phosphorous extractability and ryegrass availability from bio-waste composts in a calcareous soil. Chemosphere 174, 722–731.

Grigatti, M., Boanini, E., Bolzonella, D., Sciubba, L., Mancarella, S., Ciavatta, C., Marzadori, C., 2019. Organic wastes as alternative sources of phosphorus for plant nutrition in a calcareous soil. Waste Manage. 93, 34–46.

Grigatti, M., Cavani, L., Ciavatta, C., 2011. The evaluation of stability during the composting of different starting materials: Comparison of chemical and biological parameters. Chemosphere 83 (1), 41–48.

Grigatti, M., Cavani, L., Marzadori, C., Ciavatta, C., 2014. Recycling of Dry-Batch Digestate as Amendment: Soil C and N Dynamics and Ryegrass Nitrogen Utilization Efficiency. Waste and Biomass Valori. 5, 823–833.

Gunnarsson, A., Bengtsson, F., Caspersen, S., 2010. Use efficiency of nitrogen from biodigested plant material by ryegrass. J. Plant Nutr. Soil Sci. 173 (1), 113–119.

Guilayn, F., Jimenez, J., Martel, J-L., Rouez, M., Crest, M., Patureau, D., 2019. Valorization of non-agricultural digestates: a review for achieving added-value products. Waste Manage. 86, 67–79.

He, Z., Zhang, H., Toor, G., Dou, Z., Honeycutt, C.W., Haggard, B., Reiter, M., 2010. Phosphorus distribution in sequentially extracted fractions of biosolids, poultry litter, and granulated products. Soil Sci. 175 (4), 154–161.

Hedley, M.J., Steward, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil phosphorous fraction induced by cultivation practises and by laboratory incubations. Soil Sci. Soc. Am. J. 46, 970–976.

Jimenez, J., Aemig, Q., Doussiet, N., Steyer, J.-P., Houot, S., Patureau, D., 2015. A new organic matter fractionation methodology for organic wastes: Bioaccessibility and complexity characterization for treatment optimization. Bioresour. Technol. 194, 344–353.

Jimenez, J., Han, L., Steyer, J.-P., Houot, S., Patureau, D., 2017. Methane production and fertilizing value of organic waste: organic matter characterization for a better prediction of valorization pathways. Bioresour. Technol. 241, 1012–1021.

Lambers, H., Shane, M.W., Cramer, M.D., Pearse, S.J., Veneklaas, E.J., 2006. Root structure and functioning for efficient acquisition of phosphorus: matching morphological and physiological traits. Ann. Bot. 98 (4), 693–713.

Lashermes, G., Nicolardot, B., Parnaudeau, V., Thuriès, L., Chaussod, R., Guillotin, M. L., Linères, M., Mary, B., Metzger, L., Morvan, T., Tricaud, A., Villette, C., Houot, S., 2010. Typology of exogenous organic matters based on chemical and biochemical composition to predict potential nitrogen mineralization. Bioresour. Technol. 101 (1), 157–164.

Li, M., Tang, Y., Lu, X.-Y., Zhang, Z., Cao, Y., 2018. Phosphorus speciation in sewage sludge and the sludge-derived biochar by a combination of experimental methods and theoretical simulation. Water Res. 140, 90–99.

Li, L., Pang, H., He, J., Zhang, J., 2019. Characterization of phosphorus species distribution in waste activated sludge after anaerobic digestion and chemical precipitation with Fe3+ and Mg2+. Chem. Eng. J. 373, 1279–1285.

Mazzini, S., Borgonovo, G., Scaglioni, L., Bedussi, F., D'Imporzano, G., Tambone, F., Adani, F., 2020. Phosphorus speciation during anaerobic digestion and subsequent solid/liquid separation. Sci. Total Environ. 734.

Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. Eng. Life Sci. 12 (3), 242–257.

Morvan, T., Nicolardot, B., Péan, L., 2006. Biochemical composition and kinetics of C and N mineralization of animal wastes: a typological approach. Biol. Fertil. Soils 42, 513–522.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

Rigby, H., Smith, S.R., 2013. Nitrogen availability and indirect measurements of greenhouse gas emissions from aerobic and anaerobic biowaste digestates applied to agricultural soils. Waste Manage. 33 (12), 2641–2652.

Schiemenz, K., Eichler-Löbermann, B., 2010. Biomass ashes and their phosphorus fertilizing effect on different crops. Nutr. Cycl. Agroecosyst. 87 (3), 471–482.

Tambone, F., Orzi, V., D'Imporzano, G., Adani, F., 2017. Solid and liquid fractionation of digestate: mass balance, chemical characterization, and agronomic and environmental value. Biores. Technol. 243, 1251–1256.

Tampio, E., Salo, T., Rintala, J., 2016. Agronomic characteristics of five different urban waste digestates. J. Environ. Manage. 169, 293–302.

Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soils. Soil Sci. Soc. Am. Proc. 29, 677–678.

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Review

Modelling hydrolysis: Simultaneous versus sequential biodegradation of the hydrolysable fractions

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ABSTRACT

Hydrolysis is considered the limiting step during solid waste anaerobic digestion (including co-digestion of sludge and biosolids). Mechanisms of hydrolysis are mechanistically not well understood with detrimental impact on model predictive capability. The common approach to multiple substrates is to consider simultaneous degradation of the substrates. This may not have the capacity to separate the different kinetics. Sequential degradation of substrates is theoretically supported by microbial capacity and the composite nature of substrates (bioaccessibility concept). However, this has not been experimentally assessed. Sequential chemical fractionation has been successfully used to define inputs for an anaerobic digestion model. In this paper, sequential extractions of organic substrates were evaluated in order to compare both models. By removing each fraction (from the most accessible to the least accessible fraction) from three different substrates, anaerobic incubation tests showed that for physically structured substrates, such as activated sludge and wheat straw, sequential approach could better describe experimental results, while this was less important for homogeneous materials such as pulped fruit. Following this, anaerobic incubation tests were performed on five substrates. Cumulative methane production was modelled by the simultaneous and sequential approaches. Results showed that the sequential model could fit the experimental data for all the substrates whereas simultaneous model did not work for some substrates.

Abbreviations: ADM1, anaerobic digestion model number one; ASM, activated sludge model; BMP, biochemical methane potential (NmL CH_4.gVS^{-1}); BMP 2.0, biochemical methane potential number 2 after acclimation phase (NmL CH_4.gVS^{-1}); DOM, dissolved organic matter; COD, chemical oxygen demand (g O_2.g TS^{-1}); $F_{accessibility_i}$, switching function; f_X_{RC}_xI, inert fraction of XRC (% COD); f_X_{RC}_ch, carbohydrate fraction of XRC (% COD); f_X_{RC}_pr, protein fraction of XRC (% COD); f_X_{RC}_li, lipid fraction of XRC (% COD); f_X_{MC}_xI, inert fraction of XMC (% COD); f_X_{MC}_ch, carbohydrate fraction of XMC (% COD); f_X_{MC}_pr, protein fraction of XMC (% COD); f_X_{MC}_li, lipid fraction of XMC (% COD); f_X_{SC}_xI, inert fraction of XSC (% COD); f_X_{SC}_ch, carbohydrate fraction of XSC (% COD); f_X_{SC}_pr, protein fraction of XSC (% COD); f_X_{SC}_li, lipid fraction of XSC (% COD); f_X_{NE}_xI, inert fraction of XNE (% COD); f_X_{NE}_ch, carbohydrate fraction of XNE (% COD); f_X_{NE}_pr, protein fraction of XNE (% COD); f_X_{NE}_li, lipid fraction of XNE (% COD); f_xch_xc, ADM1 default parameters for disintegration of particular COD into carbohydrates (%COD); f_xli_xc, ADM1 default parameters for disintegration of particular COD into lipids (%COD); f_xpr_xc, ADM1 default parameters for disintegration of particular COD into proteins (%COD); f_xi_xc, ADM1 default parameters for disintegration of particular COD into inerts (%COD); K_{hyd}_XRC, contois hydrolytic biomass growth rate for XRC hydrolysis (d^{-1}); K_{hyd}_XMC, contois hydrolytic biomass growth rate for XMC hydrolysis (d^{-1}); K_{hyd}_XSC, contois hydrolytic biomass growth rate for XSC hydrolysis (d^{-1}); K_{hyd}_XNE, contois hydrolytic biomass growth rate for XNE hydrolysis (d^{-1}); K_I_XRC, switching function inhibition parameter for XMC hydrolysis (kg COD. m^{-3}); K_I_XMC, switching function inhibition parameter for XSC hydrolysis (kg COD. m^{-3}); K_I_XSC, switching function inhibition parameter for XNE hydrolysis (kg COD. m^{-3}); NIRS, near infra-red spectroscopy; VFA, volatile fatty acids; VS, volatile solids (% dried matter); XD, dead biomass variable (kg COD. m^{-3}); X_{RC}, readily biodegradable fraction (kg COD. m^{-3}); X_{MC}, moderately biodegradable fraction (kg COD. m^{-3}); X_{SC}, slowly biodegradable fraction (kg COD. m^{-3}); X_{NE}, non-extractible fraction (kg COD. m^{-3}).

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1. Introduction

In mixed substrate biological conversion, hydrolysis is used as the general depolymerisation of substrates into soluble compounds. It is dominated by the actual process hydrolysis – i.e., depolymerisation into monomers by addition of water molecules (Brock and Madigan, 1991). The process is mediated by enzymes, generally in extracellular reactions. In mixed substrate mathematical models, the hydrolysis process must be adequately described to allow predicting spatial and temporal availability of organic substrates for nutrient removal processes (Morgenroth et al., 2002). Hydrolysis is generally considered the limiting step in biodegradation of particulates and solids substrates (Vavilin et al., 2008). Process modelling kinetics is dominated by the limiting steps and hence the hydrolysis model is critical. According to a review made by Morgenroth et al. (2002), hydrolysis and kinetics in wastewater treatment and excess sludge from wastewater treatment applications are not well understood and first order processes are applied as an aggregate approximation (Eastman and Ferguson, 1981).

Hydrolysis refers to all mechanisms that make slowly biodegradable substrate available for microorganism growth (Gujer et al, 1999). In this latter definition, the key word "available" leads to consider three major concepts: bioaccessibility, bioavailability and biodegradability. Hydrolysis is mainly governed by bioaccessibility (Jimenez et al., 2015a, 2015b). Indeed, due to the complex organisation of some organic residues, bioaccessibility defines the access to the molecules. It can depend on physical structure, process duration and hydrolytic activity. Thus, a fraction can become bioavailable by crossing the membrane of the microorganism mediating the degradation (Semple et al., 2004; Aquino et al., 2008). Ultimately, the biodegradable fraction is the bioavailable organic matter consumed by the biomass.

Different hydrolysis approaches have been applied in aerobic and anaerobic models. In aerobic process models, the hydrolysis concept has been challenged several times by many authors (Sollfrank and Gujer, 1991; Gujer et al., 1999; Shimizu et al., 1993; Siegrist et al., 1993; Angelidaki et al., 1997; Spérandio and Paul., 2000; Vavilin et al., 2008; Yasui et al., 2008; Mottet et al., 2013; Garcia-Gen et al., 2015) since the well-known developed activated sludge models (ASM) (Henze et al., 1987). However, first order processes have been generally applied due to difficulties in identifying higher order models. Multiple (two) particulate biodegradable fractions have been considered not only according to the physical separation process but also to the biological response of the model in a simultaneous degradation way (Ekama and Marais, 1979; Ekama et al., 1986; Henze et al., 1987; Gujer et al., 1999). In this respect, the associated kinetics was based on a surface-limited equation and one biomass. Ekama and Marais (1979) divided the particulate fraction into two biodegradable fractions: a readily biodegradable fraction mainly consisting of soluble organic matter; and a slowly biodegradable fraction consisting of large molecules, colloids and particles that have to be hydrolysed before degradation. The distinction between these two fractions was also determined by experimental biological response analysis (Ekama et al., 1986; Spérandio and Paul., 2000).

As regards anaerobic process models such as the Anaerobic Digestion Model No. 1 (ADM1, Batstone et al., 2002), one biodegradable fraction was initially considered. Then, this biodegradable fraction is split into biochemical fractions (i.e. carbohydrates, lipids, proteins and inert) after a disintegration process (i.e., a mix of sequential and simultaneous). This approach was not supported by experiments, and was purely conceptual, and has been criticised (Batstone et al., 2015). Other previous studies (see, for instance, Shimizu et al., 1993; Siegrist et al., 1993; Angelidaki et al., 1997) have generally applied first order kinetics.

The common approach in the event of inadequate model performance is: (i) to increase the number of hydrolysable fractions (Sollfrank and Gujer, 1991; Orhon et al., 1998; Spérandio and Paul, 2000; Yasui et al., 2008; Mottet et al., 2013; Garcia-Gen et al., 2015); (ii) to replace the first order kinetics by surface limitation equations (i.e. Contois equation, Vavilin et al., 2008; Mottet et al., 2013), or (iii) to include a particle size distribution model (Dimock et al., 2006; Sanders et al., 2000; Yasui et al., 2008); (iv) to differentiate non-active and active hydrolytic bacteria in particles colonization (Ginestet et al., 2002; Benneouala et al., 2017).

In the studies considering several hydrolysable fractions, some authors considered simultaneous degradation (Sollfrank and Gujer, 1991; Lagarde et al., 2005; Orhon et al., 1998; Mottet et al., 2013; Garcia-Gen et al., 2015; Kouas et al., 2017) and others sequential degradation (Bjerre, 1996; Confer and Logan, 1997; Lagarde et al., 2005; Spérandio and Paul, 2000, Yasui et al., 2008). These approaches are inconsistent mechanistically and in basic kinetic response. A key challenge is to determine experiments to identify the most appropriate approach.

Recently, a promising methodology for organic matter characterisation has been successfully developed to describe the organic matter bioaccessibility and bioavailability of organic residues (Muller et al., 2014; Jimenez et al., 2015a, 2015b). Jimenez et al. (2014) showed that bioaccessibility could be determined for wastewater treatment sludge using sequential extractions to characterize the organic matter accessibility. This fractionation method was subsequently used to determine new input variables of a modified ADM1 model in order to predict biogas performance and digestate quality of an anaerobic digester fed with sludge (Jimenez et al., 2015a).

Since the method is a sequential chemical procedure, it is possible to isolate and consider each fraction separately and to perform biological tests on them to evaluate simultaneous or sequential behaviour.

In this paper, the use of the new fractionation methodology, describing bioaccessibility, was applied on several substrates and their respective fractions in anaerobic incubation tests. The results of the fractionation methodology were used as input variables of an anaerobic digestion model for the treatment of different organic wastes related to the two hypotheses: simultaneous and sequential concepts. Finally, the objective of this paper was to challenge the classical simultaneous concept of multi-substrates hydrolysis experimentally (i) by using the bioaccessibility characterization and anaerobic incubation tests and (ii) by comparing simulation results obtained by simultaneous approach modelling and sequential approach modelling.

2. Material and methods

2.1. Accessibility characterization

The accessibility characterization methodology was based on sequential chemical extractions that can be used as indicators to describe the biochemical molecules of a substrate. Indeed, Jimenez et al. (2015a) showed that each fraction, from the most to the least accessible one, is composed of different kinds of molecules associated to the extraction nature which impact the biodegradability. The characterisation methodology used in this study is detailed in (Jimenez et al., 2014, 2015b) and has been optimised in order to fractionate the substrate within 2 days instead of 5 days. The main optimisation was obtained by pooling the first two extractions into only one, corresponding to the most accessible fractions, which were biodegraded with same kinetics as shown by (Jimenez et al., 2014).

First, a liquid/solid phase separation was performed by sample centrifugation (18,600 g, 20 min, 4 °C) and the supernatant was filtered at 0.45 μm. The recovered filtered supernatant fraction was considered as the first fraction named Dissolved Organic Matter (DOM). It was considered as the most available fraction. The fraction retained by the filter is measured, but is not normally considered further, as it represents a negligible quantity of COD. The solid pellet was dried and milled (1 mm) and sequential chemical extractions (30 mL) were performed on 0.5 g of this dried pellet.

Based on Jimenez et al. (2014, 2015a, 2015b), three fractions were considered in this study and were obtained by performing sequential chemical extractions, as follows:

- The readily hydrolysable fraction (X_{RC}) was obtained from supernatant of a saline basic extraction (pellet suspended in 30 mL of 10 mM NaCl and 10 mM NaOH twice) and centrifuged for 15 min, at 30 °C and 300 rpm.
- The moderately hydrolysable fraction (X_{MC}) was obtained from the supernatant of 4 sequential basic extractions (30 mL of 0.1 M NaOH) of the remaining pellet for 1 h, at 30 °C and 300 rpm.
- The slowly hydrolysable fraction (X_{SC}) was obtained from the supernatant of 2 sequential strong acid extractions (25 mL 72% w/v H_2SO_4) of the remaining pellet for 3 h, at 30 °C and 300 rpm.
- The non-extractable fraction (X_{NE}) was obtained by subtraction.

2.2. Analysis on the chemical sequential extractions

The Chemical Oxygen Demand (COD) was measured in duplicate using Aqualytic kits (0–1500 mg $O_2.L^{-1}$) on substrates and extracts. Indeed, the analysis of the freeze-dried and milled (1 mm) sample was performed on a solution of 1 g TS L^{-1}).

At each extraction step, the insoluble fraction was recovered, dried and milled at 1 mm. The BMP values of each remaining fraction were obtained using an innovative and rapid FlashBMP® method developed by Ondalys (Lesteur et al., 2011). This method is based on Near InfraRed Spectroscopy (NIRS) applied to more than 600 types of wastes (agro-industrial waste, green waste, energy crops, municipal solid waste, sludge and digestates) for which classical BMP tests were performed according to Angelidaki and Sanders (2004). Samples were freeze-dried and milled at 1 mm before NIRS acquisition. Spectra were measured using a BUCHI NIRFlex N-500 (Buchi, Switzerland), with add-on vials. Results are expressed in mL $CH_4.gVS^{-1}$. The biodegradability of each fraction can be then obtained by converting the results into in mL $CH_4.gCOD^{-1}$ and divided by 350 mL $CH_4.gCOD^{-1}$, the theoretical yield (Angelidaki and Sanders, 2004).

Proteins and carbohydrates of each fraction were analysed respectively by the Lowry method (Lowry et al., 1951) and the Dubois method (Dubois et al., 1956). Lipids were analysed as heptane extractable material by gravimetry. 1 g of freeze-dried and milled sample was extracted with 25 mL of hot and pressurized heptane using an extra-Accelerated Solvent Extractor ASE 200 (Thermo Fisher Scientific®, Sunnyvale, California 94,085 USA). The extracted solution was collected in a 60 mL glass vial. The heptane was evaporated under a N_2 flow. The quantity of extracted fatty matter was measured once the remaining sample was dried at 105 °C for 2 h.

2.3. Anaerobic incubation tests

In addition to the FlashBMP measurements, two types of anaerobic incubation tests were used in the study: (i) a classical biochemical methane potential (BMP) to assess the maximum anaerobic biodegradability of a substrate, in optimal conditions for a characterization objective, and (ii) a successive batch anaerobic reactor to acclimate the microorganisms and simulate the real digester performances for modelling objective.

- Classical BMP test

Three model substrates of different composition were selected: wheat straw (i.e. lignocellulosic substrate where biodegradable material is protected by an external layer of recalcitrant tissue), apple (carbohydrates substrate) and wastewater treatment sludge from an activated sludge plant (retention time of 20 days). Wastewater treatment sludge was selected to be representative of microorganism compounds, rich in proteins and *exo*-polymeric susbtances organised in flocs (Jimenez et al., 2014). The three substrates were fractionated as described by the Jimenez et al. (2015a, 2015b) protocol. At each step of the chemical extraction protocol, the recovered pellet after centrifugation was incubated under anaerobic conditions, with the same substrate COD concentration as described in Jimenez et al. (2014). Three samples were considered:

- the initial substrate (wheat straw, apple or wastewater treatment sludge);
- the pellet recovered after the first extraction (two saline extractions) and after centrifugation;
- the pellet recovered after the first two extractions (two saline extractions and four basic extractions) and after centrifugation.

The experimental conditions were those described by Angelidaki and Sanders (2004) for the biochemical methane potential (BMP) assessment, in 500 mL bottles. The substrate/inoculum ratios were 0.5 g VS g VS^{-1}. These tests were named BMP tests in this study.

- Successive batch tests

Torrijos et al. (2015) developed a new protocol to assess the BMP value. In order to be closer to the real conditions of a continuously fed digester, successive batch tests were conducted to achieve inoculum acclimation in a 6-L lab-scale reactor. Once the methane production kinetics obtained was stable, the microorganisms were considered acclimated to the substrate. A last feed was then added. These final data were used for modelling purposes. The reactor was magnetically stirred. A temperature of 35 °C was maintained in the reactors by a double wall fed with 35 °C water from a water bath. The biogas production was measured on-line by Milligascounter MGC-1 flow meters (Ritter® gas meters) with a 4–20 mA output. Gas composition was measured using a Shimadzu GC 8 chromatography associated with a Shimadzu GC 3A integrator. The carrier gas was argon. The organic load of each batch was 1 g $VS.L^{-1}$ and the substrate/inoculum ratios were 0.08 g VS.g VS^{-1}.

In this study, successive batch tests were obtained from several experiments of anaerobic digestion of the following organic residues: wheat straw, apple, carrot, potatoes, lettuce, cauliflower and wastewater treatment sludge. Eight fed batch tests were operated before reaching the acclimation of the tested substrates. Wastewater treatment sludge kinetics data were provided by the same test but only after 30 days of batch feeding before data collection (Jimenez et al. 2015a data). Four cumulated methane production curves were obtained over four feed cycles to strengthen the model calibration.

2.4. Definition of simultaneous and sequential concepts

Regarding hydrolysis and biodegradability concepts, the bioaccessibility of a molecule needs to be considered. Indeed, according to Jimenez et al. (2015b), an organic fraction is defined as "bioaccessible" if, at some point, microorganisms have access to it. This depends on several factors, such as process duration, hydrolytic activity of the microorganisms, or the pre-treatments applied. Once bioaccessible, a fraction is biodegradable if it is able to cross the membrane of the microorganism. Semple et al. (2004) defined a minimum size of 10 kDa for molecules to cross the membrane. Therefore, hydrolysis aims at reducing the size of the molecule. Enzymatic potential of the microorganisms and the physical characteristics of the molecule (i.e. size) govern its bioaccessibility. To make a molecule bioavailable, simultaneous versus sequential hydrolysis concepts are two different ways considered in hydrolysis modelling. Fig. 1 gives a schematic overview of these definitions. In the simultaneous concept, all fractions X_{RC}, X_{MC} and the least degradable fractions $X_{SC} + X_{NE}$ are degraded simultaneously. As the most readily degradable fractions are consumed, the overall hydrolysis rate reduces, and hence the degradation kinetics parameters for each fraction are dominated by the slowest degradable fraction.

Concerning the sequential concept, the most accessible fraction (i.e. X_{RC}) is first degraded. This fraction acts as a protection layer and limits the next accessible fraction (i.e. X_{MC}) degradation. Similarly X_{MC} fraction limits the least accessible fractions (i.e. $X_{SC} + X_{NE}$) degradation. Consequently, during the first period of degradation, X_{RC} is the only fraction consumed, before the degradation of X_{MC} and the degradation of $X_{SC} + X_{NE}$.

2.5. Model implementation

The input variables of the Anaerobic Digestion model n°1 (ADM1, Batstone et al., 2002) were replaced by outputs from the fractionation method, i.e. readily hydrolysable fraction X_{RC}, moderately hydrolysable fraction X_{MC}, slowly hydrolysable fraction X_{SC} and non-extractable fraction X_{NE}. Each fraction contains proteins, lipids and carbohydrates as in ADM1. The fractions are degradable according to the parameters f_X_{RC}_xI, f_X_{MC}_xI, f_X_{SC}_xI, f_X_{NE}_xI, where (1-f_X_I) is the biodegradable fraction of each component. The sum of unbiodegradable fraction (i.e. inert in ADM1), carbohydrates, lipids and proteins ratios has to be equal to 1.

Fig. 2 shows a schematic overview of the modified model. ADM1 processes were used as in Batstone et al., (2002). Hydrolysis kinetics was replaced by the Contois (saturation) kinetics model (Vavilin et al., 2008; Mottet et al., 2013), see Eq. (1). The death-regeneration concept was kept but a new variable was introduced as the dead biomass fraction (X_D) which was hydrolysed into proteins, carbohydrates, lipids and inerts using parameters from Batstone et al., (2002) (i.e. f_xpr_xc, f_xch_xc, f_xli_xc and f_xi_xc). Indeed, the dead biomass was regenerated into the substrate fraction X_C in the initial ADM1 model. In the modified model, four particulate COD fractions were considered. The substrates fractions and the regenerated dead biomass were split to avoid confusion in their respective biochemical repartition.

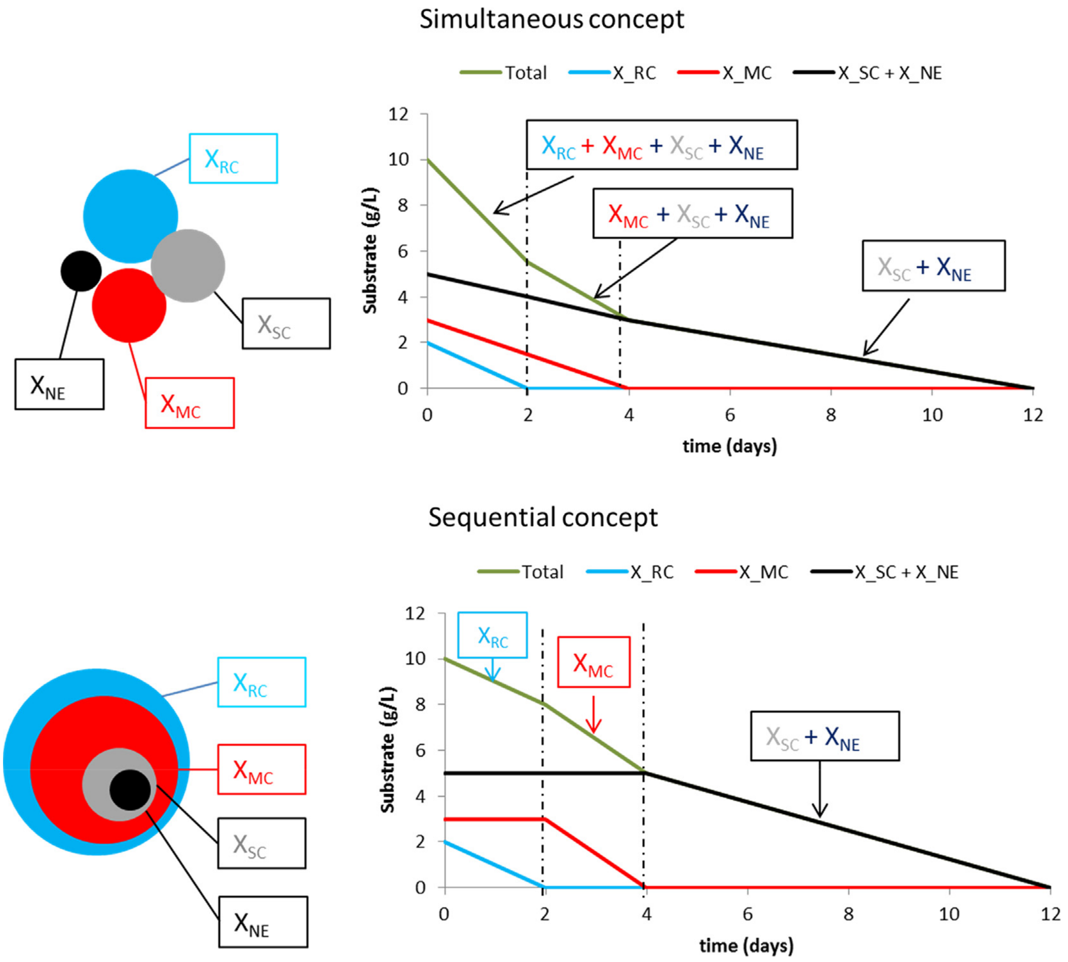

Fig. 1. Schematic definition of simultaneous and sequential concepts. Legend: readily hydrolysable fractions (X_{RC}), moderately hydrolysable (X_{MC}), slowly hydrolysable fractions (X_{SC}) and poorly hydrolysable fractions (X_{NE}).

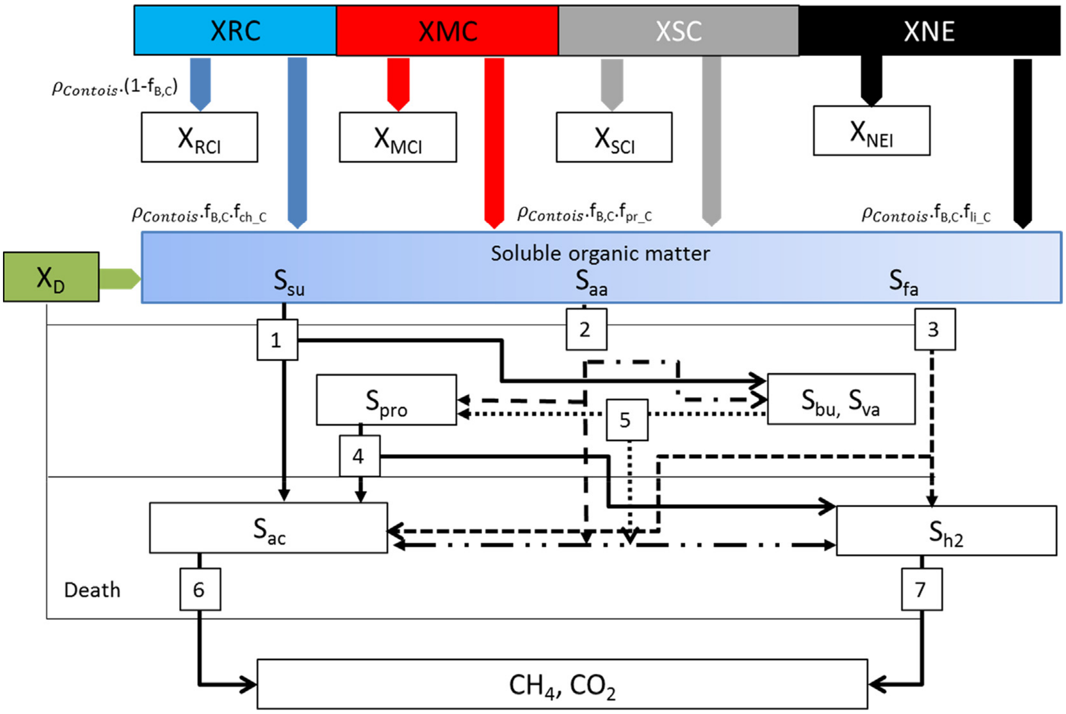

Fig. 2. Modified ADM1 model proposed, (1) acidogenesis from sugars, (2) acidogenesis from amino acids, (3) acetogenesis from long chain fatty acids, (4) acetogenesis from propionate, (5) acetogenesis from butyrate and valerate, (6) acetoclastic methanogenesis and (7) hydrogenotrophic methanogenesis. Schematic overview of the modified anaerobic digestion model based on ADM1.

A "switching" function was introduced in order to simulate sequential hydrolysis which switched off one process while switching on the next (Eqs. (2) to (4)). This function was added to each hydrolysis process by introducing three parameters $K_I\_X_{RC}$, $K_I\_X_{MC}$ and $K_I\_X_{SC}$, as limiting fractions concentrations. In the sequential hydrolysis model, the switching function was below 1. Indeed, it represented a limitation for the next accessible fraction, depending on the K_I_X parameters values. No limitation occurs in the simultaneous case where switching function parameters values ($K_I\_X_{RC}$, $K_I\_X_{MC}$ and $K_I\_X_{SC}$) were considered much higher than the fractions concentrations values.

Analyzing the switching function led to the following statements:

- *If* $S \gg K_{hyd_S}$, $F_{accessibility} \to 0$ (strict sequential concept, high limitation level)
- *If* $S\ K_{hyd_S}$, $F_{accessibility} \to 0.5$
- *If* $S \ll K_{hyd_S}$, $F_{accessibility} \to 1$ (no limitation)

$$\rho_i = K_{kyd}\_S_i \times \frac{S_i/X_i}{K_{S_i} + S_i/X_i} \times X_i \times F_{accessibility_i} \qquad (1)$$

If i = 1, S = X_{RC} and $F_{accessibility} = 1$

$$\text{If } i = 2,\ S = XMC \text{ and } F_{accessibility} = \frac{1}{1 + X_{RC}/K_{I\_X_{RC}}} \qquad (2)$$

$$\text{If } i = 3,\ S = XSC \text{ and } F_{accessibility} = \frac{1}{1 + X_{MC}/K_{I\_X_{MC}}} \qquad (3)$$

$$\text{If } i = 4,\ S = XNE \text{ and } F_{accessibility} = \frac{1}{1 + X_{SC}/K_{I\_X_{SC}}} \qquad (4)$$

where:

S is the concentration of organic matter contained in the fraction considered (kg COD/m^3)
$K_{hyd}\_S_i$ is the growth rate of hydrolytic bacteria for the fraction S_i (d^{-1})
K_S is the half-saturation coefficient of hydrolytic bacteria (–)
X_i is the hydrolytic biomass of each fraction (kg COD/m^3)
$F_{accessibility}$ is a switching function based on the accessibility degree of the substrate (–)
$K_I\_S_i$ is the switching concentration from one fraction to another in the switching function (kg COD/m^3).

The biodegradability of each fraction was obtained using the FlashBMP® analysis (Lesteur et al., 2011) for the batch tests. In the case of the semi-continuous test with sludge, Jimenez et al. (2015a) data were used. The biodegradable fractions as a percentage were calculated using each fraction mass balance between feedstock fractionation and digestate fractionation. The results allowed the calculation of the biodegradability percentage of each fraction, and thus, the inert percentage (i.e. parameters $f\_X_{RC}\_xI$, $f\_X_{MC}\_xI$, $f\_X_{SC}\_xI$, $f\_X_{NE}\_xI$).

The initial values of the state variables (i.e. microorganisms' state variables) used in the model were determined by simulating the model under continuous conditions to reach steady-state equilibrium. The steady-state values of the state variables were then used as state variable initial values. This estimation was considered as a non-linear problem. Using the modified ADM1, the hydrolysis parameters were optimised by trial and error to minimize the squared value of the difference between predicted and experimental methane production curves.

3. Results and discussion

3.1. Fraction biodegradability test: Case studies on apple, wastewater treatment sludge and wheat straw

In order to test a wide range of molecular and accessibility structures, three substrates were chosen: wheat straw (lignocellulose), apple (i.e. carbohydrates) and wastewater sludge (proteins). Indeed, the lignin protection layer from wheat straw, the floc structure from wastewater sludge and the simpler structure of apple's carbohydrates have specific characteristics to investigate the simultaneous and sequential biodegradation concepts. To reach this goal, the removal of X_{RC} and X_{MC} fraction (i.e. protection layers) was proposed to investigate the BMP kinetics of total and residual fractions. As shown by Jimenez et al. (2014), the X_{RC} extractions did not alter the chemical structure of the residual fractions. The substrates fractionations are presented in Fig. 3. The BMP tests results are presented in Fig. 4. Regarding the accessibility characterization, the method was repeatable as suggested by the standard deviations obtained (less than 5% for X_{RC} and X_{MC} and between 3 and 10% for X_{SC}). The results are consistent considering the fruits/vegetables, wheat straw and wastewater treatment sludge nature. Indeed, apple contained mainly accessible fractions (large fraction of X_{RC}, 68% of COD) while wheat straw is mainly composed of poorly accessible fractions (i.e. $X_{SC} + X_{NE} = 73\%$ of COD). The wastewater treatment sludge had intermediate values ($X_{RC} = 29\%$ and $X_{MC} = 37\%$ of COD). The saline and basic extractions allowed the ionisation of some poorly attached proteins. This extraction was based on sludge *exo*-polymeric substance extraction and on the flocs structure of activated sludge (Jimenez et al., 2014), which is why wastewater sludge was mainly composed of X_{RC} and X_{MC} fractions (Fig. 3). On the contrary, wheat straw contained more fibers such as celluloses, extracted by acid hydrolysis (X_{SC} fraction). X_{NE} was mainly composed of non-soluble lignin (Jimenez et al., 2015a). However, some lignin can be solubilised under basic conditions (Carrere et al., 2010). Wheat straw's X_{MC} fraction contained alkaline soluble lignin. On the contrary, substrate like apple was mainly composed of X_{RC} fraction, related to soluble sugar and protein. As stated by Jimenez et al (2015b), these results confirmed the ability of the extraction procedure to characterize accessibility and biochemical nature of the substrates.

For each substrate, three BMP tests associated with the biodegradation of the entire substrate, the substrate deprived of X_{RC} fraction, and the substrate deprived of X_{RC} and X_{MC} fractions, after saline and basic extractions respectively were done.

Regarding the cumulated methane production obtained for the apple (Fig. 4a), the biodegradability decreased as the accessibility decreased, similar to the rate of each remaining samples after sequential extractions. Both methane production rate and yield values were higher for the total sample than for total sample without X_{RC} and without X_{RC} and X_{MC}. The methane production rate evolution of each fraction could be obtained by subtraction and the simultaneous concept can be applied.

Concerning the wheat straw (Fig. 4b), as previously mentioned, the X_{RC} fraction was low, thus the biodegradability curves of total substrate and of total substrate minus X_{RC} were very similar. However, when the BMP test was performed on the $X_{SC} + X_{NE}$ fractions only, the rate increased (linear curve slope between 1 and 3 days calculated: 46 $mlCH_4.gCOD^{-1}\ d^{-1}$) compared to the total substrate without X_{RC} (linear curve slope between 1 and 3 days calculated: 25 $mlCH_4.gCOD^{-1}\ d^{-1}$). Finally, the specific methane productions were the same for the three experiments.

Methane production rate curves from the individual fractions X_{RC} and X_{MC} can be calculated according their fractionation percentage of COD in the substrate as explained by Equations (5) and (6).

$$\mathbf{BMP}_{(\mathbf{X_{RC}})} = \boldsymbol{BMP}_{(\mathbf{X_{RC}}+\mathbf{X_{MC}}+\mathbf{X_{SC}}+\mathbf{X_{NE}})} - \mathbf{BMP}_{(\mathbf{X_{MC}}+\mathbf{X_{SC}}+\mathbf{X_{NE}})} \times \frac{\mathbf{X_{MC}} + \mathbf{X_{SC}} + \mathbf{X_{NE}}}{\mathbf{X_{RC}} + \mathbf{X_{MC}} + \mathbf{X_{SC}} + \mathbf{X_{NE}}} \tag{5}$$

$$\mathbf{BMP}_{(\mathbf{X_{MC}})} = \mathbf{BMP}_{(\mathbf{X_{MC}}+\mathbf{X_{SC}}+\mathbf{X_{NE}})} \times \frac{\mathbf{X_{MC}} + \mathbf{X_{SC}} + \mathbf{X_{NE}}}{\mathbf{X_{RC}} + \mathbf{X_{MC}} + \mathbf{X_{SC}} + \mathbf{X_{NE}}} - \mathbf{BMP}_{(\mathbf{X_{SC}}+\mathbf{X_{NE}})} \times \frac{\mathbf{X_{SC}} + \mathbf{X_{NE}}}{\mathbf{X_{RC}} + \mathbf{X_{MC}} + \mathbf{X_{SC}} + \mathbf{X_{NE}}} \tag{6}$$

where BMP (X) is the cumulative methane production of the X fraction (NmL CH_4) and X_{RC}, X_{MC}, X_{SC}, X_{NE} the COD concentration of each fraction (kg COD m^{-3})

Fig. 5a shows the results obtained after applying Eqs. (5) and (6) on the wheat straw methane production cumulated curves. The simultaneous hypothesis requires that all the fractions are

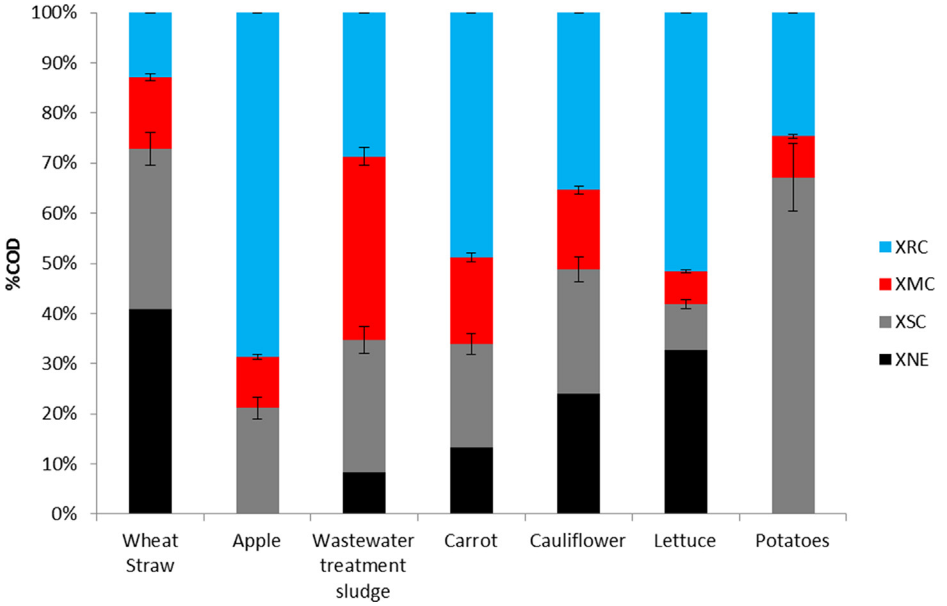

Fig. 3. COD fractionation of the studied substrates.

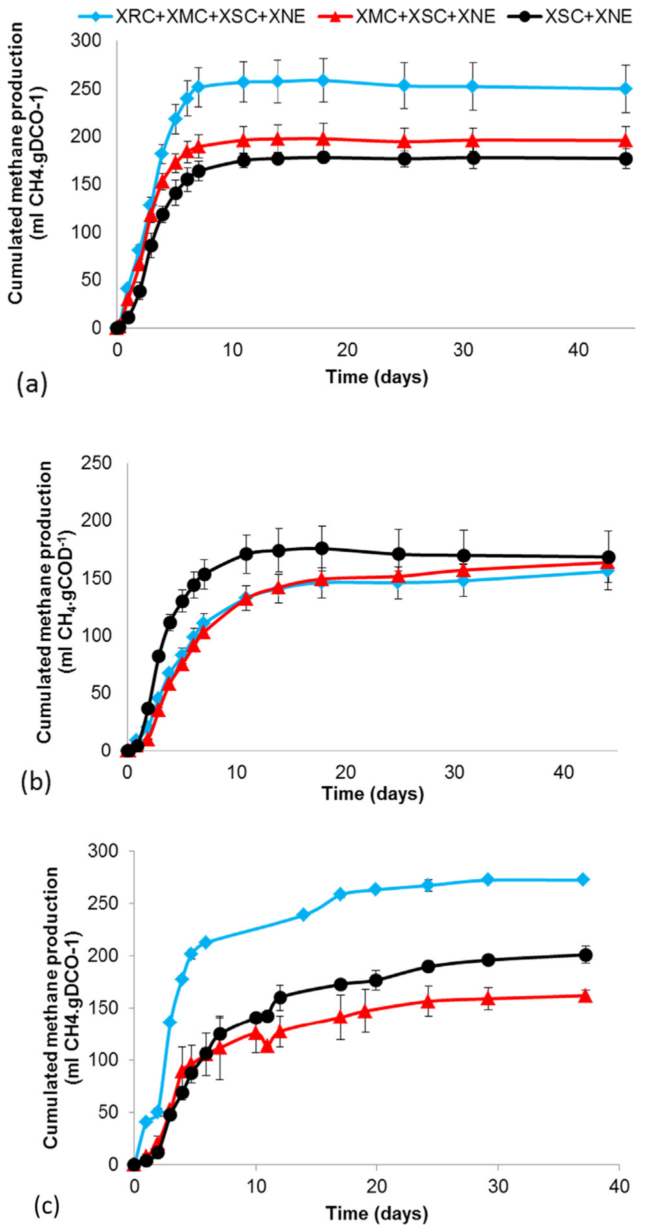

Fig. 4. Anaerobic biodegradation of the apple (a), the wheat straw (b) and the wastewater treatment sludge fractions (c).

hydrolysed at the same time (as shown by the Fig. 1). In this hypothesis, methane production rate curve associated to X_{MC} was calculated and negative values were obtained (Fig. 5a), proving that simultaneous hypothesis did not fit. If positive X_{MC} methane production rate curve is to be obtained, another approach could be to assume that a fraction *n* is not hydrolysed until the fraction $n-1$ reaches low concentrations. This second scenario was simulated using sequential modelling approach and with the switching function previously described (Fig. 5b). In the case of the sequential hypothesis, X_{MC} is always positive. Therefore, the proposed switching functions (Eqs. (2) and (3)) have to be used for modelling the hydrolysis of each fraction when applying this hypothesis.

Overall, the sequential approach is applicable for the three substrates biodegradation. Indeed, the composition and structural accessibility feature of wheat straw and sewage sludge seemed to reveal the sequential concept. Wheat straw and sewage sludge have different physical accessibility structures. Regarding wheat straw, a compact layer of wax covered the outside of the straw, which protects the straw from insects and microorganisms. At the boundary of the primary and second walls a network structure appeared, made of cellulose and hemicellulose, with some lignin localised on the surface of the network as observed by atomic force microscopy (Yan et al., 2004). Thus, the wheat straw has a lignin and wax layer which makes not accessible a part of cellulose and hemicellulose and the sewage sludge contains exocellular-polymeric substances which were probably extracted and made accessible by alkali extraction.

Moreover, the alkaline X_{MC} extraction step acts as a pre-treatment for both substrates. It allows the partial solubilisation of recalcitrant material wheat straw. Plant stems have a recalcitrant shell which protects the degradable interior. Alkali treatments induce depolymerisation and cleavage of lignin-carbohydrates linkages (Zhen et al., 2017). In the case of wheat straw, the wax layer protects another layer containing cellulose and pectin (pectin is water soluble). The X_{MC} extraction removes the wax layer and allows a quicker biodegradation of the X_{SC} fraction (i.e. hemicellulose and cellulose).

This means that the poor accessibility of X_{SC} limits hydrolysis despite the high biodegradable potential of X_{SC} which is consistent with the sequential concept. Similar results were obtained by Rincker et al. (2013) after pre-treatments applied on lignocellulose-like substrates. According to the authors, the lag phase could correspond to a colonisation process. This colonisation phase was also observed for cellulosic fibres with low lignin content (toilet paper) found in primary sludge (Ginestet et al., 2002). In the case of the apple, this phenomenon is not occurring because the fruit was pulped before feeding the reactor and physical structure is lost in the crushed apple.

Regarding the sewage sludge (Fig. 4c), similar results were obtained as wheat straw. Biological sludge is organized in flocs with cells coated with *exo*-polymeric substances. This three-dimensional gel-like biopolymer provides protective shielding and prevents cell rupture and lysis influencing flocculation and dewaterability. The cell membranes are also composed of glycan strands crosslinked by peptides acting as barriers to anaerobic digestion (Zhen et al., 2017). After X_{MC} removal, the flocs were disrupted. Sequential hypothesis fits better than simultaneous hypothesis (i.e. negative results obtained, as for wheat straw).

The methane production rate slowed down at day 6 (Fig. 4) before increasing at day 12. Yasui et al. (2008), Mottet et al. (2013) and Jimenez et al. (2014) also observed such a deceleration phenomenon between readily and slowly biodegraded fractions of organic matter from primary and biological sludge. As no inhibition phenomenon was noticed, the authors proposed to use this observation to assess both readily and slowly biodegradable fractions.

These results showed that sequential biodegradation concept could be revealed in cases where the accessibility was limited like wheat straw and biological sludge. In those cases, a part of X_{MC} fraction has to be degraded before to have access to the X_{SC} fraction. However, some aspects have to be investigated such as the impact of the chemical extraction procedure on the molecular structure of X_{SC}. Even if the lignin barrier of the wheat straw was solubilised by the alkaline extraction, one issue not solved was about the initial molecular structure of X_{SC} alteration by alkaline extraction. Jimenez et al. (2014) compared the methane production rate curves obtained with whole wastewater treatment sludge and with the sludge after saline + alkaline extraction (10 mM). The results showed that the methane production rate curve of the remained pellet overlaid the least biodegradable fraction observed for the whole substrate. This means that the X_{RC} extraction seemed not to alter the X_{SC} fraction degradation kinetics. However, despite the fact that alkaline condition targets lignin whereas acid condition targets holocelluloses, no similar test has been performed after stronger alkaline extraction.

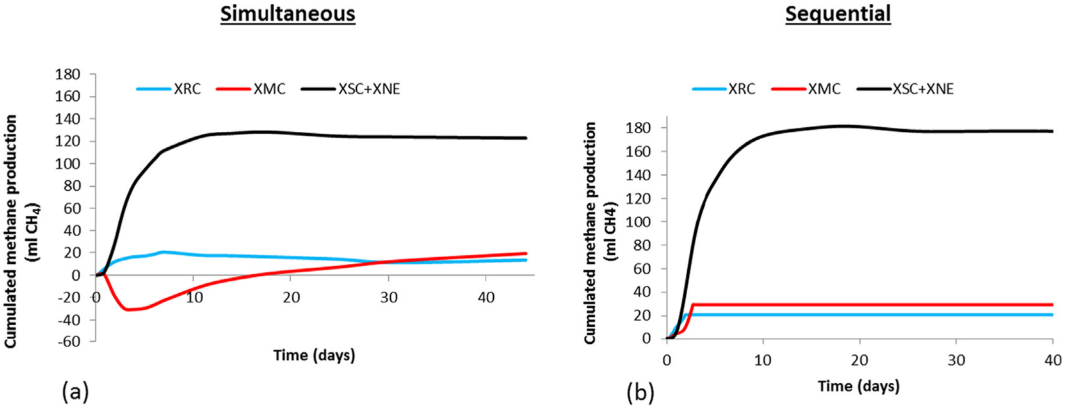

Fig. 5. Fraction kinetics calculation based on experimental data using simultaneous concept (a) and fraction kinetic simulation based on sequential concept model (b) of the digestion of wheat.

In the apple case, the X_{SC} biodegradation kinetics was below than those of X_{MC} and X_{RC}. It seems that there was no structural accessibility limitation as for wheat straw. Thus, both sequential and simultaneous concepts fit.

Based on these results, other substrates were characterized in terms of sequential chemical extraction and anaerobic incubation tests to test the two hypotheses (i.e. simultaneous versus sequential) by comparing the two associated modified models.

3.2. Results obtained on several substrates

Five substrates were tested with the successive batch test method. They represent a large range of biochemical characteristics as shown by the measured parameters and variables in Table 1. Results of biogas production measured during these batch tests are summarised in Fig. 6. Simulations obtained with the simultaneous (i.e. the switching function equal to 1 in the model) and sequential models are also presented in Fig. 6. Table 1 presents the measured parameters and variables used in the models and the calibration data parameters, all others parameters of ADM1 being equal to their standard values from Batstone et al. (2002). Fractions and stoichiometric parameters were measured as described in material and methods. The hydrolytic biomass growth rate from sequential and simultaneous kinetics and the switching function parameter value were calibrated using the cumulated methane production rate by trial and error methodology. Table 2 presents the simulated methane production rate vs experimental data errors. The sum of squared errors J can be used as a criterion (Dochain et al., 2001) to calibrate the model and estimate the prediction model quality.

From the results obtained, the J values were always lower in the sequential model in comparison with the simultaneous model for the 5 substrates considered. During calibration step of methane production rate, the lowest errors were obtained with switching function parameter $K_{I\_XRC\ \mathrm{or}\ XMC}$ values equal to 0.05 g m^{-3} except for carrot (0.01 g m^{-3}). These values were low compared to the substrates fractions concentrations meaning that the sequential approach limitation was high (i.e. switching function low).

Table 1
Calibration parameters of simultaneous and sequential model for the five substrates.

			Carrot	Cauliflower	Lettuce	Wheat straw	Potato	Wastewater treatment sludge
Measured variables and parameters	COD per fraction (kgDCO.m^{-3})	X_{RC}	0.59	0.47	0.63	0.51	0.33	0.29
		X_{MC}	0.21	0.21	0.08	0.56	0.11	0.37
		X_{SC}	0.25	0.33	0.11	1.26	0.90	0.26
		X_{NE}	0.16	0.32	0.40	1.61	0.00	0.08
	Fractions content into inert, proteins, lipids and carbohydrates (%COD)	f_XRC_xI	0.00	0.20	0.24	0.51	0.00	0.21
		f_XRC_ch	0.73	0.55	0.37	0.02	0.77	0.19
		f_XRC_pr	0.05	0.19	0.09	0.04	0.23	0.46
		f_XRC_li	0.22	0.01	0.18	0.43	0.00	0.14
		f_XMC_xI	0.21	0.70	0.66	0.80	0.19	0.65
		f_XMC_ch	0.31	0.16	0.03	0.06	0.19	0.08
		f_XMC_pr	0.05	0.13	0.03	0.02	0.00	0.20
		f_XMC_li	0.43	0.01	0.28	0.12	0.62	0.07
		f_XSC,NE_xI	0.43	0.18	0.42	0.46	0.03	0.80
		f_XSC,NE_ch	0.33	0.52	0.17	0.40	0.79	0.18
		f_XSC,NE_pr	0.01	0.14	0.06	0.01	0.01	0.02
		f_XSC,NE_li	0.23	0.16	0.35	0.13	0.17	0
Calibrated parameters	Experimental data used		BMP 2.0	BMP 2.0	BMP 2.0	BMP 2.0	BMP 2.0	Fed batch reactor
	Sequential Switch (kg.COD.m^{-3})	K_I	0.01	0.05	0.05	0.05	0.05	0.05
		Khyd_XRC	3.50	4.00	4.00	10.00	5.00	11
	Kinetics (d^{-1})	Khyd_XMC	2.50	2.00	1.00	3.50	1.00	9
		Khyd_XSC	2.50	1.00	9.00	3.50	1.50	9
		Khyd_XNE	0.50	0.50	7.00	0.80	0.50	9
	Simultaneous kinetics (d^{-1})	Khyd_XRC	2.00	4.00	4.00	5.00	2.00	9
		Khyd_XMC	1.67	2.00	2.00	0.50	1.00	6
		Khyd_XSC	1.67	1.00	1.00	0.80	0.75	6
		Khyd_XNE	0.33	0.50	1.00	0.50	0.25	6

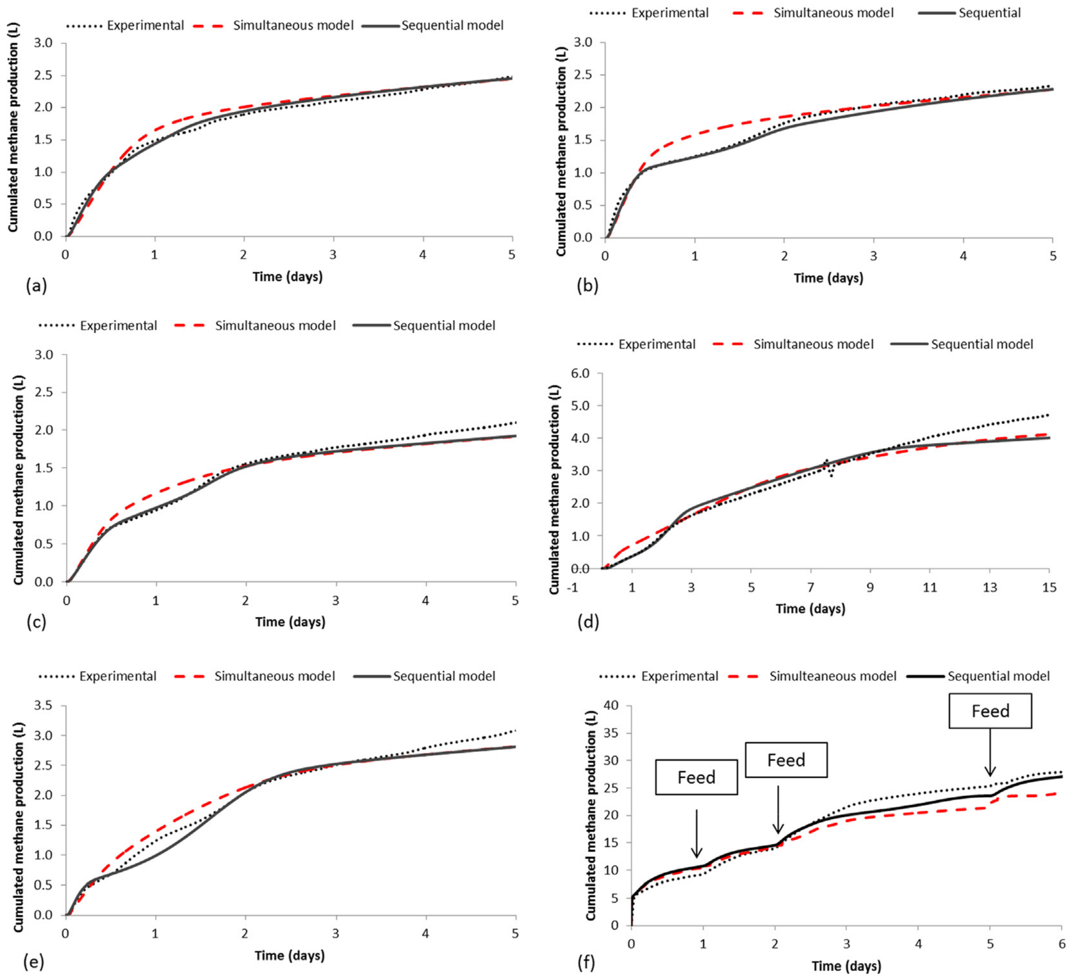

Fig. 6. Cumulated methane production curves obtained experimentally (black dot line) and by simulations with the simultaneous model (red dashed lines) and with the sequential model (black line) (a: carrot, b: cauliflower, c: lettuce, d: wheat straw, e: potato and f: wastewater treatment sludge). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Estimation of the quality of each model by the sum of squared errors.

	N	J simultaneous model	J sequential model
Carrot	5219	58	15
Potato	5001	93	75
Cauliflower	4999	124	23
Lettuce	5219	78	35
Wheat Straw	5000	182	97
Wastewater treatment sludge	145	955	309

J is the sum of squared errors and N the number of data points to fit.

Concerning the potato biodegradation (Fig. 6e), both models did not perfectly fit with the experimental behaviour. However, the sequential model gave less error than the simultaneous model. Consequently, the use of the sequential concept for all substrates would be applied to all the substrates to reach a better fit of all methane production rate curves.

3.3. Potentials and limitations of the sequential approach

The sequential chemical extraction methodology was successfully used to simulate bioaccessibility in this study as in previous studies (Jimenez et al., 2014; Jimenez et al., 2015a; 2015b). Jimenez et al. (2014) used the fractionation combined with 3D fluorescence spectroscopy to predict readily and slowly hydrolysable fractions of wastewater treatment sludge. Indeed, the authors showed that the first extractions were associated to the readily hydrolysable fractions whereas the poorly extractible fractions were associated to the slowly hydrolysable fraction. Spectroscopy was used to describe the complexity of each fraction in terms of non-biodegradable molecules. To go further on organic matter biodegradation modelling, this study used the sequential aspect of the protocol to challenge the simultaneous hydrolysis concept and to propose an alternative. The biodegradability study on three substrates after each extraction step revealed that (i) the decrease of accessibility led to a decrease of biodegradability and (ii) the alkaline extraction of two substrates led to an increase of the remaining fraction. Indeed, this extraction can act as a pretreatment (Carrere et al., 2010). It can solubilise bounded proteins, lipids and lignin. Thus, the protection layer of the wheat straw made of wax and lignin could be solubilised and the flocs from wastewater treatment sludge could be disrupted. According to these results, we hypothesized that the protection structure of both substrates led to reveal the sequential approach. Indeed, X_{SC} evolution kinetics was then calculated. Negative values were obtained showing that simultaneous approach could not fit the data. However, the impact of chemical extraction on the molecular structure of the remaining fraction was not evaluated.

Moreover, the chemical extraction procedure applied seemed to not alter the bioaccessibility of X_{MC} components after the first alkaline extraction as shown (Jimenez et al., 2014). However, this statement was not proven for X_{SC} and X_{NE} fractions. After strong alkaline and acid extractions, the molecular structure could indeed be altered, affecting the sequential model parameters. This issue could be a limitation of the use of this technique to represent the reality and should be deeply investigated.

More generally, the introduced switching function decreased the errors between experimental and simulated data on methane production curves for all the studied substrates. However, this function consists on a limitation concept that relies on a specific concentration of the considered variable. When the concentration is above the calibrated K_I_X parameter value, sequential degradation occurs and only the first accessible fraction is degraded. Then, the value becomes equal to or below the calibrated K_I_X parameter value. In this case, the next accessible fraction begins to be degraded and the model leads to a simultaneous degradation model. Does this mean that hydrolysis is a mixture of sequential and simultaneous processes as suggested by Morgenroth et al. (2002)? Clearly more in-depth research is required to answer this.

4. Conclusions

The objective of this paper was to use an organic matter characterization method based on accessibility assessment to compare two hydrolysis modelling concepts: simultaneous versus sequential degradation. This comparison revealed that the sequential hydrolysis concept is applicable to all the substrates studied (protein-like and carbohydrates to fibrous-like substrates). The simultaneous model scenario did not fit to all the experimental curves of methane production as highlighted by the study of wastewater treatment sludge and wheat straw biodegradation. However, some issues about the experimental fractionation methodology and its impact on fraction biodegradation kinetics, and on calibrated model parameters values have been raised. Further investigation on this topic should be done to validate the proposed model.

References

Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Bio/Technol. 3, 117–129.

Angelidaki, I., Ahring, B., 1997. Anaerobic digestion in Denmark. Past, present and future. In: III Curs d'Enginyeria Ambiental. Lleida, pp. 336–342.

Aquino, S.F., Chernicharo, C.A.L., Soares, H., Takemoto, S.Y., Vazoller, R.F., 2008. Methodologies for determining the bioavailability and biodegradability of sludges. Environ. Technol. 29 (8), 855–862.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T. M., Siegrist, H., and Vavilin, V. A., 2002. Anaerobic Digestion Model No.1. (ADM1). IWA Scientific and Technical Report No. 13. IWA, ISBN:1-900222-78-7.

Batstone, D.J., Puyol, D., Flores-Alsina, X., Rodríguez, J., 2015. Mathematical modelling of anaerobic digestion processes: applications and future needs. Rev. Environ. Sci. Biotechnol. 14, 595–613.

Benneouala, M., Bareha, Y., Mengelle, M., Bounouba, M., Sperandio, M., Bessiere, Y., Paul, E., 2017. Hydrolysis of particulate settleable solids (PSS) in activated sludge is determined by the bacteria initially adsorbed in the sewage. Water Res. 125, 400–409.

Bjerre, H.L.,1996. Transformation of wastewater in an open sewer: The Emscher River, Germany. Ph.D. dissertation, Aalborg University, Denmark.

Carrère, H., Dumas, C., Battimelli, A., Batstone, D.J., Delgenès, J.P., Steyer, J.P., Ferrer, I., 2010. Pretreatment methods to improve sludge anaerobic degradability: a review. J Hazard Mater 183 (1-3), 1–15.

Brock, T.D., Madigan, M.T., 1991. Biology of Microorganisms. Prentice Hall, New Jersey.

Confer, D.R., Logan, B.E., 1997. Molecular weight distribution of hydrolysis products during biodegradation of model macromolecules in suspended and biofilm cultures. 1. Bovine serum albumin. Water Res. 31, 2127–2136.

Dimock, R., Morgenroth, E., 2006. The influence of particle size on microbial hydrolysis of protein particles in activated sludge. Water Res. 40, 2064–2074.

Dochain, D., Vanrolleghem, P.A., 2001. Dynamical Modelling and Estimation in Wastewater Treatment Processes. IWA Publishing, London, UK.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28 (3), 350–356.

Eastman, J.A., Ferguson, J.F., 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. J. Water Pollut. Control Fed. 53, 352–366.

Ekama, G.A., Marais, G.v.R., 1979. Dynamic behaviour of the activated sludge process. J. Water Pollut. Control Fed. 51, 534–556.

Ekama, G.A., Dold, P.L., Marais, G.v.R., 1986. Procedures for determining influent COD fractions and the maximum specific growth rate of heterotrophs in activated sludge systems. Water Sci. Technol. 18 (6), 91–114.

Garcia-Gen, S., Sousbie, P., Rangaraj, G., Lema, J.M., Rodriguez, J., Steyer, J.-P., Torrijos, M., 2015. Kinetic modelling of anaerobic hydrolysis of solid wastes, including disintegration processes. Waste Manage. 35, 96–104.

Ginestet, P., Maisonnier, A., Sperandio, M., 2002. Wastewater COD characterization: biodegradability of physico-chemical fractions. Water Sci. Technol. 45 (6), 89–97.

Gujer, W., Henze, M., Mino, T., van Loosdrecht, M.C.M., 1999. Activated sludge model no. 3. Water Sci. Technol. 39 (1), 183–193.

Henze, M., Grady, C.P.L., Gujer, W., Marais, G.v.R. and Matsuo, T., 1987. Activated Sludge Model No. 1. IAWPRC Scientific and Technical Reports, No.1. IWA Publishing, London, UK.

Jimenez, J., Gonidec, E., Cacho Rivero, J.A., Latrille, E., Vedrenne, F., Steyer, J.-P., 2014. Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: towards ADM1 variables characterization. Water Res. 50, 359–372.

Jimenez, J., Espinoza Cavajal, C., Aemig, Q., Houot, S., Steyer, J.-P., Vedrenne, F., Patureau, D. 2015a. Organic matter characterization: towards a unified methodology for biological treatments modelling. Presented at 14. World Congress on Anaerobic Digestion (AD14), Viña del Mar, CHL (2015-11-15 - 2015-11-18).

Jimenez, J., Aemig, Q., Doussiet, N., Steyer, J.-P., Houot, S., Patureau, D., 2015b. A new organic matter fractionation methodology for organic wastes: bioaccessibility and complexity characterization for treatment optimization. Bioresour. Technol. 194, 344–353.

Lagarde, F., Tusseau-Vuillemin, M.H., Lessard, P., Heduit, A., Dutrop, F., Mouchel, J. M., 2005. Variability estimation of urban wastewater biodegradable fractions by respirometry. Water Res. 39, 4768–4778.

Kouas, M., Torrijos, M., Sousbie, P., Steyer, J.P., Sayadi, S., Harmand, J., 2017. Robust assessment of both biochemical methane potential and degradation kinetics of solid residues in successive batches. Waste Manage. 70, 59–70.

Lesteur, M., Latrille, E., Maurel, V.B., Roger, J.M., Gonzalez, C., Junqua, G., Steyer, J.P., 2011. First step towards a fast analytical method for the determination of biochemical methane potential of solid wastes by near infrared spectroscopy. Bioresour. Technol. 102, 2280–2288.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 (1), 265–275.

Morgenroth, E., Kommedal, R., Harremoes, P., 2002. Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment—a review. Water Sci. Technol. 45 (6), 25–40.

Mottet, A., Ramirez, I., Carrère, H., Déléris, S., Vedrenne, F., Jimenez, J., Steyer, J.P., 2013. New fractionation for a better bioaccessibility description of particulate organic matter in a modified ADM1 model. Chem. Eng. J. 228, 871–881.

Muller, M., Jimenez, J., Antonini, M., Dudal, Y., Latrille, E., Vedrenne, F., Steyer, J.P., Patureau, D., 2014. Combining chemical sequential extractions with 3D fluorescence spectroscopy to characterize sludge organic matter. Waste Manage. 34, 2572–2580.

Orhon, D., Cokgor, E.U., Sozen, S., 1998. Dual hydrolysis model of the slowly biodegradable substrate in activated sludge systems. Biotechnol. Tech. 12, 737–741.

Rincker, M.N., Diara, A., Peu, P., Badalato, N., Girault, R., Carrère, H., Bassard, D., Pauss, A., Ribeiro, T., Béline, F., 2013. Anaerobic respirometry as a tool to evaluate the effect of pretreatment on anaerobic digestion efficiency. 13th world Congress on Anaerobic Digestion, Santiago de Compostela, Spain.

Sanders, W.T.M., Geerink, M., Zeeman, G., Lettinga, G., 2000. Anaerobic hydrolysis kinetics of particulate substrates. Water Sci. Technol. 41 (3), 17–24.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., Harms, H., 2004. Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environ. Sci. Technol. 38, 228A–231A.

Shimizu, T., Kudo, K., Yoshikazu, N., 1993. Anaerobic waste-activated sludge digestion–a bioconversion mechanism and kinetic model. Biotechnol. Bioeng. 41, 1082–1091.

Siegrist, H., Renggli, D., Gujer, W., 1993. Mathematical modelling of anaerobic mesophilic sewage sludge treatment. Water Sci. Technol. 27 (2), 25–26.

Sollfrank, U., Gujer, W., 1991. Characterization of domestic wastewater for mathematical modeling of the activated sludge process. Water Sci. Technol. 23 (4–6), 1057–1066.

Spérandio, M., Paul, E., 2000. Estimation of wastewater biodegradable COD fractions by combining respirometric experiments in various So/Xo ratios. Water Res. 34, 1233–1246.

Torrijos, M., 2015. Assessment of BMP and kinetics in 6L-batch reactors with successive feedings. Presented at Workshop on the Conundrum of Biomethane Potential Tests, Leysin, CHE (2015-06-10 - 2015-06-12).

Vavilin, V.A., Fernandez, B., Palatsi, J., Flotats, X., 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: an overview. Waste Manage. 28, 939–951.

Yan, L., Li, W., Yang, J., Zhu, Q., 2004. Direct visualization of straw cell walls by AFM. Macromol. Biosci. 4, 112–118.

Yasui, H., Goel, R., Li, Y.-Y., Noike, T., 2008. Modified ADM1 structure for modelling municipal primary sludge hydrolysis. Water Res. 42, 249–259.

Zhen, G., Lu, X., Kato, H., Zhao, Y., Li, Y.-Y., 2017. Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: current advances, full-scale application and future perspectives. Renew. Sustain. Energy Rev. 69, 559–577.

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Methane production and fertilizing value of organic waste: Organic matter characterization for a better prediction of valorization pathways

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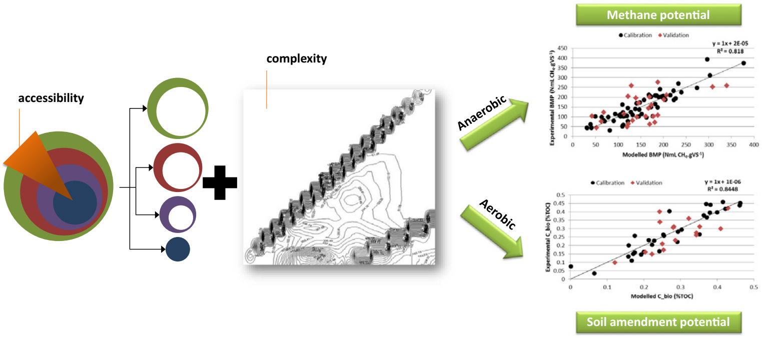
HIGHLIGHTS

- Fractionation and fluorescence data allowed the best prediction of biodegradability.
- Differences and similarities are observed on the variables impact on predictions.
- Humic-like fraction has a negative impact on BMP but not on soil mineralized carbon.
- Actuators identification can be found to control valorization ways.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic wastes are potential sources of both energy as well as crop production fertilizers. Correlations and models, involving organic matter characterization, have been previously described by several authors although there is still a lack in knowledge on the potential of simultaneous predictions of methane and organic fertilizer quality to optimize the wastes treatments. A methodology combining chemical accessibility and fluorescence spectroscopy was used to characterize 82 different organic wastes. Characterization data were compared with the biochemical methane potential (BMP), and with the biodegradable organic carbon obtained by soil incubation (C_bio). High correlations values were observed (R^2 of 0.818 for BMP and 0.845 for C_bio). Model coefficients highlighted the differences and similarities between anaerobic and aerobic soil biodegradation, suggesting that anaerobic recalcitrant molecules could enhance soil fertility. This is a first step in the development of a tool for optimising both types of valorisation according to agrosystem needs and constraints.

1. Introduction

The aim of environmental biorefinery is to establish a complete valorisation of organic wastes, which should turn into valuable organic resources. Indeed, process treatments involved in biorefinery include anaerobic digestion, fermentation, aerobic digestion, composting, thermal conversion, etc. The choice of these treatments depends on the characteristics of the organic residues (OR) and on the targeted services (i.e. energy production, high added value molecules or organic fertilizers).

In this study, focus was put on two main services: (i) the production of methane from anaerobic digestion of OR, which could potentially be subsequently used for electricity or heat production and (ii) the use of OR as organic fertilizer on cropped soils whether

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Nomenclature

ADM1	anaerobic digestion model N°1
BMP	biochemical methane potential Nml $CH_4 \cdot gVS^{-1}$
C_bio	biodegradable organic carbon in soil incubation %TOC
COD	chemical oxygen demand $gO_2 \cdot g\ DS^{-1}$
DS	dried solids % fresh matter
FFMSW	fermentescible fraction of municipal solid wastes
HCA	hierarchical clustering analysis
IROC	indicator of residual organic carbon % TOC
NIRS	near infraRed spectroscopy
NEOM	non extractible organic matter % COD
OM	organic matter
OR	organic residues
PCA	principal component analysis
PEOM	poorly extractible organic matter % COD
PLS	partial least square regression
$P_f(i)$	fluorescence percentage of a zone i %
R^2	correlation coefficient from PLS
REOM	readily extractible organic matter % COD
RMSE	root mean square error unit of the predicted variable
RMSEP	root mean square error of prediction unit of the predicted variable
Q^2	percent of variation of Y predicted by model in cross-validation
SEOM	slowly extractible organic matter % COD
S(i)	area of a zone i from fluorescence spectra Nm^2
SPOM	soluble from particular extractible organic matter % COD
TOC	total organic carbon mg $C \cdot g\ DS^{-1}$
$V_f(i)$	normalized fluorescence volume of a zone i U.A.
$V_{f\_raw}(i)$	raw fluorescence volume of a zone i U.A.
VS	volatile solids % DS

treated or not, thanks to their content in nutrients and organic carbon.

In both cases, the potential interest is related to the biodegradation potential of the organic matter during anaerobic digestion (the higher the biodegradation potential, the greater the methane production) or during aerobic soil incubation (the lower the biodegradation potential, the greater its value as an organic soil amendment).

Until present, the quality of digestate for agronomic valorisation has been poor. Management and prediction of anaerobic digestion for methane production would allow for digestate quality control and the availability of this knowledge would improve the optimisation of anaerobic digestion for both targeted services. The main issue to achieve this goal would be to identify relevant actuators for controlling both potentials. These two potentials depend on the chemical characterization of OR as well as its chemical and biodegradability/stability (Angelidaki and Sanders, 2004; Lashermes et al., 2009; Peltre et al., 2010; Godin et al., 2015).

Indeed, during the past two decades, several authors observed significant correlations between certain indicators based on the chemical composition of OR and the studied potentials (Buffiere et al., 2006; Gunaseelan, 2007, 2009; Lashermes et al., 2009; Lesteur et al., 2011; Thomsen et al., 2014; Peltre et al., 2010; Thomsen et al., 2014; Godin et al., 2015; Bekiaris et al., 2015a,b; Kafle and Chen, 2016; Fitamo et al., 2017). These correlations were used for predicting biological incubation tests which can be resource and time consuming (Godin et al., 2015).

Usually, the anaerobic biodegradation potential of an OR is assessed by performing a Biochemical Methane Potential test (BMP) (Angelidaki and Sanders, 2004). During this test, the methane production related to anaerobic biodegradation is measured for at least 30 days (depending on the OR) on a mixture of OR with an anaerobic inoculum under optimal conditions (35 °C, addition of metals and nutrients, appropriate substrate: inoculum ratio and bicarbonate buffer). At the end of the incubation period, the cumulated production is assumed to be the methane potential production of the OR. A similar biological incubation test is widely used to assess the aerobic organic carbon degradation of OR during soil incubation under controlled conditions of moisture and temperature, over at least 91 days (Lashermes et al., 2009). A number of authors have applied a chemical characterisation of OR for BMP and soil carbon mineralization prediction. For aerobic biodegradation, an indicator of residual organic carbon (IROC) has been developed by Lashermes et al. (2009) and has been standardized as mentioned by Peltre et al. (2011). It was based on fiber fractionation (Van Soest, 1963) and on the organic carbon fraction mineralized after 3 days of incubation. It has been applied to predict the evolution of soil OM after repeated applications of various organic residues in long-term field experiments (Peltre et al., 2012). Van Soest fractionation has also been successfully used for BMP prediction of lignocellulosic residues (Buffiere et al., 2006; Gunaseelan, 2007, 2009; Thomsen et al., 2014; Kafle and Chen, 2016). However, it cannot be applicable to all OR as demonstrated by Mottet et al. (2010) who applied a Van Soest fractionation to wastewater treatment sludge for BMP prediction. Bayard et al. (2015) applied similar approach on agricultural and forest residues. Although the BMP and some lignocellulosic characteristics of substrates were correlated, its prediction was low. Furthermore, some of the results obtained concerning the impact of cellulose and hemicellulose on BMP were contradictory (Thomsen et al., 2014). In the case of OR containing a mixture of all the biochemical groups and a low fiber content, the first "Soluble" Van Soest fraction represented more than 80% of VS (Mottet et al., 2010; Jimenez et al., 2015) and gathered a large diversity of molecules that could not characterize the biodegradability. According to Lashermes et al. (2009), Van Soest fractionation was not sufficient for the prediction of the IROC on a large panel of residues. The addition of the organic carbon fraction mineralized after 3 days of incubation can be associated to the readily biodegradable fraction which is generally high in animal waste excluding litter and wastewater sludge.

Another common methodology for predicting BMP and carbon mineralisation on soil is Near InfraRed Spectroscopy (NIRS). The use of the NIRS to quantify BMP (Lesteur et al., 2011; Godin et al., 2015; Fitamo et al., 2017) has been successfully investigated, and subsequently led to its commercial use at a later stage. A similar study was applied to the IROC (Peltre et al., 2011). A few outliers were observed and mainly associated to wastewater sludge, liquid manure and organo-mineral fertilizers. However, the NIRS method provides limited qualitative information about chemical composition of OR due to overlapping overtones and combination bands (Bekiaris et al., 2015b). This tool, therefore, does not facilitate a more mechanistic interpretation.

More recently, a Fourier transform mid-infrared photoacoustic spectroscopy technique (FTIR-PAS) was applied to predict BMP on plant biomass (Bekiaris et al., 2015a) and the mineralisable fraction of carbon in soils from several OR (Bekiaris et al., 2015b). This technique allowed for (i) the influence of sample particle size and absorption of dark and opaque samples to be ignored and (ii) for more information to be produced on the composition of OM than with NIRS (Bekiaris et al., 2015a,b). In these two different studies, although the authors demonstrated the impact of the composition of OR on biodegradability prediction, they did not confront the results from both studies. Nonetheless, differences and similarities did appear, as for example the ability of certain carboxylic acids to produce both a negative effect on BMP on one hand, and a positive impact on mineralisable soil carbon.

Few studies have dealt with anaerobic versus aerobic biodegradability correlations (Bayard et al., 2015; Maynaud et al., 2017). However, no studies have yet focussed on the impact that OM characterisation may have on both biodegradation pathways, and on the differences and similarities between these impacts, in particular for BMP and soil incubation tests. Despite the fact that some authors applied similar characterization tools for BMP test predictions in one hand and soil carbon mineralization test on another hand, not a single study has yet addressed the simultaneous prediction of both potentials.

Moreover, biodegradability of OR not only depends on the quantity of OM but it also relies on the accessibility and complexity of the OM (Jimenez et al., 2015). Given the complex organisation of certain organic residues, the term "accessibility" defines the potential access to the molecules by microorganisms responsible for their degradation. It can depend on the physical characteristics of the OR (particle size, porosity of the OR), on the distribution of the organic fraction within the OR matrices in relation with the process duration, the hydrolytic activity or the applied pretreatment. Molecules with a weight below 1000 Da can pass through the cell wall (Aquino et al., 2008). Finally, once the OM becomes accessible, it can be more or less easily biodegradable by microorganisms, depending on its chemical nature or complexity. Cardeña et al. (2017) demonstrated that ozonation enhanced the accessibility of certain microalgae which have structural and physical barrier to biodegradation, allowing, at the end, their biodegradability. Furthermore, both complexity and accessibility have not yet been considered together in any existing indicator. The chemical nature of extracted organic fractions have been observed to vary during treatments such as composting, which thus explains the change in biodegradability of given organic fractions (Peltre et al., 2012).

One study did successfully predict the biodegradability of sewage sludge under anaerobic conditions by considering both accessibility and complexity (Jimenez et al., 2014). The methodology developed was based on the combination of a sequential chemical extraction to assess accessibility and fluorescence spectroscopy to assess the complexity of each extracted organic fraction. Since, this sequential extraction methodology has been updated for applications over a wider spectrum of OR (Jimenez et al., 2015). Maynaud et al. (2017) used this combined approach in order to characterize solid and composted digestates. The authors also performed anaerobic and aerobic tests without inoculum in order to characterize the stability of the studied digestates. One main conclusion based on a statistical study highlighted the strong potential of this methodology to predict digestate biodegradability.

According to the present literature review, the improvement of mechanistic knowledge and optimisation of anaerobic digestion valorisation pathways are still limited by the lack of studies dealing with the comparison between aerobic and anaerobic predictions from OM characterisation. The updated methodology (Jimenez et al., 2015) has therefore been applied to predict both anaerobic biodegradability thanks to BMP measurements and aerobic carbon biodegradation in soil. The main objectives of this study are (i) to predict both energy production and soil organic carbon potential using a combination of accessibility and complexity characterisation performed on the same OR, (ii) to use the OM characterization to analyse the similarities and differences between anaerobic biodegradability and soil organic carbon mineralization and thus (iii) to identify OM characterization actuators in order to optimize anaerobic digestion for methane production and organic amendments quality requirements.

2. Material and methods

To assess the accessibility (i.e. sequential chemical extractions) and complexity (i.e. fluorescence spectroscopy) of OM, eighty-two OR were characterised with the recently developed methodology. They were also incubated under anaerobic conditions during BMP and 44 of them were incubated under aerobic conditions with crop soil. These incubation tests led to the evaluation of the methane potential and potential residual organic carbon. Statistical relations were investigated between the characterisation and the degradation kinetics parameters.

a. Sample set

Eighty-two samples were used for anaerobic incubation. The samples covered a large range of types of OM. Indeed, the OR considered were: 2 biowastes (e.g. food waste); 3 Fermentable Fractions of Municipal Solid Wastes (FFMSW); 3 green wastes, 4 initial composting mixtures (2 sludge-green waste mixtures and 2 digested sludge-green waste mixtures), 22 composts (9 from sludge, 6 from digested sludge, 2 from biowaste digestates, 2 from FFMSW digestates and 3 from manure digestates),1 potting mix (e.g. commercial medium for plant growth), 3 types of manure, 6 secondary wastewater sludge and 38 digestates obtained after anaerobic digestion of sludge (11), manure (13), territorial waste mixtures (8), FFMSW (3) and crop residues (3).

From these 82 samples used for anaerobic incubation, 44 samples were used for aerobic incubation tests: these include 21 digestates (5 from sludge, 7 from manure, 3 from territorial wastes, 3 from crop residues and 3 from FFMSW), 8 composts (4 from sludge digestates, 1 from manure digestate and 3 from sludge), 4 urban wastewater treatment sludge, 3 FFMSW, 3 manures, 2 green wastes, 2 initial composting mixtures (digested sludge and green waste mixtures) and 1 potting mix. In addition, 3 samples were used for aerobic incubation only (i.e. 1 soil, 1 territorial waste, 1 crop residue digestate).

b. Physico-chemical characterization

The Dried Solids (DS) were analysed by gravimetry after 24 h at 105 °C and the Volatile Solids (VS) were analysed by gravimetry after 2 h at 550 °C. The VS was obtained by subtraction of the mineral matter obtained after 550 °C and of the DS.

The Chemical Oxygen Demand (COD) was measured in duplicate using Aqualytic® kits (0–1500 mg $O_2 \cdot L^{-1}$). Units were in $mgO_2 \cdot L^{-1}$ for the liquid phases and $mgO_2 \cdot gDS^{-1}$ for the solid and total phases. Indeed, the analysis of the freeze-dried and grinded (1 mm) sample was performed on a solution of $1gDS \cdot L^{-1}$.

The Total Organic Carbon (TOC) was performed on grinded freeze-dried samples by catalytic combustion at 900 °C using a TOC-V-SSM-500A Shimadzu device. The units were in $mgC \cdot gDS^{-1}$.

c. Chemical fractionation

The application of a recently published method (Jimenez et al., 2015) was extended to a wider range of organic residues. This methodology, based on chemical extractions that indicate the chemical accessibility of organic residues, is a modified version of the protocol in (Jimenez et al., 2014). The main modification consists in the addition of a sulfuric acid extraction step from the Van Soest protocol (Van Soest, 1963) for determining the carbohydrates (i.e. cellulose and hemicellulose). Sequential extractions (30 mL of each extractant) were performed on 0.5–1 g of freeze-dried and grinded (1 mm) samples. Each extraction stage was followed by a centrifugation step (18600g, 20 min, 4 °C) and a 0.45 μm filtration step. The resulting fractions were:

1. Extractable Soluble from Particulate Organic Matter (SPOM) (milli-Q water solution containing 10 mM of $CaCl_2$, 15 min × 4, 30 °C, 300 rpm), which represents the most accessible fraction and essentially contains water-soluble proteins and sugars.
2. Readily Extractable Organic Matter (REOM) (NaOH 10 mM, 15 min × 4, 30 °C, 300 rpm), which represents an accessible fraction of mainly proteins and lipids.
3. Slowly Extractable Organic Matter (SEOM) (NaOH 0.1 M, 4 h × 4, 30 °C, 300 rpm), which mainly contains humic-like and fulvic-like acids and complex proteins (i.e. glycolated proteins) as well as certain lignocellulosic compounds which can be soluble under strongly basic conditions.
4. Poorly Extractable Organic Matter (PEOM) (25 mL H_2SO_4, 72%, 3 h × 2, 30 °C, 300 rpm), which targets holocelluloses (i.e. hemicellulose and cellulose).
5. Non-Extractable Organic Matter (NEOM), which contains lignin-like compounds and non-extractable humic-like acids (i.e. humin).

Results are expressed in COD ($gO_2 \cdot gDS^{-1}$).

d. 3D fluorescence spectroscopy

The acquisition of 3D fluorescence spectra was performed on the extracted fractions, allowing for OM complexity to be qualified (Jimenez et al., 2014, 2015). The complexity of OM could be assessed through the proportions of fluorescence volumes of the most recalcitrant molecules such as humic-like substances, fulvic-like acids, lipofuscin-like (i.e. a lignocellulosic marker) as well as amino acids and less complex molecules (Muller et al., 2014).

The fluorescence spectrometer was a Perkin Elmer LS55. Excitation wavelengths varied between 200 and 600 nm with a 10 nm incrementation. Based on the work of Jimenez et al. (2014) and Muller et al. (2014), spectra were decomposed into seven zones, each associated to a particular biochemical family. The simplest molecules (i.e. amino acid fluorescence) are located in zones 1–3 while the more complex molecules are located in zones 4–7. The proportion of fluorescence for a given zone “i” $P_f(i)$ was calculated using the fluorescence zone volumes V_f (i) according to Eqs. (1) and (2):

$$V_f(i)(\mathrm{U.A./mg \cdot COD \cdot L^{-1}}) = \frac{V_{f\_raw}(i)}{COD_{sample}} \times 1/\frac{S(i)}{\sum_{i=1}^{7} S(i)} \qquad (1)$$

$$P_f(i)(\%) = \frac{V_f(i)}{\sum_{i=1}^{7} V_f(i)} \times 100 \qquad (2)$$

with:

$V_f(i)$ (U.A. $mg \cdot O_2 \cdot L^{-1}$): the normalized volume of the zone i,
$V_{f\_raw}(i)$ (U.A. $mg \cdot O_2 \cdot L^{-1}$): the raw volume of the zone i,
COD_{sample} ($mg\ O_2 \cdot L^{-1}$): the COD concentration of the sample,
$S(i)$ (nm^2): the area of a zone i,
$P_f(i)$ (%): the fluorescence proportion of a zone i.

e. Biodegradation tests

Biochemical methane production (BMP) tests

- The BMP values were obtained using an innovative and rapid FlashBMP® method developed by Ondalys (Lesteur et al., 2011) and commercialized by Buchi. This method is based on Near InfraRed Spectroscopy (NIRS) applied to more than 600 types of OR (agro-industrial waste, green waste, energy crops, municipal solid waste, sludge and digestates) for which classical BMP tests were performed according to Angelidaki and Sanders (2004). Samples were freeze-dried and grinded to 1 mm before NIRS acquisition. Spectra were measured using a BUCHI NIRFlex N-500 (Buchi, Switzerland), with add-on vials, allowing for immediate analysis. Results are expressed in $mL \cdot CH_4 \cdot gVS^{-1}$.

Soil incubation tests

- Freeze-dried and 1 mm grinded organic residues were incubated with 100 g of cropped soil, (substrate: soil ratio between 0.002 and 0.01 g TOC product/g TOC dried soils) in hermetically closed bottles (1 L) during, at least 91 days under moisture and temperature controlled conditions. For unlimited microbial growth conditions, mineral nitrogen was added. Incubation of the soil without the addition of OR was also performed so as to take into account the mineralization of soil organic carbon. The soil was provided by a control treatment sampled during the QualiAgro field experiment (Ile de France, France) which began in 1998 to study the effects of various organic fertilizers (Houot et al., 2002). Soil moisture content was adjusted to a water content equivalent to 75–100% of the soil water holding capacity. Carbon mineralization was monitored using a 20 mL trap solution of NaOH (0.5 N) for CO_2 which was periodically replaced during the incubations. The CO_2 was analysed by titration of the remaining NaOH with HCl (1 M) or measured by gas chromatography (Perkin Elmer CLARUS 480) following the acidification of the NaOH trap solution with HCl (1 M) in hermetic jars. Cumulated mineralized carbon was obtained as a percentage of initial TOC.
The mineralised TOC was identified as C_bio and results were expressed in $gC \cdot gC^{-1}$.

f. Statistical analysis

- In order to determine the correlations between biodegradability and indicators from OM characterization, principal component analysis (PCA), hierarchical clustering analysis (HCA) and partial least square (PLS) regressions were performed using SIMCA software from UMETRICS.
In its simplest form, a linear model specifies the (linear) relationship between a dependent (response) variable *Y*, and a set of X predictor variables, the *X*'s. The dataset was split into two subsets: one for the calibration step (3/4 of the data) and one for the validation step (1/4 of the data). Indeed, for the validation of the model set up to be robust, the dataset used for calibration should not be the same as the dataset used for validation. In order to cover and validate the whole range of data values, (i) the dataset was sorted by increasing the Y-variable values and (ii) one out of four data points were selected for the validation step, as in Jimenez et al. (2014).
The parameters from the PLS models used to assess model robustness are the following:
  - Correlation coefficient R^2.
  - Root Mean Square Error (RMSE), used as an accuracy measurement of differences between predicted values and measured model values.
  - RMSEP is the RMSE for the prediction of validation samples.
  - Q^2: percentage of variation of Y predicted by the PLS model according to cross-validation. This parameter indicates how well the model predicts the data. A large Q^2 (>0.5) indicates good predictivity. Moreover, it is a compromise between root mean square error and R^2. Q^2 also represents the criteria

for the choice of the component number. In others words, when the cumulated Q^2 reaches its maximum value, the corresponding component is selected.

3. Results and discussion

- Anaerobic biodegradability prediction

Based on the methodology of Jimenez et al. (2015), the 82 samples were first characterised (fractionation and 3D fluorescence) and BMP measurements were performed. Samples covered a large range of OM quality. Indeed, BMP values ranged from 31 to 394 Nml $CH_4 \cdot gVS^{-1}$. The characterization data (X variables) were compared to the BMP data (Y variable).

On one hand, PLS regression was applied using 33 explicative variables (X variables) composed of $P_f(i)$ (i = 1–7), the fluorescence percentage of each zone and the COD percentage of each fraction (e.g. SPOM, REOM, SEOM, PEOM, NEOM). This model is identified as Model n°1. As suggested by Thomsen et al. (2014), the sum of the five fractions was equal to 1. The residual non extactible OM (e.g. NEOM) was included in the PLS to remove misinterpretations of regression coefficients weight due to relative nature of compositional data. On another hand, Model n°2 was built using 5 explicative variables composed of the five fractions. Out of the 82 observations, 56 were used to set up a first calibration for both models. The validation samples were selected according to the increasing BMP values. One sample out of four was used for validation (i.e. a total of 26 samples for validation).

Table 1 presents the quality parameters of the evaluated PLS models. Results clearly point out that Model n°1 has the best prediction potential (i.e. $Q^2 = 0.689$ for Model 1 and 0.250 for model 2), highest correlation coefficient (i.e. $R^2 = 0.818$ for Model 1 and 0.410 for Model 2) and a low RMSE value. The most significant and positive variables in Model 2 were the accessible SPOM and REOM fractions. However, the lack of description in the quality fraction did not allow for certain OR and OR digestates to be distinguished. Indeed, although some digestates had high SPOM, they also contained non-biodegradable material that was not considered by fractionation only. Consequently Model n°1 was selected. The quality of this model is illustrated in Fig. 1. The distribution of experimental Y-variables versus predicted Y-variables was similar to the line of perfect fit (y = x) for BMP values. This result points to the closeness between the model and experimental data. Three components were sufficient enough to explain 61% of the X-variables (i.e. characterisation data) and 82% of the Y-variables (i.e. BMP data). The Root Mean Square Error (RMSE) was 34 Nml $CH_4 \cdot gVS^{-1}$ and the Root Mean Square Error of Prediction (RMSEP) was 66 Nml $CH_4 \cdot gVS^{-1}$. These results could allow for an accurate prediction of the methane potential, with values ranging between 31 and 394 mL $CH_4 \cdot gVS^{-1}$.

According to the PLS scores and loading results (Fig. 2a–c), the BMP values were positively correlated to the fluorescence zones 1–3 (i.e. protein-like compounds) and to the SPOM fraction (i.e. the most accessible fraction). They were negatively correlated to the SEOM fraction and to the complex fluorescence zones (4–7) from all fractions. Therefore, the first component was essentially characterised by the complexity of the OR (Fig. 2b), the second component was characterised by the SPOM fraction, while the third one was negatively correlated to the SEOM (Fig. 2c) and positively correlated to the SPOM. Finally, these second and third components were rather related to the OR accessibility.

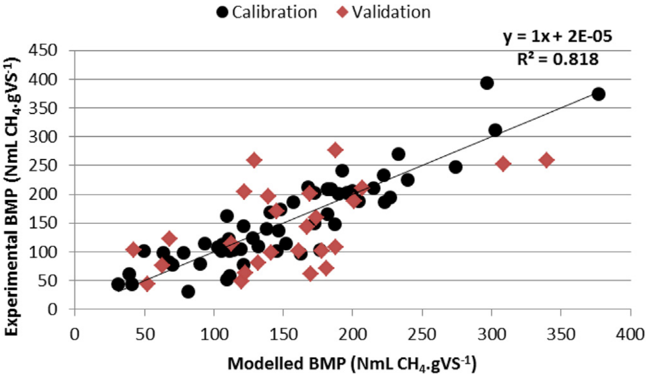

Fig. 1. Observed versus predicted BMP value obtained with the PLS model n°1 *(black dots: calibration data and red dots: validation data).* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2 highlights the impact of the most significant X-variables affecting the prediction of Y-variables derived from the calculation of the weight of each variable. Positive and significant variables comprised the SPOM fraction and the fluorescence zone 3 related to protein-like molecules from all organic fractions. Bayard et al. (2015) observed similar results with good correlations between BMP values and water soluble COD applied on biowastes, residual municipal solid waste and their digestates. Moreover, with the use of principal component analysis, Maynaud et al. (2017), pointed out that SPOM and fluorescence zones 1–3 were correlated with BMP values of digestates.

Conversely, the negative and significant variables were associated to the SEOM fraction (i.e. the moderately accessible fraction) and to the complex fluorescence zones 4 and 6 from REOM, related to complex molecules such as humic-like and fulvic-like acids. Bayard et al. (2015) also demonstrated that the humic substance index (i.e. obtained using a similar NaOH extraction as for SEOM followed by an acid precipitation to recover fulvic acids) had a negative impact on BMP values of biowastes and their digestates.

This result implies that the combination of accessibility and fluorescence-derived complexity could allow for the prediction of organic residue biodegradability under anaerobic conditions.

A Hierarchical Clustering Analysis (HCA) was performed simultaneously with the PLS. This analysis highlighted 6 observation groups, as illustrated by the scores plot in Fig. 2a. According to this analysis, the highest BMP values are positively correlated to the fluorescence zones 1–3 (i.e. protein-like) and to the SPOM fraction (i.e. the most accessible fraction). They are associated with Group n °4 samples that are mainly composed of sludge, biowaste and vegetables (e.g. potatoes). Conversely, the lowest BMP values are negatively correlated to the complex fluorescence zones (4–7). They are associated with Groups n°1 and 2, according to their content in accessible fractions and are essentially composed of well

Table 1
Quality parameters of the PLS models for BMP and C_bio prediction.

Y-variables	X-variables	Model	Number of component	RMSE	RMSEP	R^2X	R^2Y	R^2Y (calibration + validation)	Q^2
BMP ($NmlCH_4 \cdot gVS^{-1}$)	33 (Fractions and fluorescence data)	1	3	33.950	65.788	0.608	0.818	0.637	0.689
BMP ($NmlCH_4 \cdot gVS^{-1}$)	5 (Fractions)	2	2	60.542	61.599	0.583	0.41	0.366	0.250
C_bio (% COT)	33 (Fractions and fluorescence data)	3	2	0.051	0.073	0.528	0.845	0.738	0.745
C_bio (% COT)	5 (Fractions)	4	2	0.078	0.098	0.58	0.632	0.549	0.456

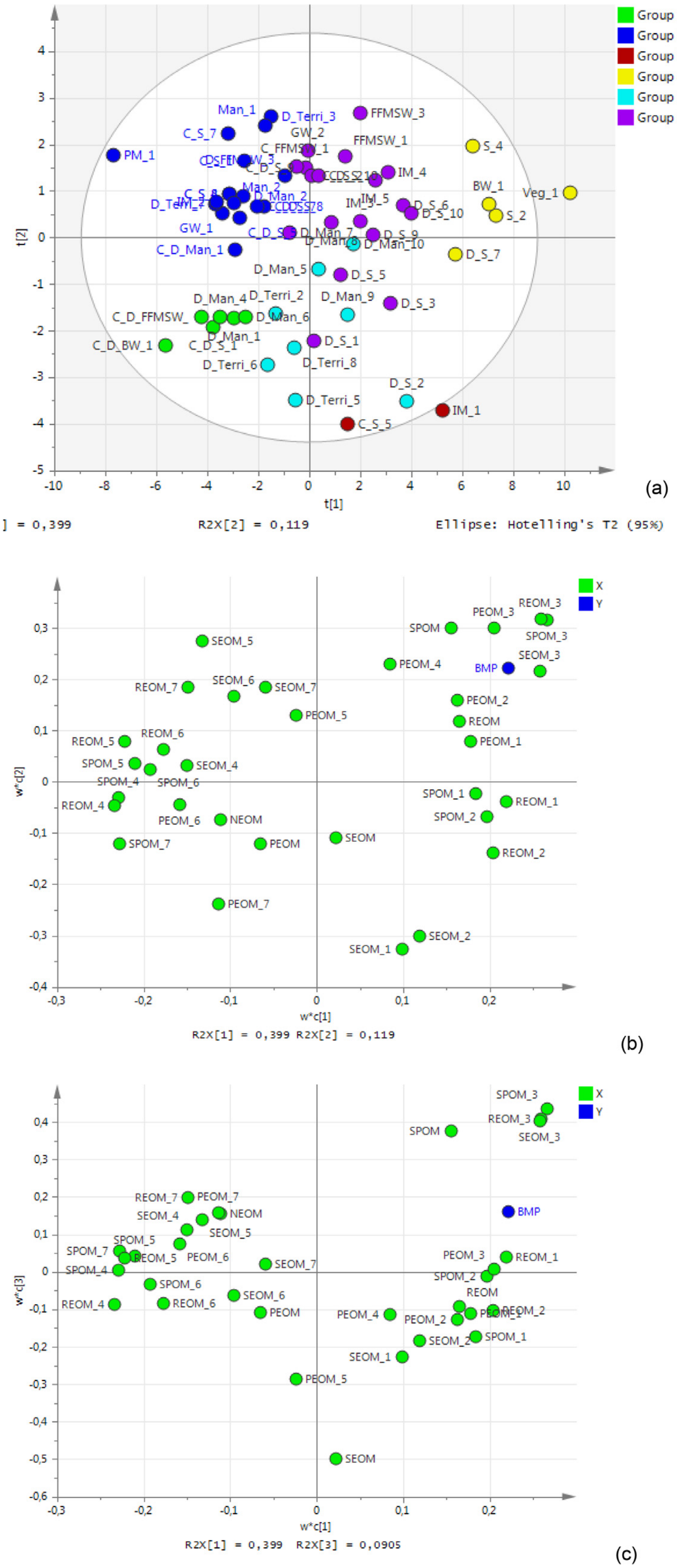

Fig. 2. Scores obtained on calibration data and HCA analysis (a) and loadings plots (b) and (c) for BMP prediction model n°1.

stabilized manure/territorial digestates and biowaste/FFMSW composts, digestate composts and potting mix. These results are consistent: the more the OR are complex and poorly accessible, the lower the biodegradability. In terms of BMP, intermediate groups could be formed: Group 5 mainly contains digestates with high SEOM fractions (sludge and manure) while Group 6 is mainly

Table 2
Significant positive and negative impacts of the X-variables on Y-variables prediction obtained from coefficients weight and variable importance for projection plots.

X-Variables\Y-Variables	BMP	C_{bio}
SPOM	+++	+
Fluorescence Zones 1–3	+++	++
Fluorescence Zones 4–7	0	– –
REOM	0	+
Fluorescence Zones 1–3	+++	+
Fluorescence Zones 4–7	–	– –
SEOM	– –	+
Fluorescence Zones 1–3	+++	0
Fluorescence Zones 4–7	0	+
PEOM	0	++
Fluorescence Zones 1–3	++	0
Fluorescence Zones 4–7	0	0
NEOM	0	–––

Legend: +: positive impact; –: negative impact; +++/–––: extremely sensitive (weight > 0.15); ++/––: very sensitive (0.1 < weight < 0.15); +/–: sensitive (0.05 < weight < 0.1); 0: not sensitive;

composed of FFMSW, manure and lignocellulose-like compounds with higher BMP than the digestate groups. Group 3, comprising two samples (sludge compost and initial mixture between green waste and digestate sludge), is close to the rich SEOM Group n°5. Jimenez et al. (2015) demonstrated that this characterisation, based on the two concepts of accessibility and complexity, could help to categorise organic residues. Indeed, this study confirms that the anaerobic biodegradability of a large panel of OR can be concurrently categorized and predicted thanks to this methodology.

- Prediction of organic carbon biodegradability

As for BMP prediction, the correlation between OR characteristics and aerobic mineralisation results was investigated thanks to a PLS regression using the same 33 explicative variables as for BMP (i.e. Model n°3), and using the 5 fractions (i.e. Model n°4). A first calibration model was set-up for 31 observations and 16 samples were used for validation, in the same manner as for BMP prediction.

The Y-variable was the aerobic carbon biodegradability (C_bio) fraction of TOC measured during soil incubation tests. The dataset was obtained from a huge diversity of organic residues with a high range of biodegradability (from 7 to 46% TOC).

From Table 1, results implied that the Model n°3 had the best prediction quality ($Q^2 = 0.745$ for Model 3 and 0.456 for Model 4), a high correlation coefficient ($R^2 = 0.845$ for Model 3 and 0.632 for Model 4) and a low RMSE value. The most significant variables in Model 4 include the accessible fraction REOM (positively correlated) and NEOM (negatively correlated). Despite the promising prediction potential of Model 4, the poor quality description of the fraction did not allow for certain OR (i.e. sludge and manure) and their respective digestates to be distinguished as for BMP prediction. Consequently, Model n°3 was selected for C_bio prediction.

As illustrated by Fig. 3, the plot of measured values versus predicted values points to the ability of this model to predict biodegradable organic carbon (i.e. closest to the perfect fit line). Two components were required to explain 53% of X-variables. The RMSE accounted for 5.1% of TOC while the RMSEP accounted for 7.3% of TOC.

The scores and loadings of the PLS model are plotted in Fig. 4. According to this plot, the first component is defined by the fluorescence-derived complexity, while the second component is defined by the accessibility axis. Indeed, as for BMP prediction, C_bio was positively impacted by the most accessible fraction SPOM and by the simplest fluorescence zones represented by the protein-like in SPOM and REOM. Conversely, the least accessible fraction NEOM was negatively related to the organic carbon mineralisation. This is contrary to the BMP model for which NEOM was not significant. However, again as for BMP prediction, the most complex fluorescence zones of the 3D fluorescence spectra of SPOM and REOM represented by humic-like, fulvic-like acids and lignocellulose-like molecules also had a strong negative impact on carbon mineralization.

The HCA comprised 6 groups of observations, as presented by the scores in Fig. 4. Group n°1 was mainly composed of sludge and sludge digestate and characterised by the highest C_bio values. Sludge samples were characterised by a large REOM fraction and the simplest fluorescence zones from REOM and SPOM while, similarly to BMP prediction, sludge digestates were rather characterised by a large SEOM fraction. Nevertheless, sludge digestate was part of a separate group in BMP prediction and not part of the same group as for C_bio (i.e. the most biodegradable samples). This confirms that, depending on their OM characteristics, aerobic and anaerobic biodegradation potentials cannot be similar for all substrates. Some digestates could be more biodegradable under aerobic soil incubation than in anaerobic BMP tests. In contrast, similarly to the soil and to the potting mix, Group n°4 contained the poorest biodegradable OR. They were characterised by a high NEOM fraction and complex fractions of SPOM. This latter characteristic was also observed in the group characterised by low BMP values (i.e. potting mix, close to stabilized composts).

Other groups were categorised as intermediate according to their nature. Indeed, Group n°2 was composed of FFMSW, initial composting mixtures of green waste and sludge, and vegetables, which represented a mixture of compounds that were rich in either protein-like fractions, or lignocellulose-like fractions. Group n°3 was composed of digestates from territorial OR from crop residues. They were associated to a large PEOM fraction and to complex fluorescence zones in this fraction. Groups n°5 and 6 contained manure digestates, wooden structures and composts in the lower part (rather associated with a high NEOM fraction and manure, i.e. a lignin-like content), and FFMSW digestates and green waste in the upper part (mainly associated with a large PEOM fraction, i.e. a cellulose and hemicellulose content). All these samples were also characterised by complex fluorescence zones from PEOM fraction.

As for the BMP prediction model, Table 2 highlights the impact of the most significant X-variables on the Y-variable prediction and confirms all the previous observations. Concerning the prediction of C_bio, the most significant positive variables were PEOM, SPOM, REOM and SEOM with fluorescence zones 1–3 (i.e. protein-like) from SPOM and REOM and fluorescence zones 5 and 6 from SEOM.

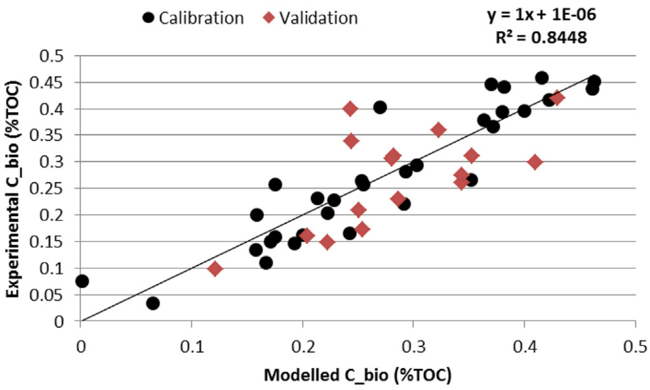

Fig. 3. Observed versus predicted Cbio value obtained with the PLS model n°3 (*black dots: calibration data and red dots: validation data*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

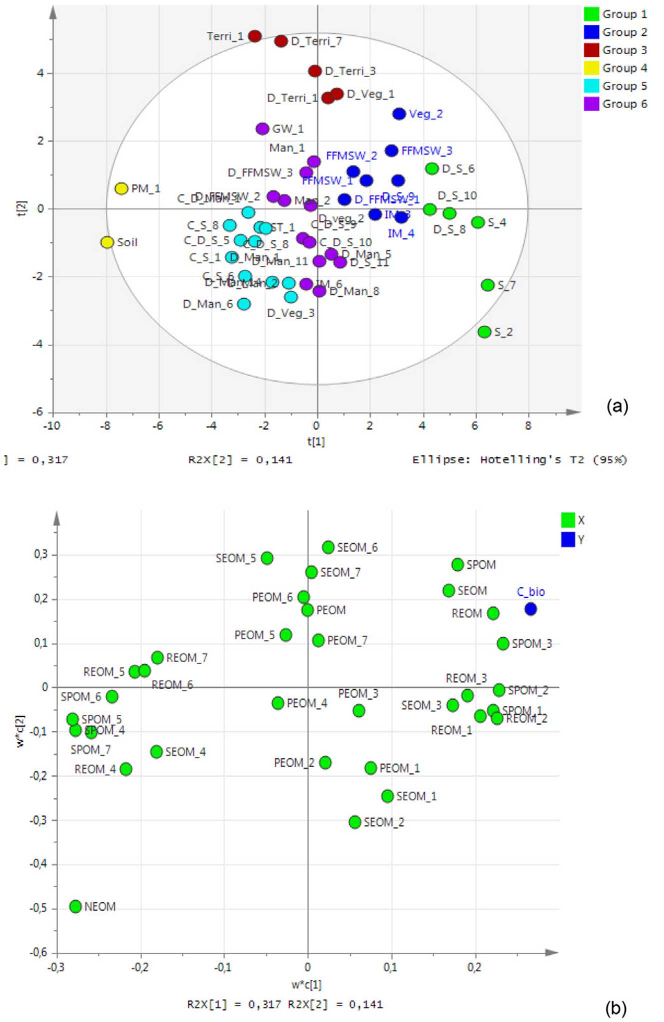

Fig. 4. Scores (a) and loadings (b) related to the C_{bio} variable prediction applied on all the data.

The most significant negative variables were NEOM and certain complex fluorescence zones from SPOM and REOM.

- Anaerobic versus soil aerobic biodegradability: impact of OM quality

In Table 2, the weight of the model variables revealed existing similarities and differences between anaerobic biodegradability and aerobic soil incubation. Both types of biodegradability were positively impacted by the SPOM fraction and the simplest fluorescence zones.

However, contrary to the BMP value prediction, SEOM showed a positive influence on aerobic biodegradability, namely its fluorescence zones 5 and 6. Indeed, the SEOM fraction and its fluorescence zone 6 were related to humic-like substances, which are mostly found in digestates. This fraction and the associated molecules which tend to be recalcitrant under anaerobic conditions showed a positive influence in soil incubation conditions. This is consistent with the nature of the molecules which can affect the production of humus.

Consequently, combining anaerobic digestion (by optimising the anaerobic recalcitrant SEOM-like production) with the digestate landspreading could entail a higher organic fertilizing value. Digestate characterisation results from a statistical analysis of nuclear magnetic resonance data (Tambone et al., 2010), showed how anaerobic digestion is capable of preserving recalcitrant molecules (i.e. lignin-like, steroids or complex lipids) known as humus precursors.

The PEOM and NEOM fractions presented a significant impact on C_bio predictions while these same fractions had no significant effect on BMP. This is consistent with the results obtained by Lashermes et al. (2009) involving a positive impact of the cellulose fraction and a negative impact of the lignin fraction, both being obtained by Van Soest fractionation.

Moreover, even though the most biodegradable samples contain a large fraction of SPOM and a high content of protein-like fluorescence zones, aerobic biodegradation appears to be further advanced than anaerobic biodegradation. In both cases, the characterisation protocol was sufficiently detailed for both mineralization potentials to be predicted. In addition, the differences between both pathways could lead to a balance between methane and organic amendment production. For example, sludge digestates and certain manure digestates that are rich in the moderately accessible SEOM fraction, presented high C_bio values. These are more appropriate for fertility purposes while NEOM-rich digestate composts and complex molecules with low C_bio values should be more appropriate for long-term organic amendment.

Characterisation data used in common for both models (e.g. BMP and aerobic soil biodegradation) have been used to investigate the correlations between the BMP and C_bio variables. Fig. 5 illustrates the resulting correlation. C_bio and BMP were positively and significantly correlated, with a Pearson coefficient of R = 0.787. In addition, a linear regression between BMP and C_bio data was established with the following equation: BMP = 474.98 × C_bio + 15.462, with a correlation coefficient of $R^2 = 0.619$, meaning that only 62% of the samples variance is explained by the linear regression.

In some cases, two samples had similar BMP values but very different and lower C_bio values (for example this could be the case for green waste and sludge; potting mix and territorial digestate). Despite the fact that, in many cases, the biodegradable state of OM is generally stable under both anaerobic and aerobic conditions, the structural and functional diversity of soil microbial communities tend to enhance OM degradation under aerobic conditions.

In the literature, many authors have tried to correlate BMP test values with rapid aerobic test results (i.e. respirometry). They observed reliable and positive correlations between these two tests that were performed on the same types of organic residues. Cossu and Raga (2008) and Scaglia et al. (2010) found such correlations on MSW landfill ($R^2 > 0.80$ and 0.89 respectively); positive correlations between BMP and Biological Oxygen Demand tests were also observed by Liu et al. (2015) with agricultural and forest residues ($R^2 > 0.94$) and Bayard et al. (2015) with biowaste and digested

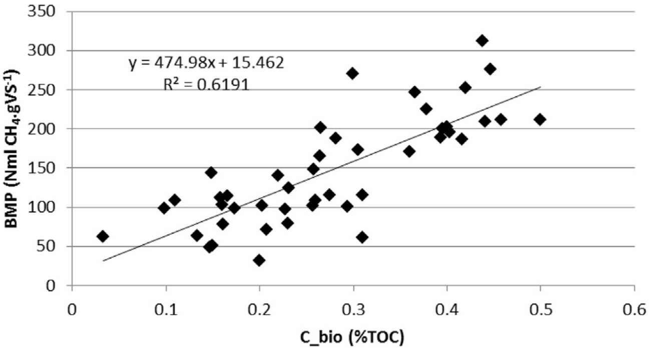

Fig. 5. Correlation between BMP experimental values and C_bio experimental values.

biowaste ($R^2 = 0.81$). Maynaud et al. (2017) also demonstrated a good correlation between anaerobic biodegradability and aerobic biodegradability obtained on solid and composted digestates without inoculum addition ($R^2 = 0.8$). In the present work, aerobic biodegradability is achieved by soil incubation. This main difference with other studies reported from the literature suggests that aerobic inoculum from soil was completely different in terms of diversity and biodegradation ability.

As previously mentioned, the organic carbon stability and potential efficiency of increasing soil OM were usually estimated with the IROC calculated as a function of the fractions provided by the Van Soest methodology and a 3-day carbon mineralisation test (C3d) (Lashermes et al., 2009). According to the authors, the models built with the Van Soest fractionation variables were not sufficiently robust and accurate to predict the IROC whereas the addition of the C3d favoured a better prediction. The C3d variable was the most significant variable of the IROC model, positively correlated with biodegradable TOC and associated with the most readily biodegradable OR (i.e. sludge, liquid manure, animal wastes without litter). Besides, the soluble fraction used for the IROC calculation is positively correlated to the residual TOC obtained at the end of the incubation (Lashermes et al., 2009).

This soluble fraction is very large for a lot of protein-like OR (i.e. sludge, liquid manure, some animal wastes, digestates) according to Lashermes et al. (2009), Mottet et al. (2010), Peltre et al. (2011), and Jimenez et al. (2015). Despite its accessible chemical feature, this fraction can contain both biodegradable and non-biodegradable molecules (Peltre et al., 2011). Prediction of biodegradable TOC still needs to be improved due to a lack in complexity information. Moreover, in contrast to the fractionation procedure applied in this study, the humic-like fraction was absent in the Van Soest fractionation. Indeed, thanks to the SEOM fraction and 3D fluorescence spectroscopy, it is possible to identify and include humification in organic waste characterisation. According to the type of sample, the SEOM fraction contains mainly complex protein-like molecules or/and humic-like substances (Jimenez et al., 2015). Also, the fluorescence signal of this fraction reveals the degree of complexity which can then be associated to its biodegradability. As PLS models have proved, the SEOM fraction represents a key variable for both soil carbon mineralization and BMP predictions. Humification, being a key process in organic carbon stabilisation, could account for a more efficient capacity in increasing soil organic carbon stocks after spreading. Unlike the IROC indicator, this methodology has the capacity to better take into account the chemical nature of the extracted fractions and their evolution during treatment for the prediction of the potential efficiency of increasing soil organic matter after residue application. The same OM fractionation combined with 3D-fluorescence spectroscopy has enabled the prediction of both methane production and mineralisable soil carbon: these input variables could be used in future investigations to combine dynamic models of carbon fate in conditions ranging from anaerobic digestion to soils. Results have shown how anaerobic digestion and soil incubation can be complementary regarding OM biodegradability for methane production and OM stability for soil amendment purposes. Depending on the soil constraints, this approach is a first step in optimising the whole processing chain from anaerobic digestion to soils.

Indeed, the main issue for this kind of modelling is the difference between the considered input/output variables of the models (ADM1, Batstone et al., 2002; Garnier et al., 2003; Sole-Mauri et al., 2007; Oudart et al., 2012; Zhang et al., 2012; Denes et al., 2015). The next step should be to use these fractions as common variables in mechanistic models in order to simulate OM accessibility throughout the whole processing chain of OR until soil utilisation.

4. Conclusions

Chemical accessibility and fluorescence spectroscopy have proved to predict both methane potentials and soil mineralized carbon, implying that the methodology can be used for aerobic and anaerobic decomposition models if plant-wide modelling is considered. Moreover, humic–like acids fraction and its associated fluorescence zone appeared to be recalcitrant for BMP but positive for C_bio prediction. These differences could be used as actuators since these recalcitrant molecules can be precursors of humus production, thus promoting the choice of digestate spreading. It is the first step towards improving the quality control of OR for their valorisation to be most profitable.

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References

Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Biotechnol. 3, 117–129.

Aquino, S.F., Chernicharo, C.A., Soares, H., Takemoto, S.Y., Vazoller, R.F., 2008. Methodologies for determining the bioavailability and biodegradability of sludges. Environ. Technol. 29, 855–862.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A., 2002. Anaerobic Digestion Model No.1. (ADM1). IWA Scientific and Technical Report No. 13. IWA, ISBN:1-900222-78-7.

Bayard, R., Gonzalez-Ramirez, L., Guendouz, J., Benbelkacem, H., Buffiere, P., Gourdon, R., 2015. Statistical analysis to correlate bio-physical and chemical characteristics of organic wastes and digestates to their anaerobic biodegradability. Waste Biomass Valorization 6 (5), 759–769.

Bekiaris, G., Triolo, J.M., Peltre, C., Pedersen, L., Jensen, L.S., Bruun, S., 2015a. Rapid estimation of the biochemical methane potential of plant biomasses using Fourier transform mid-infrared photoacoustic spectroscopy. Bioresour. Technol. 197, 475–481.

Bekiaris, G., Bruun, S., Peltre, C., Houot, S., Jensen, L.S., 2015b. FTIR–PAS: a powerful tool for characterising the chemical composition and predicting the labile C fraction of various organic waste products. Waste Manage. 39, 45–56.

Buffiere, P., Loisel, D., Bernet, N., Delgenes, J.P., 2006. Towards new indicators for the prediction of solid waste AD properties. Water Sci. Technol. 53 (8), 233–241.

Cardeña, R., Moreno, G., Bakonyi, P., Buitrón, G., 2017. Enhancement of methane production from various microalgae cultures via novel ozonation pretreatment. Chem. Eng. J. 307, 948–954.

Cossu, R., Raga, R., 2008. Test methods for assessing the biological stability of biodegradable waste. Waste Manage. 28 (2), 381–388.

Denes, J., Tremier, A., Menasseri-Aubry, S., Walter, C., Gratteau, L., Barrington, S., 2015. Numerical simulation of organic waste aerobic biodegradation: a new way to correlate respiration kinetics and organic matter fractionation. Waste Manage. 36, 44–56.

Fitamo, T., Triolo, J.M., Boldrin, A., Scheutz, C., 2017. Rapid biochemical methane potential prediction of urban organic waste with near-infrared reflectance spectroscopy. Water Res.

Garnier, P., Neel, C., Aita, C., Recous, S., Lafolie, F., Mary, B., 2003. Modelling carbon and nitrogen dynamics in a bare soil with and without straw incorporation. Eur. J. Soil Sci. 54, 555–568.

Godin, B., Mayer, F., Agneessens, R., Gerin, P., Dardenne, P., Delfosse, P., Delcarte, J., 2015. Biochemical methane potential prediction of plant biomasses: comparing chemical composition versus near infrared methods and linear versus non-linear models. Bioresour. Technol. 175, 382–390.

Gunaseelan, V.N., 2007. Regression models of ultimate methane yields of fruits and vegetables solid wastes, sorghum and napier grass on chemical composition. Bioresour. Technol. 98, 1270–1277.

Gunaseelan, V.N., 2009. Predicting ultimate methane yields of *Jatropha curcas* and *Morus indica* from their chemical composition. Bioresour. Technol. 100 (13), 3426–3429.

Houot, S., Clergeot, D., Michelin, J., Francou, C., Caria, G., Ciesielski, H., 2002. Agronomic value and environmental impacts of urban composts used in agriculture. In: Insam, H., Riddech, N., Klammer, S. (Eds.), Microbiology of Composting. Springer-Verlag, Berlin Heidelberg, pp. 457–472.

Jimenez, J., Gonidec, E., Cacho Rivero, J.A., Latrille, E., Vedrenne, F., Steyer, J.-P., 2014. Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: towards ADM1 variables characterization. Water Res. 50, 359–372.

Jimenez, J., Aemig, Q., Doussiet, N., Feurgard, I., Steyer, J.-P., Patureau, D., Houot, S., 2015. Towards a unified organic waste characterization of organic matter accessibility and complexity. Bioresour. Technol. 194, 344–353.

Kafle, G.K., Chen, L., 2016. Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. Waste Manage. 48, 492–502.

Lashermes, G., Nicolardot, B., Parnaudeau, V., Thuriès, L., Chaussod, R., Guillotin, M. L., Linères, M., Mary, B., Metzger, L., Morvan, T., Tricaud, A., Villette, C., Houot, S., 2009. Indicator of potential residual carbon in soils after exogenous organic matter application. Eur. J. Soil Sci. 60, 297–310.

Lesteur, M., Latrille, E., Maurel, V.B., Roger, J.M., Gonzalez, C., Junqua, G., Steyer, J.P., 2011. First step towards a fast analytical method for the determination of biochemical methane potential of solid wastes by near infrared spectroscopy. Bioresour. Technol. 102 (3), 2280–2288.

Liu, X., Bayard, R., Benbelkacem, H., Buffière, P., Gourdon, R., 2015. Evaluation of the correlations between biodegradability of lignocellulosic feedstocks in anaerobic digestion process and their biochemical characteristics. Biomass Bioenergy 81, 534–543.

Maynaud, G., Druilhe, C., Daumoin, M., Jimenez, J., Patureau, D., Torrijos, M., Pourcher, A.M., Wery, N., 2017. Characterisation of the biodegradability of post-treated digestates via the chemical accessibility and complexity of organic matter. Bioresour. Technol. 231, 65–74.

Mottet, A., François, E., Latrille, E., Steyer, J.P., Déléris, S., Vedrenne, F., Carrère, H., 2010. Estimating anaerobic biodegradability indicators for waste activated sludge. Chem. Eng. J. 160, 488–496.

Muller, M., Jimenez, J., Antonini, M., Dudal, Y., Latrille, E., Vedrenne, F., Steyer, J.P., Patureau, D., 2014. Combining chemical sequential extractions with 3D fluorescence spectroscopy to characterize sludge organic matter. Waste Manag. 34, 2572–2580.

Oudart, D., Paul, E., Robin, P., Paillat, J.M., 2012. Modeling organic matter stabilization during windrow composting of livestock effluents. Environ. Technol. 33 (19), 2235–2243.

Peltre, C., Dignac, M.F., Derenne, S., Houot, S., 2010. Change of the chemical composition and biodegradability of the Van Soest soluble fraction during composting: a study using a novel extraction method. Waste Manage. 30 (12), 2448–2460.

Peltre, C., Thuriès, L., Barthès, B., Brunet, D., Morvan, T., Nicolardot, B., Parnaudeau, S., Houot, S., 2011. Near infrared reflectance spectroscopy: a tool to characterize the composition of different types of exogenous organic matter and their behaviour in soil. Soil Biol. Biochem. 43, 197–205.

Peltre, C., Christensen, B.T., Dragon, S., Icard, C., Kätterer, T., Houot, S., 2012. RothC simulation of carbon accumulation in soil after repeated application of widely different organic amendments. Soil Biol. Biochem. 52, 49–60.

Scaglia, B., Confalonieri, R., D'Imporzano, G., Adani, F., 2010. Estimating biogas production of biologically treated municipal solid waste. Bioresour. Technol. 101 (3), 945–952.

Sole-Mauri, F., Illa, J., Magrí, A., Prenafeta-Boldú, F.X., Flotats, X., 2007. An integrated biochemical and physical model for the composting process. Bioresour. Technol. 98 (17), 3278–3293.

Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. Chemosphere 81 (5), 577–583.

Thomsen, S.T., Spliid, H., Østergård, H., 2014. Statistical prediction of biomethane potentials based on the composition of lignocellulosic biomass. Bioresour. Technol. 154, 80–86.

Van Soest, P.J., 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Anal. Chem. 46, 829–835.

Zhang, Y., Lashermes, G., Houot, S., Doublet, J., Steyer, J.P., Zhu, Y.G., Barriuso, E., Garnier, P., 2012. Modelling of organic matter dynamics during the composting process. Waste Manage. 32, 19–30.

Instrumentation and control of anaerobic digestion processes: a review and some research challenges

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REVIEW PAPER

Instrumentation and control of anaerobic digestion processes: a review and some research challenges

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Abstract To enhance energy production from methane or resource recovery from digestate, anaerobic digestion processes require advanced instrumentation and control tools. Over the years, research on these topics has evolved and followed the main fields of application of anaerobic digestion processes: from municipal sewage sludge to liquid—mainly industrial—then municipal organic fraction of solid waste and agricultural residues. Time constants of the processes have also changed with respect to the treated waste from minutes or hours to weeks or months. Since fast closed loop control is needed for short time constant processes, human operator is now included in the loop when taking decisions to optimize anaerobic digestion plants dealing with complex solid waste over a long retention time. Control objectives have also moved from the regulation of key variables—measured on-line—to the prediction of overall process performance—based on global off-line measurements—to optimize the feeding of the processes. Additionally, the need for more accurate prediction of methane

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production and organic matter biodegradation has impacted the complexity of instrumentation and should include a more detailed characterization of the waste (e.g., biochemical fractions like proteins, lipids and carbohydrates) and their bioaccessibility and biodegradability characteristics. However, even if in the literature several methodologies have been developed to determine biodegradability based on organic matter characterization, only a few papers deal with bioaccessibility assessment. In this review, we emphasize the high potential of some promising techniques, such as spectral analysis, and we discuss issues that could appear in the near future concerning control of AD processes.

Keywords Anaerobic digestion · Organic matter · Characterization · Instrumentation · Control · Diagnosis

Abbreviations

AD	Anaerobic digestion
ADM1	Anaerobic digestion model No 1
AFM	Atomic force microscopy
BCA	Bicinchonic acid
BD	Ultimate anaerobic biodegradability
BMP	Biochemical methane potential
BOD	Biochemical oxygen demand
CH_4	Methane
CLSM	Confocal laser-scanning microscopy
CO_2	Carbon dioxide
COD	Chemical oxygen demand
Da	Dalton
EPS	Extracellular polymeric substances
FOG	Fats, oils, and greases
FTIR	Fourier transform infrared spectroscopy
GASDM	General activated sludge and digestion model
GC/MS	Gas chromatography coupled with mass spectroscopy
GISCOD	Eneral integrated solid waste co-digestion model
HA	Humic acids
HPLC	High performance liquid chromatography
HRT	Hydraulic retention time
ICA	Instrumentation, control and automation
IWA	International water association
LCFA	Long chain fatty acids
MPR	Methane production rate
MSW	Municipal solid waste
NIRS	Near infra-red spectroscopy
NMR	Nuclear magnetic resonance spectroscopy
OLR	Organic load rate
PLS	Partial least square
R^2	Regression coefficient
RI_4	Respiration index 4 days
SEM	Scanning electron microscopy
STP	Standard conditions of temperature and pressure
S/X	Substrate to biomass ratio
TEM	Transmission electron microscopy
TKN	Total kjeldahl nitrogen
TOC	Total organic carbon
TS	Total suspended solids
VFA	Volatile fatty acids
VS	Volatile solids
XPS	X-ray Photoelectron spectroscopy
3D-EEM	3D emission excitation matrix

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1 Introduction

One of the key issues for global sustainable development is the energy consumption, particularly as fossil fuels, which represents up to 80 % of the global energy consumption. Moreover, fossil fuels are considered the main source of acidifying contaminants and greenhouse gasses, as well as the main factor contributing to global warming and climate change. Hence, one big challenge for this century is to develop new competitive sources of renewable energy, capable of replacing fossil fuels with a minimum impact on both the environment and society, while maintaining energy (electricity or gas) grid stability (Szarka et al. 2013). In this respect, alternative energy sources such as methane from organic residues must be considered.

Anaerobic Digestion (AD) is a biological process in which the organic carbon is converted through oxidation–reduction reactions to both its most oxidized state (CO_2) and its most reduced form (CH_4). The methane produced is an energy source that can be valorized as electricity, heat, biofuel or can be injected into the natural gas grid. In the context of a widely perceived energetic and climatic crisis, AD has become a very interesting alternative for organic waste disposal. For example, in France, wastewater treatment plant (WWTP) energy consumption is about 20 kWh per year per person equivalent, based on a 100,000 person equivalent plant. From these observations and the fact that wastewater sludge potentially contains a high amount of energy that can be recovered, it is clear that WWTPs of the future—or water resource reclamation facilities (WRRFs) as they are now called—should aim at a positive energy balance (Cao and Pawlowski 2012).

1.1 From municipal wastewater solids to industrial and agricultural wastes

AD has been used to stabilize municipal wastewater solids for over 80 years, probably with the first heated, mixed system being employed in Germany in 1927 (Imhoff 1938). During the last 30 years, the total number of papers on AD and industrial applications increased rapidly, mainly due to a favorable environmental policy: the Kyoto protocol (2005), national or international legislation promoting AD, special rates for selling electricity produced from biogas. The evolution of the market also led to a higher complexity of the substrates considered for AD valorization.

In the eighties, industrial wastewater treated by AD began to grow and worldwide, the overall number of anaerobic reactors treating industrial wastewater reached 2266 references in 2007 (van Lier 2008) and kept on increasing since then. The main focus of AD optimization has been about kinetics of soluble substrates, considering acetogenesis and methanogenesis as the limiting steps (Mata-Alvarez et al. 2000).

At the end of the eighties, AD applications focused on the conversion of solid waste began to increase. Solid wastes then included mainly municipal solid waste (MSW) and green wastes. The increasing production of solid waste combined with waste management policies aiming at reducing long-term environmental impacts of landfill disposal have created a need for alternative treatment. The use of AD to treat the organic fraction of municipal solid waste became a reality (De Baere 2000, 2008): from three plants in 1990 to 55 plants referenced in 2010 in Europe and at least 4 in North America today, for example. From a process control standpoint, the disintegration/hydrolysis step received considerable attention for solid waste since it is the rate-limiting step for substrates containing mainly particulates (Mata-Alvarez et al. 2000; Lauwers et al. 2013).

Concomitantly, farmers have become increasingly interested in the AD process, both as an additional source of revenue and as an alternative energy source without greenhouse gas emission to the atmosphere. AD is indeed one of the technologies that fulfil European criteria for second generation biofuel production (fuels manufactured from various types of complex organic carbon sources such as lignocellulose biomass or agricultural residues, e.g., manure). The case of Germany where more than 7850 plants generate over 3.5 GW of electricity is an example or in China where more than 35 million household digesters and 25,000 digesters for agricultural residues have been deployed (Fang 2012).

1.2 Biodegradability, bioavailability and bioaccessibility

Hydrolysis rate of complex substrates has been identified for a long time as an important factor for AD modelling and process optimization (Vavilin et al. 1997), especially considering substrate characterization and hydrolysis kinetics. Modern dynamical models of AD are very useful for optimization of biogas production. For example, the IWA Anaerobic Digestion Model No 1 ADM1 (Batstone et al. 2002) has a detailed pathway description, but the model's main drawback is that it also needs detailed input variables and data that may not be available for a specific application (Astals et al. 2013a). Indeed, a key-point for the successful description of a bioprocess is appropriate influent characterization data (Huete et al. 2006; Buffiere et al. 2006; Kleerebezem and van Loosdrecht 2006).

Lately, three major concepts have been shown to be of prime importance to characterize organic matter biodegradation: biodegradability, bioavailability and bioaccessibility (Jimenez et al. 2014). Biodegradability is the ability of a substrate to be broken down by a microorganism into simpler compounds but this biodegradation is limited by molecule's bioavailability, complexity and/or toxicity. Bioavailability is defined as the direct access to the molecule to be degraded while Aquino et al. (2008) defined bioaccessibility as the possible access to the molecule depending on several factors such as the contact time between the substrate and the microorganism, the efficiency of hydrolytic activity or ultimately any pre-treatment applied to the waste. There is thus a notion of physical accessibility as in the case of the cellulose protection by lignin or vegetal walls acting as a barrier and needing chemical or physical break-up to make cellulose accessible to microorganisms (Motte et al. 2014, Reilly et al. 2015). Consequently, the bioavailable organic matter is included in the bioaccessible fraction such as the organic fraction able to be degraded by secreted exo-cellular enzymes (Jimenez et al. 2014).

In parallel, the control problem associated with anaerobic biological waste or wastewater treatment processes must involve—like in any aerobic processes—process configurations that remain robust against unpredicted perturbations (e.g., physicochemical, mechanical, etc.) and uncertainties in relation to: (a) initial conditions, (b) kinetic and hydrodynamic parameters, (c) yield coefficients, and (d) input concentrations. All these aspects strongly influence the overall objectives of instrumentation and control and are currently profoundly impacting the technical challenges and optimization criteria applied to AD processes.

2 Instrumentation of anaerobic digestion processes

The following section first focuses on classical instrumentation that is very often encountered in practice. On-line instruments that can be used in fast closed-loop control scheme and have proven to be very useful for monitoring any type of digester will be presented first (See also Spanjers and van Lier 2006 for additional information). Next, because of the development of the solid AD process—with long residence time—some techniques that are not yet available in an on-line context will be discussed. They can indeed provide very informative measurements that can help to optimize AD plants with long solid retention time, such as those dealing with municipal or agricultural waste. Sensor dynamics are likely to be less important than static characteristics and other cost benefit considerations in most cases, as the process dynamics are seldom challenging to the sensor technologies used.

2.1 On-line instrumentation

2.1.1 Flow, temperature, pH and ORP

Instruments to monitor gas and liquid flows are ubiquitous in wastewater treatment. For example, Harremoës et al. (1993) provided an extensive overview of liquid flow measurement techniques and pointed out the importance of proper installation for guaranteed accuracy. Measurements are based on pressure differentials resulting from restrictions (venturi, orifice plates, and meshes) placed in the flow path. In addition, electromagnetic and ultrasonic sensors can also be applied.

Temperature is a rather important variable for anaerobic digesters and temperature control is often implemented. Three commonly used types of process measuring instruments are available for measuring temperature: resistance thermometer, thermo-element, and thermistor.

It is normal practice to install pH electrodes in a treatment plant. Immersion of these probes in 'sticky' sludge has encouraged the development of different cleaning strategies: hydraulic (water spray), mechanical (brush), chemical (rinsing with cleaning agent) or ultrasonic cleaning. With these techniques, longer periods without maintenance can be attained. Poor or no automatic cleaning may indeed cause problems and self-diagnosis has been integrated in advanced systems. More sophisticated set-ups include automated checks of the impedance of the diaphragm and the glass electrode, while tests performed during (automatic) calibration may be used to indicate other sensor deficiencies. Although pH is a variable that is important in all biological processes, its value is especially critical in anaerobic digestion, eventually leading to acidification and process failure. Hence, its measurement and control are important. However, in the case of wastewaters with high buffering capacity, pH measurements may be rather insensitive to indicate process changes and are therefore not advisable for process supervision and control. In such cases, they may be replaced with bicarbonate and/or alkalinity measuring systems (Di Pinto et al. 1990; Hawkes et al. 1993 and Guwy et al. 1997—see also Sect. 2.1.3).

Oxidation–reduction potential (ORP) sensors are also sometimes installed since an increase in ORP indicates a possible presence of oxygen in the process. In this respect, it is recommended to maintain an ORP potential below -300 mV relative to a standard hydrogen electrode (depending on the wastewater characteristics) in order to not adversely affect anaerobic methanogenic archaea activity. ORP is also sometimes used to monitor sulfate reduction in digesters and H_2S in the biogas through micro-aeration (Nghiem et al. 2014).

As biogas formation rate is one of the most commonly monitored variables in anaerobic digestion processes, gas flow sensors are very often part of digester instrumentation. Pressure measurements can be found in AD plants as well, especially for alarm functions.

2.1.2 Biogas composition

Gas composition measurements are also required in lab processes and full-scale plants. Typically, specific gas analyzers monitor the content of a component directly and infrared absorption measurements are used to determine carbon dioxide and methane concentrations. There are several of such sensors available today in the market. It has to be kept in mind that, although not always straightforward to predict from measurements in the gas phase, the corresponding concentrations of gasses in the liquid phase are important as they represent the environment the microorganisms operate in. It is possible to use Henry's law to calculate equilibrium aqueous concentration, however it is necessary to know the gas composition and the Henry's constant for each component at the required temperature and in aqueous solutions of variable ionic strength. Also, gas–liquid partitioning in digesters is very dynamic and equilibrium conditions may not be present.

The presence of hydrogen sulphide in the gas and the explosive character of biogas also require careful precautions. Hydrogen sulphide measurement in the gas phase may be performed by monitoring the reaction of sulphide with a Pb-strip. Subsequently, the black PbS that is produced is quantified by colorimetry. No direct on-line measurement of hydrogen sulphide in the liquid phase has been reported though. Membrane inlet mass spectrometry (Ryhiner et al. 1992) is another method to directly measure a large number of dissolved gasses and volatile compounds. The MS membrane probe response is often linear over very large concentration ranges. For application of thin membranes—that are required for sufficiently fast response and high sensitivity—the analyzer should be protected because of the rather high risk of membrane rupture. A fast safety shut-off system including fast pressure measurement is thus advised to be installed.

Specific hydrogen (H_2) analyzers have been developed—mainly in laboratories—based for example on electrochemical cells (Mathiot et al. 1992). Immersible sensors have been developed to measure dissolved hydrogen concentrations directly in the liquid phase down to partial pressures of 1 Pa (10^{-5} atm). Their reliability and long-term stability have been reported (Pauss and Nyns 1993). An inexpensive amperometric dissolved hydrogen probe has been used to determine the onset of digester failure by substrate overloading (Cord-Ruwisch et al. 1997). The measuring principle is based on the oxidation of hydrogen at a platinum black electrode at an adjusted potential. The current flowing to the electrode is directly related to the hydrogen concentration in the bulk liquid but H_2S has to be trapped and removed before the biogas flows into the hydrogen monitor. Björnsson et al. (2001a) applied a hydrogen-sensitive palladium–metal oxide semiconductor (Pd-MOS) sensor in combination with a Teflon membrane for liquid-to-gas transfer for the detection of dissolved hydrogen and the monitoring of a laboratory-scale anaerobic digestion process, employing mixed sludge containing mainly food/industrial waste. The sensor gave valuable information about approaching process overload, and can serve as a good alternative for volatile fatty acids (VFA) monitoring. The sensor was stable and robust during 3 months of operation, and therefore it was concluded that hydrogen sulfide, which is known to poison the Pd-MOS sensor, could not penetrate the Teflon membrane.

2.1.3 *Alkalinity*

The incentive to measure the bicarbonate content of the mixed liquor indeed originates from the fact that imbalance in anaerobic digestion (due to the accumulation of volatile fatty acids, VFA) cannot easily be detected on the basis of pH measurements, especially when the alkalinity of the mixed liquor is high (Hawkes et al. 1993). Because the alkalinity is often mainly due to the bicarbonate buffer, it has been proposed since the early sixties that its measurement can be used in control strategies for anaerobic digesters (McCarty 1964). One way to do so is by titration. Such methods involve titrating the sample down to pH 3.5 to determine the bicarbonate content with a correction for the volatile fatty acids present [see for example Ripley et al. (1985) or Anderson and Yang (1992)]. The method is based on quantifying the gaseous carbon dioxide evolved from the sample as it is acidified. The volume of gas may be measured in two different ways. The overpressure in a closed constant volume vessel can be measured, or the gas volume produced can be measured with a sensitive gas flow meter in a constant pressure system. During titration, interferences from other weak acid/base constituents cannot be excluded and overestimation of VFA may sometimes occur (Purser et al. 2014).

2.1.4 *Volatile fatty acids*

Total VFA concentrations have been monitored for a long time as process performance indicators. It gives fast and reliable information of process status compared to other common indicators such as pH, alkalinity, gas production, and gas composition (Ahring et al. 1992; Björnsson et al. 2001b; Boe et al. 2007). Automated bicarbonate and total VFA instruments based on titrimetry have been developed and applied in practice for some years—see for example Feitkenhauer et al. (2002) or Ruiz et al. (2005).

Compared to total VFA concentration, individual VFA (acetate, propionate, butyrate etc.) can provide more information of the process status. Several studies have highlighted the importance of individual VFA as an early warning of process imbalance (Boe et al. 2010; Pind et al. 2003; Pratt et al. 2003, 2012; Van Ginkel and Logan 2005). Ahring et al. (1992) suggested the overall level of n-butyric and iso-butyric was the best indicator of process stress. Boe et al. (2010) advised propionate as the most persistent parameter which was effective indicator of stress status of the process. Individual VFA are easily measured off-line using GC or HPLC, provided that all particulate matter has been removed from the sample.

However, only a few studies reported the development of an on-line individual VFA monitoring system because when dealing with anaerobic waste treatment, the presence of particulate matter is often high. Ryhiner et al. (1993) used GC for on-line analysis of acetic, propionic, butyric, valeric, and iso-valeric in a UASB reactor treating whey powder solution. The sample was purified by membrane filtration, acidified by phosphoric acid, and injected into the GC column by an auto-sampler with a specially constructed flow-through vial. However, no performance data was shown for this system. Zumbusch et al. (1994) used a HPLC for VFA monitoring in a UASB reactor treating baker's yeast wastewater using an ultra-filtration module for sample purification. The main problem of this process was membrane fouling requiring a high level of maintenance of the filtration system. Pind et al. (2003) used a GC for on-line analysis of VFA in a CSTR reactor treating manure and sample purification employed a three step filtration; pre-filtration by a rotating filter inside the reactor, ultra-filtration by a membrane cartridge, and a mini-filter for final purification. The system showed good correlation with the off-line measurement. However, membrane fouling was still the crucial problem and the membrane needed to be cleaned every 15–18 h to obtain sufficient flow. Boe et al. (2007) developed a new method to measure individual VFA based on headspace gas chromatography (HSGC). The method applies ex situ VFA stripping with variable headspace volume and gas analysis by gas chromatography-flame ionization detection (GC-FID). In each extraction, digester sample was acidified with H_3PO_4 and $NaHSO_4$, and then heated to strip the VFA into the gas phase. The system has been tested for on-line monitoring of a lab-scale CSTR reactor treating manure for more than 6 months and has shown good agreement with off-line analysis.

2.1.5 *Spectral sensors*

Spectral techniques—UV/visisible spectroscopy (UV/vis), Mid InfraRed spectroscopy (MIR), Near Infra-Red spectroscopy (NIRS)—are beginning to provide very useful information about the complexity of organic matter.

UV/vis spectroscopic probes in the range of 190–750 nm are often used in wastewater treatment plants to measure COD, TOC and NO_3-N (Sarraguça et al. 2009). Wolf et al. (2013) developed a UV/vis spectroscopic system for VFA measurement (1.1–3 g L^{-1}) in AD plants. An UV/vis probe from S::CAN was used in combination with a custom-built dilution system to monitor the absorption of fully fermented sludge. To validate the approach, on-line measurements have been taken at a full-scale 1.3 MW industrial biogas plant. Results showed that VFA concentrations can be predicted with an accuracy of 87 %. Nevertheless, the necessary dilution system is a disadvantage compared to NIR and MIR spectroscopic systems.

NIRS presents great potential for monitoring the AD process. Holm-Nielsen et al. (2008) evaluated the use of NIRS technology on-line (Transflexive Embedded Near Infra-Red Sensor or TENIRS) to monitor a thermophilic digester treating manure and organic food industrial waste. Good correlation was obtained between on-line NIRS measurement of glycerol and VFA content in the anaerobic digester. Further works documented the potential to monitor VFA as well as VS in on-line installations at lab-scale and full-scale plants (Krapf et al. 2013; Jacobi et al. 2009).

Mid InfraRed (MIR) spectroscopy is another interesting technique to characterize waste organic matter. One major advantage against existing NIR sensors is that process variables such as VFA, total alkalinity (TA), NH_4-N and TS show distinctive peaks in the MIR spectrum between 1800 and 800 cm^{-1}, which makes it easier to correlate peak intensity to actual concentrations. Provenzano et al. (2014) used Fourier Transform InfraRed (FTIR) and fluorescence spectroscopy to characterize the organic matter evolution during AD and composting of pig slurry. Steyer et al. (2002) also used for several years a FTIR spectrometer for on-line measurements of COD, TOC, VFA, total and partial alkalinity of an AD fixed bed treating industrial wine distillery wastewater. Spanjers et al. (2006) applied the same technique at a full scale plant for the on-line monitoring of VFA, COD, alkalinity, sulphate, and, since aerobic post-treatment was considered, total nitrogen, ammonia and nitrate concentrations. Based on these studies, Wolf et al. (2014) developed an on-line MIR system with an FTIR probe using Polychristalline-Infrared (PIR) fibres that allow for higher signal to noise ratio (S/N) ratios as well as longer fibres. Furthermore, a fully automated process interface for cleaning and recalibration was used in order to reduce maintenance to a minimum. Good calibration results were obtained for VFA ($R^2 = 0.97$, RMSE 0.372 g L^{-1}), TA ($R^2 = 0.99$, RMSE = 0.259 g L^{-1}) and NH_4-N ($R^2 = 0.99$, RMSE = 0.11 g L^{-1}). In spite of all advantages and advances in infrared spectroscopic on-line measurement systems, two main challenges remain: (1) despite the great interest in infrared spectroscopy on organic matter characterization, this technique is not sensitive enough for structural interpretation of complex molecules and does not account for the bioaccessibility of organic constituents; (2) prices for infrared spectroscopic measurement systems, NIR and MIR, are still far too expensive to be widely used in AD plants, so that financial feasibility is mostly not provided.

2.1.6 *Other on-line instrumentation*

Other examples of advanced instrumentation can be seen in electronic tongues and noses and microwave or acoustic chemometrics (Madsen et al. 2011). A gas chromatograph or mass spectrometer coupled to a sample preparation unit can also be used, but so far no full-scale applications for these methods have been reported.

Liquid phase electrical conductivity is defined as the ability of a solution to conduct electrical current and is directly proportional to ion concentrations. Moreover, it can be easily monitored on-line: a cell formed by two electrodes is placed in the sample and the current between both electrodes is measured by means of the application of a potential difference

(Colombié et al. 2007). Conductivity measurements could bring very informative measurements for monitoring and control of AD processes since ion concentrations are mainly affected by both VFA and bicarbonate concentrations (Hawkes et al. 1994), two of the most reliable indicators of AD process performance. Several studies have been published on the feasibility of electrical conductivity sensors for bioprocess monitoring (see, for instance, Hoffmann et al. 2000; Varley et al. 2004; Aguado et al. 2006; Ellison et al. 2007). However, there is still a lack of knowledge regarding its applicability to AD processes, despite some applications in dark fermentation processes for H_2 production (Aceves-Lara et al. 2010).

2.2 Off-line instrumentation

With long HRTs or SRTs, off-line characterization of the waste and biomass can be considered as a way to provide operators with useful information to optimize AD plants, even though the data are yet not on-line. Several techniques exist and they are presented below.

2.2.1 Global characterization methodologies

From an analytical point of view, the performance of AD in wastewater or waste treatment is traditionally evaluated using parameters such as chemical oxygen demand (COD), total organic carbon (TOC) and biochemical oxygen demand (BOD). In order to optimize plant design and operation, Raunkjær et al. (1994) proposed to link COD fractions and biodegradability. Kayhanian (1995) showed that the content of biodegradable volatile solids (VS) impacted the prediction of biogas production rate and the computation of the organic loading rate and the carbon/nitrogen (C/N) ratio. Since the seventies, the most widely used indicator to assess the performance of digesters has been the amount of methane produced per unit of total solid (TS) or volatile solids (VS) of any given substrate (Chynoweth et al. 1993).

2.2.2 Biodegradability and organic matter characterization

One of the key issues in operating and optimizing AD plants is to assess the quantity of methane that can be produced from an organic residue. To this end, the most commonly used method to measure anaerobic biodegradability is the biochemical methane potential (BMP) test (ISO 11734 1995).

2.2.2.1 BMP data and use for process modeling The BMP assay is a procedure developed to determine the methane yield of an organic material during its anaerobic decomposition by a mixed microbial community in a defined medium. The procedure was developed for a serum-bottle technique by Owen et al. (1979). Angelidaki and Sanders (2004) described the procedure and the calculations. The test ends when the cumulative biogas curve closely approaches an asymptote, usually after 30 days of incubation but it may be much longer for non-easily degradable material such as fibers. Therefore, the main inconvenience of the test is the long time required in its execution. Other negative points are the variability of the results obtained through the BMP tests and their ability to predict continuous digester performances. Concerning the first point, several studies made inter-laboratory assays to compare the BMP test results. Kinetic rates were widely different among different participating laboratories, standard deviations ranged from 57 to 68 % (Jensen et al. 2009). The relative standard deviation of BMP values ranged from 15 to 24 % and decreased to 10 % when outliers were not considered (Raposo et al. 2011). Currently, only one inter-laboratory (French Inter-laboratory assay 2013–2014) proposes new guidelines and protocol after 2 test rounds achieved on solid substrates. This last study has shown good intra-laboratory repeatability (equal to 4 %), reproducibility (between 5 and 7 %) and reproducibility (between 13 and 21 %)—see Cresson et al. (2014).

Concerning the second drawback, according to Jensen et al. (2009), the biodegradability and the bioaccessibility of hydrolysis-limited substrates could be defined by the parameters B_0 and k calculated from the Gompertz equation applied to a BMP curve (cumulative methane production vs. time), $B = B_0 \times (1 - e^{-kt})$, where B is the cumulative methane production, B_0 is the maximal methane production and k is the hydrolysis rate constant. However, the authors discuss the conservative feature of these parameters measured in a BMP test. Several opinions are found in the literature concerning the use of B_0 and k parameters obtained in batch tests in order to model continuous digesters (see, for example, Val del Rio et al. 2011; Nielfa et al. 2015; Strömberg et al. 2015). Batstone et al. (2009) found that the BMP test's parameters should not be used for dynamic modelling of continuous digesters. While the final value of BMP was found to be consistent with continuous data, these authors found that the hydrolysis rate parameter value was lower in a BMP test than in a continuous digester treating thermally a waste activated sludge (i.e., 0.15–0.25 vs. >5 day^{-1}). According to Labatut et al. (2011), the BMP test is not suitable for predicting methane production kinetics for continuous digesters because it is conducted under diluted conditions, so preventing any inhibition response from being observed. Nevertheless, Jensen et al. (2009) found that the batch test was slightly conservative in terms of estimating degradability and rate, when applied to slowly degradable substrates such as waste activated sludge. Fannin et al. (1987) concluded that the maximum theoretical methane yield determination was useful to evaluate digester performance and to provide basis for experimental work. On the other hand, biodegradation tests performed sequentially in batch reactors using a slightly different protocol than the one used in BMP tests (Ganesh et al. 2013) were shown to be very informative in assessing the biodegradation kinetics of a broad spectrum of biowaste (García-Gen et al. 2015).

2.2.2.2 More rapid prediction of methane potential Over the years, several authors developed relationships between the organic matter composition and the methane production or the anaerobic biodegradability. Static models are correlations (obtained by linear regression or partial least square (PLS) regression) where the parameters of interest are expressed as a function of one or more variables based on some analytical composition of the given substrate. Static implies neither kinetic equation nor variation over time. Three kinds of static models appeared in the literature to predict biodegradability of solid organic waste. Table 1 summarizes the comparative analysis, including benefits and drawbacks, of the different characterization methodologies involved in the integrative tools.

Initial biogas production modelling Some authors used the initial rate of biogas production modelling in order to predict the final value of BMP (Donoso-Bravo et al. 2011; Strömberg et al. 2015). For example, based on a database, Strömberg et al. (2015) proposed an algorithm to predict the BMP value from incubation experimental data operated during 6 days with an error less than 10 %. Donoso-Bravo et al. (2011) used similar technique with incubation during 3–4 days. However, the modelled methane production of a continuous digester was underestimated by 20 % with these parameters.

Organic matter characterization Over the last two decades, several authors also tried to build other static integrative tools based on organic matter characterization but they were mainly applied to municipal solid waste (Buffiere et al. 2006), kitchen, fruits and vegetables wastes (Gunaseelan 2007, 2009). Few studies dealt with municipal sludge although the methodologies used on solid waste can be transposed to sludge. The most recent publications have been presented by Mottet et al. (2010), Appels et al. (2011) and Jimenez et al. (2014).

First, the theoretical BMP obtained from the empirical formula has been calculated since 1930 using the Buswell equation (Neave and Buswell 1930). This stoichiometric equation is based on the elemental composition ($C_nH_aO_b$) where organic matter is reduced to methane and oxidized into carbon dioxide, with the assumption of a total conversion. However, these relationships remain theoretical and they assume that organic matter is fully converted. They did not consider (i) the fraction of substrate used for bacterial growth, (ii) the refractory organic matter (such as lignin) contained in the substrate, (iii) the fraction of the organic matter remains inaccessible due to binding within particles and (iv) the limitation of nutrients (Angelidaki and Sanders 2004). Several authors showed that biodegradability was overestimated using this technique (Shanmugam and Horan 2009, Labatut et al. 2011). Additionally, when applied to municipal solid waste, Davidsson et al. (2007) showed that theoretical methane potential is more realistic when the calculation is based on biochemical composition (lipids, carbohydrates, proteins) rather than on elemental composition analysis.

From Table 1, correlations obtained depend on the nature of different waste molecules. For example, fiber characterization would be more suitable for lignocellulose-like substrates such as green wastes, fruits and vegetables wastes (Buffiere et al. 2006) than for sewage sludge. Indeed, Mottet et al. (2010) applied the Van Soest fractionation (Van Soest 1963) to characterize organic matter from municipal sludge in order to build a biodegradability indicator. The error for the

Table 1 Summary of the different methodologies used in integrative tools found in the litterature

Integrative tools	Characterization methods	Benefits	Drawbacks	References
Static model PLS, correlations Stoechiometric reaction	Biochemical characterization Proteins, carbohydrates, lipids COD/TOC, TOC soluble	Analytical simple and rapid methods	Model validation not yet achieved Based on one type of sludge (secondary) Care to be taken of the accuracy of methods used Not take into account complexity and accessibility	Mottet et al. (2010)
	CHNOS elemental analysis	Fast and practical method	Consideration of the whole organic matter degradation: the biodegradable fraction is not used Over-estimation of BMP tests	Shanmugam and Horan (2009)
	Van Soest and fibers analysis	Faster and practical method Validation on several solids wastes Accessibility taking into account with growing extraction power	Not suitable for sewage sludge in terms of protocol (porosity) Model validation not conclusive	Chandler et al. (1980) Gunaseelan (2007) Mottet et al. (2010)
	Aerobic respiration rate	Faster than a BMP test (4 days instead of 21–30 days) Promising on solid wastes	Only readily substrate taken into account No accessibility taken into account Assumption on the same biodegradability under aerobic and AD	Cossu and Raga (2008) Scaglia et al. (2010)
	Initial rate technique	Faster method than BMP Maximum production rate and affinity constant determined	Extrapolation in continuous digester underestimate methane production Not information on substrate bioaccessibility	Donoso-Bravo et al. (2011) Strömberg et al. (2015)
	Biochemical characterization Bioaccessibility compartiment	Bioaccessibility taken into account Biochemical fractions calculated from practical analysis	Necessity of long batch test for fractions assessment	Yasui et al. (2006, 2008) Mottet et al. (2010)
	NIRS	Biodegradability assessment Fast Various type of substrates	Necessity of drying and freezing the sample Bioaccessibility not tkan into account	Lesteur et al. (2010) Doublet et al. (2013)
	3D fluorescence spectroscopy combined with accessibility characterization	Bioaccessibility taken into account Both biodegradability and bioaccessibility predicted Fast method	Calibrated on sludge-like samples	Jimenez et al. (2014)

validation of the Partial Least Square (PLS) model was about 35 %. Van Soest fractionation targets fibers and carbohydrates (i.e., cellulose, hemicellulose, lignin) but sewage sludge are also composed of proteins, humic acids and lipids (Jimenez et al. 2013). In the second part of their work, the authors found a better correlation between anaerobic biodegradability and the specific biochemical fractions of organic matter, such as proteins, carbohydrates, lipids and the degree of oxidation of organic molecules. Only Gunaseelan (2007, 2009) considered fibers, carbohydrates, lipids and proteins.

Concerning biomolecules characterization, several methods exist and are summarized in the Table 2. Initially conceived to analyze proteins, lipids and carbohydrates in serum samples, colorimetric methods have been applied in environmental engineering to characterize organic fractions. They are now coupled with analytical improvements such as organic matter extraction techniques (Park and Novak 2007; Ras et al. 2008). Table 2 summarizes some of the available methods used to determine the main components of organic matter. Depending on the nature of the substrate (total sludge or EPS solubilized in an extracting agent) the methods are more or less adequate (Jimenez et al. 2013). Recently, several reported works used a more advanced methodology: gas chromatography with mass spectroscopy (GC/MS) was used in order to determine the detailed composition of carbohydrates, proteins and lipids present in the sample. Huang et al. (2010) used this technology for wastewater characterization.

Aerobic tests Indirect correlations between aerobic activity tests and anaerobic tests such as BMP are also

Table 2 Analytical protocols for biochemical compounds determination

Organic fraction	Method type	Concentration (mg/L)	Reagent used	Standard	Reference
Proteins	Colorimetric	0–200	Folin reagent Copper sulfate 0.5 % (w/w)	Bovine albumin serum	Lowry et al. (1951) Frølund et al. (1996)
	Colorimetric	0–200	Bicinchonic acid		Smith et al. (1985)
	Colorimetric	0–100	Gornall biuret reagent and NaCl		Gornall et al. (1949)
	Colorimetric	2–120	Coomassie brilliant blue G-250 reagent		Bradford (1976)
	Standard method for TKN assessement	N content × 6.25 g proteins/gN	Mineralisation and ammonia dosage	None	Kjeldahl (1883)
Humic acids like	Colorimetric	0–200	Folin reagent	Humic acids (Aldrich)	Frølund et al. (1996)
Polysaccharides	Colorimetric	0–100	Phenol 5 % (w/w) Sulfuric acid 95 %	Glucose	Dubois et al. (1956)
	Colorimetric	0–100	Anthrone 0.125 % (w/v) Sulfuric acid 95 %		Dreywood (1946) Raunkjær et al. (1994)
Fibers	Extractions	–	Weende method Van Soest	None	Henneberg and Stohmann, (1860) Van Soest (1963)
Lipids	Colorimetric	0–1000	Vanillin 0.6 % (w/w) Phosphoric acid 85 % Sulfuric acid 95 %	Commercial olive oil	Frings and Dunn (1970)
	Extraction Infrared spectroscopy	–	CCl_4, Uvasol, Al_2O_3, Na_2SO_4, HCL 6M	Cornoil	APHA (2005)
	Extraction Gravimetry		Organic solvent	–	APHA (2005)

often proposed. Aerobic tests are less time consuming than anaerobic tests and they can be easier from a practical point of view (e.g., no need for anaerobic conditions and precautions working in an air environment). Although the respirometric test takes less time than the BMP test, there are some limitations in using it to determine the BMP. First, only the readily biodegradable organic matter is considered (the more complex organic matter, such as cellulose, are degraded more slowly and are not measured in the short-term test) (Lesteur et al. 2010). The second limitation is the assumption that the organic matter in sludge presents the same biodegradability under aerobic and anaerobic conditions (Ekama et al. 2007). Buendía et al. (2008) used long anaerobic and aerobic batch tests in order to estimate readily and slowly biodegradable fractions and found a good correlation between the anaerobic and the aerobic readily biodegradable fraction. However, the slowly biodegradable fraction was underestimated by the aerobic batch testing. In the same way, Park et al. (2008) showed some proteins bound to divalent cations were bioaccessible only under aerobic conditions but were not bioaccessible under anaerobic conditions. Higher volatile solids removal was observed under aerobic conditions (48 %) compared to AD (39 %).

2.2.2.3 Emerging techniques for organic matter characterization Progress in analytical chemistry has led to the development of new instruments and techniques to characterize organic matter. Among them, NIRS and 3D fluorescence spectroscopy are the most promising for instrumentation and biodegradability measurement.

Recently, NIRS is used for BMP assessment following two different approaches. The first approach is to determine the composition of the input material using NIRS and to calculate the BMP value by regression using static models. The second approach to predict the biodegradability uses directly the spectra through a dedicated calibration. Jacobi et al. (2012) used both approaches for the determination of the biogas production from maize, which is commonly used in Germany. The calibration allowed errors for volatile solids of 0.74 % fresh matter and for biogas production of 5.26–11.14 l/kg fresh matter. Application of the technique for off-line prediction of continuously gathered data allowed, together with first order degradation kinetics, the prediction of the biogas production of a full-scale biogas plant over several months. Zhang et al. (2009) succeeded in building PLS models between NIRS results and ethanol, acetate, propionate and butyrate concentrations in a H_2 producing reactor fed on synthetic wastewater. Lignin concentration has also been correlated to NIRS measurement by Brinkmann et al. (2002). However, so far NIRS has not yet found its way into practical implementation at biogas plants. One obstacle seems to be the transfer of calibrations of a given sample set to new samples and the reliability of the predicted values.

Lesteur et al. (2011), Doublet et al. (2013) and Triolo et al. (2011) have successfully developed PLS models for BMP prediction of different waste organic matter BMP values using Near InfraRed Spectroscopy (NIRS). Lesteur et al. (2011) and Doublet et al. (2013) found a direct correlation between the NIRS analysis and the biodegradability provided by the BMP tests for municipal solid waste. The prediction demonstrated good accuracy (standard deviation of 28 $mLCH_4/gVS$ and relative error of 13 % respectively). However, NIRS measurement for biodegradability assessment is still performed on dried-frozen samples and does not consider accessibility of the organic matter.

Another promising technique is the fluorescence spectroscopy. Fluorescence allows the characterization of the analyzed organic material in both liquid and solid phases. The technique gives a topographic map of the organic matter complexity. Identification of molecular-like groups is possible based on the excitation and emission wavelength coordinates (Jimenez et al. 2014). It is indeed a selective and sensitive method since fluorescence characteristics are related to the structure and the functional groups in the molecules. Some studies have revealed the potential of fluorescence spectroscopy to link to the complexity of a substrate and its biodegradability (Tartakovsky et al. 1996; Reynolds and Ahmad 1997) and results on establishing a link between complexity, sludge stabilization degree and accessibility, were encouraging (He et al. 2011; Wan et al. 2012). Recently, Jimenez et al. (2014) proposed a sewage sludge characterization methodology to assess both biodegradability and bioaccessibility needed for modified ADM1 input variables and thus for further optimization of AD plants. These authors combined basic chemical extractions with 3D fluorescence spectroscopy in a

5 days long methodology and predicted successfully both parameters using a PLS regression model. A wide range of biodegradability (0–60 %) and of readily/slowly biodegradable fractions (0–46 %), representing bioaccessibility, were predicted with errors of 6 % for both. However, this technique was specific to sewage sludge, as far as proteins compose the main part of the organic matter in this organic waste.

2.3 Dynamical models and software sensors

As previously presented, static models have been proposed as an alternative solution to predict biodegradability with several kind of organic matter characterization as explicative variables. However, all the static models were not able to predict simultaneously the bioaccessibility and the biodegradability as the digester dynamics.

Dynamical models accounts for evolution in kinetic equation and biomasses. This leads to more complex models generally based on ordinary differential equations representing mass balance within the process. The first dynamical AD digestion models were proposed in the mid-sixties by Andrews and Pearson (1965) and Andrews and Graef (1971). Only a single stage was considered gathering acidogenesis and methanogenesis. A Haldane kinetic equation was proposed to account for acetoclastic methanogenesis inhibition at high concentration of acetate. Mosey (1983) and Hobson (1985) extended the model with hydrogenotrophic methanogenesis. The models were then extended depending on the different substrates (wastewater, sludge or manure). More than 10 years ago, the IWA Task Group on Mathematical Modelling of Anaerobic Digestion Processes proposed the Anaerobic Digestion Model No1 (ADM1), as a consensual modelling of anaerobic digestion (Batstone et al. 2002). The biochemical reactions represented in the model describe: (i) an extracellular disintegration step converting composite particulate matter into carbohydrates, lipids, proteins and inert compounds, (ii) an extracellular enzymatic hydrolysis step that converts the degradation products into their chemical building blocks, i.e., LCFA, monosaccharides and amino acids, (iii) acidogenesis or fermentation into hydrogen, acetate and VFA, (iv) acetogenesis of VFA into acetate and (v) acetoclastic and hydrogenotrophic methanogenesis. The extracellular reactions are assumed to be of first-order, while the intracellular biochemical reactions use Monod-type kinetics for substrate uptake and biomass growth. Variants to the ADM1 model given by Batstone et al. (2002) are available for plant wide modelling (Rosén and Jeppsson 2006; Grau et al. 2007; De Gracia et al. 2009; Barat et al. 2012). Many applications of the ADM1 model have been published for a wide variety of substrates (see e.g., Batstone et al. 2009; Lauwers et al. 2013) and some models account for both the biodegradability and the bioaccessibility of the waste (Mottet et al. 2013; García-Gen et al. 2015). On the other hand, simpler models have been developed, more suitable to support monitoring or control strategies. For example, the model of Bernard et al. (2001a) includes two reactions and turns out to approximate efficiently the ADM1 model (Bernard et al. 2005b) for modeling AD processes treating industrial wastewater.

In many occasions, on-line or off-line measurements are not enough to evaluate and to assess the operating conditions of AD plants but, when combined with dynamical models, these measurements can lead to very useful additional information about non measured variables. This methodology leads to the so-called "software sensors". It is possible to distinguish the approaches based on data sets, those founded on expert knowledge (in the broad sense of the term) and those founded upon an analytical—mathematical—description of the system. In this section, we focus particularly on the estimation for the efficient development and implementation of state estimation schemes. These estimation schemes are called estimators, state observers, software sensors, or simply observers, and they can be used for design or optimization strategies in a wide class of biochemical processes. As underlined, these algorithms are able to estimate both state variables, that are normally not measured, and unknown parameters from the available measurements. In biological processes, observers are mainly useful in on-line estimations for control purposes. The most popular approaches used in the past have been the well-known classical extended Kalman filters (EKF) and extended Luenberger observers (ELO). One of the reasons for the popularity of EKF/ELO is that they are easy to implement since the algorithms can be directly derived from the state space model. However, since these estimators are based on a linearized model of the process, the stability and convergence properties are essentially local; it is difficult to guarantee its stability over a wide operating range. As a matter of fact, very few works deal with the observability of nonlinear biochemical processes (e.g., Gauthier and Kupka 1994) and they are usually concerned with particular process applications. Another problem is that the theory for EKF/ELO is developed assuming a perfect knowledge of the system model and parameters, in particular of the process kinetics, and as a consequence, it is difficult to develop error bounds to take into account the large uncertainty of these parameters.

In order to overcome these drawbacks, several other approaches have been proposed from the early seventies (Misawa and Hedrick 1989; Perrier et al. 2000; Dochain 2003; Alcaraz-González and González-Álvarez 2007). For example, adaptive observers (Bastin and Dochain 1990; Dochain 2003) belong to the class of observers allowing the estimation of both kinetic parameters and states. As in the EKF, the poorly known (or unknown) parameters are considered to be extra states with no dynamics. One of the original features of the adaptive observer is to consider a nominal process model, i.e., a model with nominal values of the poorly known parameters (Chen 1990). The design of nonlinear observers in general has been a very active research area. Most of the nonlinear approaches are placed in the category of ''high gain'' observers (HGO) since they tend to split the dynamics into a linear part and a nonlinear part and to choose the gain of the observer so that the linear part dominates the nonlinear one (Gauthier et al. 1992; Gauthier and Kupka 1994; Dochain 2003).

Several linearization methods also have been proposed (Baumann and Rugh 1986). Nevertheless, like EFK/ELO, only local behavior can be guaranteed as they miss practical results on performance and stability. Other approaches are sliding observers based on the theory of variable structure systems (Xiong and Mehrdad 2003) but their design involve conditions that must be assumed a priori or that are usually hard to verify (Misawa and Hedrick 1989). All these approaches solve some of the problems described above but in most of the cases, the complexity of the resulting estimating algorithms is a limitation for real time computation. Indeed, monitoring algorithms can prove to be efficient if they are able to incorporate the important well-known information on the process while being able to deal with the missing information in a robust way. They include the lack of on-line measurements and the uncertainty on the process dynamics.

Two relatively new robust nonlinear observers have found a wide acceptation in biological process, including of course anaerobic digestion. Such robust observers are capable of coping simultaneously with the aforementioned problems while remaining easy to implement with a minimum number of straightforward conditions to verify. The first one, the asymptotic observer (Bastin and Dochain 1990; Alcaraz-González and González-Álvarez 2007), although requiring the knowledge of the process inputs, has the main advantage that it permits the exact cancellation of the nonlinear terms of the systems, and so facilitates its design, stability analysis and implementation. The second one, the interval observer, allows for the reconstruction of a guaranteed interval on the unmeasured states instead of reconstructing their precise numerical values assuming that only guaranteed lower and upper limits on the process inputs and model parameters are available (Gouzé et al. 2000; Alcaraz-González et al. 2005a; Rapaport and Dochain 2005; Moisan et al. 2009).

The main disadvantage of the aforementioned asymptotic observer is that the process operational conditions (mainly the hydraulic retention time) establish its convergence properties and it is not possible to modify the convergence rate by choosing a gain like in the classical observers or the HGO. However, adapting the design features of the HGO and adaptive observers, a Tunable Asymptotic Observer (TAO) has been proposed for AD processes (Bernard and Gouzé 2004, Alcaraz-González et al. 2005b). Furthermore, in a more diverse sense, super-twisting observers have also been demonstrated recently to be very useful in achieving a very fast convergence without loss of robustness, (Sbarciog et al. 2012).

Concerning the drawback of influent uncertainty—very common in AD plants—, the general problem of simultaneous estimation of unmeasured state variables and inputs for nonlinear systems has been addressed from a number of different robust approaches. With respect to AD processes, Theilliol et al. (2003) proposed a simultaneous input-and-state concentrations observer that required the full knowledge of the process kinetics. Also, Aceves-Lara et al. (2010) simultaneously estimated state space variables and the input concentrations in a biohydrogen production process in which input and state estimations were performed using a state transformation and an asymptotic observer. More recently, Jáuregui-Medina et al.

(2009) proposed an observer-based estimator, named the "Virtually Controlled Observer" (VCO) because one of the observer's inputs (the hypothetical—unmeasured—influent substrate concentration) is updated by a feedback control that regulates the estimation error of a measured output. In a fixed bed configuration, several of these approaches have also been applied to distributed parameter systems (see e.g., Delattre et al. 2004; Aguilar-Garnica et al. 2009).

3 Control of anaerobic digestion processes

Because of the inherent complexity and necessity for safety in biotechnological processes, efficient monitoring and decision support systems are required in order to optimize their operation. Indeed, even in normal operational conditions, several types of disturbances may occur with serious consequences in the performance of the process. Fluctuations in the influent to be treated is an illustration and a typical example would be an integrated dairy producing 100 different products that, over the course of a week, result in a wastewater stream with flow/total COD/TSS/FOG/temperature variations of 20×/10×/5×/3×/1.5×, some of these changes taking place in a matter of hours. Hence, the last two decades have seen an increasing interest to improve the operation of AD processes by applying advanced control schemes. Optimized and stable performances are indeed required to be guaranteed consistently and this has major consequences for instrumentation, control and automation (Huntington 1998; Olsson and Newell 1998). Two main factors (which can be interpreted as both, incentives and constraints) have contributed to this new paradigm: (1) the need for optimally controlled plants due to environmental regulatory norms and (2) the need to reduce cost. In order to fulfill these requirements, the optimal control of AD processes faces important uncertainties arising from the intrinsic complexity of plant design. Among others, the main disturbances that can be observed are the following: acidification, inhibition and toxicant exposure (McCartney and Oleszkiewicz 1991, 1993; O'Flaherty et al. 1998; Hao 2003; Appels et al. 2008; Chen et al. 2008; Cirne et al. 2008), overload (Waewsak et al. 2010; Wijekoon et al. 2011), alkalinity, variability of inputs, water content and rheology, foaming, stirring and mixing problems (McMahon 2001; Dalmau et al. 2010) and lack of macro- and micro-nutrients (Speece 2008).

By far, the most developed control laws in the literature use the dilution rate as manipulated variable (see Fig. 1) but it is mainly in simulation and only few full-scale applications are available. Manipulating the dilution rate is indeed difficult in practice and AD processes are facing the problem of the lack of actuators. Examples for other manipulated variables

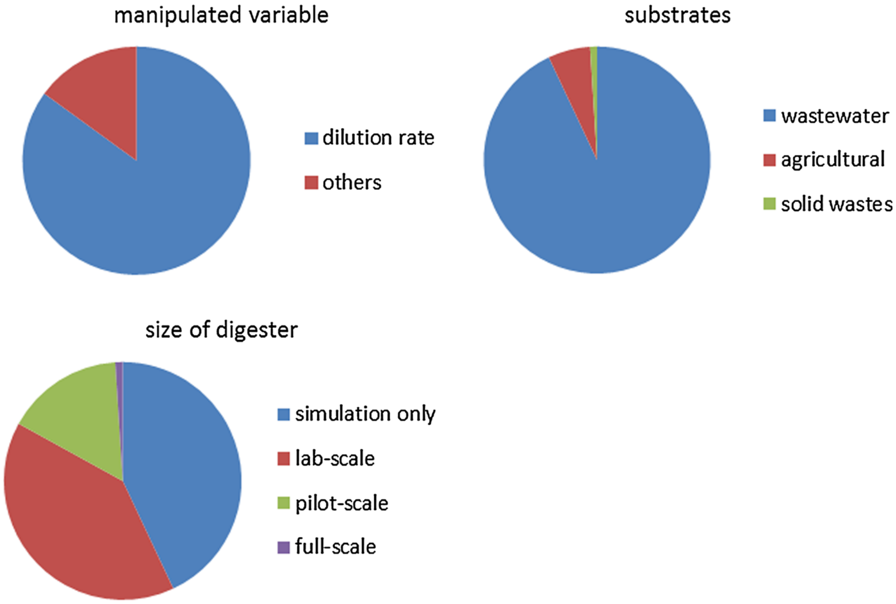

Fig. 1 Percentage distribution of manipulated variable (121 publications), size of digester (134 publications) and substrates (109 publications) of the reviewed publications

are liquid recirculation rates and the addition of bases to stabilize the process. In case of a co-digestion plant, only one substrate or a constant substrate mix is usually controlled using the dilution rate as manipulated variable. The other substrates then must be calculated based on boundary conditions such as hydraulic retention time, organic loading rate or restrictions defined by funding schemes (Zhou et al. 2012).

Whilst experimentation is required for the tuning of regulators, either on the plant itself or within a simulation environment, design techniques have been developed that allow devising the optimal controller for a particular process model and performance index. Certain constraints imposed on the control action, such as a minimization of the control effort, can be accommodated during the design.

3.1 Classical control in AD

PID and on/off controllers belong to classical control methods. Table ESM.1 and ESM.2 in Online Resource 1 illustrates some examples of application of these control methodologies in AD.

The first application of on/off control in AD was reported in the 70 s (see Table ESM.1 in Online Resource 1), which aimed at setting the manipulated variable to a binary value depending on predefined threshold values. They were followed by PID controls including P, PI, and PID controls. For instance, Marsili-Libelli and Beni (1996) applied PID control for stabilising alkalinity and pH by manipulating the addition of bicarbonate. On the other hand, von Sachs et al. (2003) proposed a PI structure for controlling biogas flow rate by modifying the dilution rate in a two-phase AD system.

PID cascade controls (see Table ESM.2 in Online Resource 1) are a simple but effective approach for feed control. Their advantages are that two possibly conflictive set-points can be simultaneously controlled whilst the set-point of the master loop can be set by an expert system. Approaches such as Liu et al. (2004a, b), Alferes et al. (2008), and Alferes and Irizar (2010) are dedicated to control biogas production at a given set-point or to operate the digester at high organic load. Therefore, these approaches try to maximize the economical benefit of the digester, whereas the set-point is established in order to avoid digester overloads.

As regards adaptive control, Zhou et al. (2012), for instance, proposed a PID aimed at controlling the methane flow rate based on measurements of VFA and VFA/TA.

Another control strategy lies on minimizing the COD or VFA content in the effluent (see e.g., Alvarez-Ramirez et al. 2002; Mu et al. 2007). The key goal of control strategies of this type is to stabilize digester performance whilst maximizing COD degradation. On the other hand, García-Diéguez et al. (2011) proposed an approach capable to maximize methane flow rate whilst tracking a set-point for effluent VFAs.

3.2 Advanced control in AD

Since classical PID controllers are usually limited to single–input–single–output control loops and to linear, simple cases, different advanced control approaches have been theoretically analyzed and experimentally validated in order to control AD processes.

3.2.1 Expert systems

Expert systems can be classified in rule-based and fuzzy systems (Tables ESM.3 and ESM.4 in Online Resource 1) and systems extended with a surrogate model such as an artificial neural network or special fuzzy systems (Table ESM.5 in Online Resource 1).

Applying nonlinear control methods comes quite natural since biogas plants are nonlinear processes. Such expert systems are quite popular for AD control because of: (1) their intuitive design based on rules, and (2) their non-linearity coping with the non-linearity of the plant. The first approach is performed by rule-based systems such as the well-known fuzzy control, whilst the latter one is performed by the use of neural networks. Furthermore, expert systems can easily incorporate all measured variables and are easily extensible if an additional process value is measured in the future.

Fuzzy logic is a problem-solving tool that can achieve a definite conclusion from imprecise information, allowing intermediate values rather than simple yes/no evaluations (García-Gen 2015). The main benefit of this approach is that it can be used to control non-linear systems. A fuzzy-logic controller (Zadeh 1965) is indeed capable of optimizing different kinds of processes under dynamic operating and loading conditions by applying valuable expert knowledge (Verbruggen and Bruijn 1997). Moreover, fuzzy-logic control does not require a large amount of data and/or a rigorous mathematical model, and allows for the development of multiple-input-multiple-output control schemes. Hence, fuzzy logic is a powerful tool for AD control (Olsson et al. 2005).

Different examples of rule-based and fuzzy-logic-based systems for AD control can be found in literature (see Tables ESM.3 and ESM.4). For instance, Pullammanappallil et al. (1991, 1998) developed an expert system aimed to control methane production by switching between different control strategies (set-point control, constant yield control, batch operation and constant dilution rate) based on a *t* test. Puñal et al. (2003) proposed a PI-based fuzzy logic system for monitoring the effluent VFA concentration in anaerobic wastewater treatment plants, using the dilution rate as manipulated variable. Murnleitner et al. (2002) and Grepmeier (2002) proposed expert systems based on fuzzy theory for overload avoidance in AD process. Different inputs were used for such purpose: H_2 concentration, CH_4 concentration, biogas flow rate, pH, and filling level of the buffer tank.

Table ESM.5 in Online Resource 1 summarises different examples of expert systems for AD control consisting of neural networks and special fuzzy systems. For instance, Steyer et al. (1997) proposed a hierarchical fuzzy control for VFA concentration which used the control error of pH, temperature and biogas flow rate as input variables. Holubar et al. (2002, 2003) used a neural network to maximize methane production and COD degradation by modifying OLR on the basis of different inputs: pH, VFA, and biogas production and composition. Carlos-Hernandez et al. (2007) developed a fuzzy supervisory controller to optimise process performance by regulating the addition of base and the dilution rate; whilst this control system was later modified (Carlos-Hernandez et al. 2010) following a neural fuzzy structure for estimating methanogenic biomass performance.

3.2.2 *Model-based and linearizing control*

Linearizing approach is popular for feed control purposes in AD (see Table ESM.6 in Online Resource 1). Moreover, much effort has been applied to develop new model-based control laws that will achieve suitable process performances (Méndez-Acosta et al. 2010). In this context, simple models like AM2 (Bernard et al. 2001b) are preferred to more complex ones like ADM1 (Batstone et al. 2002).

Linearizing control is based on a non-linear controller, which is precisely designed to achieve linear closed-loop dynamics (Isidori 1989; Ignatova et al. 2008). The main aim of linearizing control is to take advantage of available mathematical models. They allow controlling very efficiently the functioning of a plant and may allow the achievement of finer actions than those controllers that decide only upon the difference between measurements and set points (Olsson et al. 2005). Linearizing controllers are designed by a two-step procedure (Kurtz and Henson 1997). First, a non-linear process model is used in order to synthesize the non-linear state feed-back controller that linearizes the map between a "new" manipulated input and the controlled output. In the second step, a linear pole placement controller is designed for the feed-back linearized system. However, due to the strongly non-linear relationships existing between both inlet and outlet of an anaerobic process, linearizing controllers only attain proper results when the process dynamics are bounded by a defined linear zone (Simeonov and Queinnec 2006).

Applications of adaptive linearizing control have been presented for anaerobic digestion (Renard et al. 1988). However, an important problem with adaptive control systems is the necessity for on-line identification of the process model while the plant is in closed-loop operation. An approach to deal with the identification problem consists of considering that the process model belongs to a bounded class of possible models with fixed parameters. The identification is then reduced to the choice of the correct model, or, as in the Model Weighting Adaptive Control (MWAC) approach (Gendron et al. 1993), by weighting the different models into a composite process model.

Another method in this category is the interval-based approach. Concerning Interval Observers, a recent control approach that uses the partial information provided by this kind of observers has been designed to exponentially stabilize a regulated variable in a neighborhood of a predetermined set-point (Rapaport and Harmand 2002). As for observers, these approaches have been also applied to distributed parameter systems applied to fixed-bed bioreactors (e.g., Dochain et al. 1997; Babary et al. 1999; Antoniades and Christofides 2001; Aguilar-Garnica et al. 2009).

Some other recent approaches for control of this kind of processes have been derived from the theory based on differential geometry (Isidori 1989; Henson and Seborg 1997). Control approaches based on differential geometry allow for the transformation of a nonlinear system into a partially or totally linear one, by means of a nonlinear state transformation, which is obtained from directional derivatives of the output. It is important to remark that geometric control differs totally from the linear approximation of dynamics by calculation of the Jacobian. Either state-space (Hunt and Su 1983) or input–output linearization (Méndez-Acosta et al. 2004, 2005, 2008) have been employed.

More recently, sliding mode approaches have been also used mainly to control Anaerobic Sequential Batch Reactors (ASBR), (Vargas et al. 2008), as well as in continuous bioreactors (Lara-Cisneros et al. 2015). In general, the sliding mode approaches are widely used due to robustness with respect to uncertainties.

3.2.3 Other advanced controllers

Table ESM.7 in Online Resource 1 summarizes other advanced control approaches, including, for instance, disturbance monitoring, non-linear, adaptive, and robust control.

A nonlinear adaptive control law for bioreactors which is robust in the face of unknown kinetics has been proposed recently for the global stabilization of bioreactors and then applied to the regulation of anaerobic digestion processes (Mailleret et al. 2004). Similar to linearizing control, different interval-based approaches have been used to exponentially stabilize a regulated variable in a neighborhood of a predetermined set-point (Alcaraz-González et al. 2005a).

On the other hand, most of the controllers reviewed before were developed to regulate known set-points or to track well defined trajectories. However, in AD operation, the control objective could be to optimize a criterion that is dependent of unknown parameters in order to keep a performance criterion at its optimal value. Also, it is well known that the explicit form of the performance function in AD processes is highly uncertain (e.g., the growth rate of methanogenesis or growth rate of acidogenesis) (Lara-Cisneros et al. 2015). Extremum-Seeking-Control (ESC) and probing control are two techniques to handle these kinds of dynamic optimization problems (Dochain et al. 2011; Guay et al. 2004; Liu et al. 2006; Marcos et al. 2004a, b; Steyer et al. 1999). The goals of ESC schemes and probing control is to find the operating setpoints, a priori unknown, such that a performance function reaches its extremum value. Steyer et al. (1999) developed a probing control approach based on the analysis of disturbances added on purpose to the influent flow rate. By increasing the influent flow rate for a short period of time, the increased biogas yield was compared to the expected one. Overloading or inhibition could be interpreted as a negative effect of the disturbance (i.e., an unsatisfactory gas yield). Liu et al. (2006) developed a cascade controller system that is embedded into a rule-based supervisory system based on ESC. This controller was applied to intensify biogas production in an anaerobic up-flow fixed bed reactor at laboratory scale and achieved good performance, especially during the early startup and during rejection of disturbances. In particular, the process was operated at maximum productivity and had safety margins adequate to ensure reliable operation, react fast on disturbances and avoid unstable process conditions. Lara-Cisneros et al. (2015) proposed an ESC scheme with sliding mode to achieve the dynamic optimization of methane outflow rate in anaerobic processes. The control law was designed to regulate VFA concentration at the optimal value whilst maximizing methane production. However, only numerical experiments illustrated the performance and robustness of the proposed control approach.

Concerning the need of sensors for control purposes, even if there now exists a large variety of devices for measuring almost all key variables, they still remain relatively expensive for medium and small enterprises, mainly in developing countries. In this sense, the challenge to control AD processes is to do it with a minimum of information, even if it is obtained off-line. In this context, discrete control approaches are beginning to be used (Méndez-Acosta et al. 2011).

3.3 Control in anaerobic co-digestion (AcoD)

Anaerobic co-digestion (AcoD) presents higher potential energy recovery than conventional single substrate AD. Therefore, high effort has been focussed on AcoD in order to: (1) enhance process performance thus maximising biogas production; (2) navigate into the use of new co-substrates; and (3) increase process feasibility by the application of digestates for agricultural purposes (Mata-Alvarez et al. 2014).

For instance, biogas production has been classically improved by co-digesting manure and organic waste (see, for instance, Ahring et al. 1992; Tafdrup 1994). Since manures are often associated with poor methane yields, AcoD of manure with other organic wastes has been identified as a cost-effective alternative for improving process efficiency (Mata-Alvarez et al. 2011; Frigon et al. 2012; Astals et al. 2013b). This co-digestion process is usually optimised when biogas yield is above 30 m^3 biogas per m^3 biomass treated, which normally requires a 25 % organic waste ratio (Boe 2006). Nevertheless, lower ratios may be enough when treating concentrated wastes (Gregersen 2003).

Other classical AcoD process is the co-digestion of sewage sludge with the organic fraction of municipal solid waste (OFMSW). Besides the biowaste composition (food waste, market waste, etc.), biogas production during the co-digestion of sewage sludge and biowaste highly depends on several factors such as sewage sludge composition (primary, secondary or mixed), OLR, reactor configuration, operating temperature or mixing conditions (Mata-Alvarez et al. 2011). For instance, Silvestre et al. (2015) assessed the effect of OFMSW loading rate and particulate size on sewage sludge mesophilic anaerobic co-digestion in a CSTR operating at 20 days of SRT. This study revealed that sewage-sludge–OFMSW mixture composed by 54 % of inlet volatile solids (OLR of 3.1 kg COD m^{-3} day^{-1}; 1.9 kg VS m^{-3} day^{-1}) resulted in an increased in volumetric methane production and methane yield of up to 200 and 59 %, respectively.

Recent literature has reported increasing interest by the scientific community on the applicability of AcoD to new biowastes. For instance, co-digesting sewage sludge and microalgae is considered one promising technology for energy production, whilst representing a key step for recycling nutrients for algal cultivation (Ward et al. 2014). Recent research has shown that AcoD can increase anaerobic degradability of algae by improving substrate composition. Nevertheless, further research is needed since the quantity and quality of the produced biogas vary considerably depending on anaerobic inocula, waste composition and operating conditions (Ajeej et al. 2015).

The control of AcoD processes can be addressed following the same strategies used for classical AD processes. However, it is crucial to characterise comprehensively the co-substrates and to choose adequately the blend of substrates to be treated (García-Gen 2015).

Alvarez et al. (2010) developed a methodology for optimising feed composition in AcoD of agro-industrial wastes. This optimisation protocol was based on a linear programming method aimed to set up different blends for maximising the total substrate biodegradation potential (L_{CH4} kg^{-1} substrate) or the biokinetic potential (L_{CH4} kg^{-1} substrate day^{-1}). To this aim, the controller defined restrictions on several characteristics of the mixture, such as NH_4^+, lipids or C/N ratio. The methodology was validated using three types of agro-industrial biowaste: pig manure, fish waste and biodiesel waste. Validation results were related to the mixture of biowaste to be feed to the AcoD process in order to maximise biodegradation potential and methane production. Linear programming was proved to be a powerful, useful and easy-to-use tool to estimate methane production in co-digestion units where different substrates can be fed (Alvarez et al. 2010).

Wang et al. (2012) proposed optimizing the feeding composition and the carbon/nitrogen (C/N) ratio for improving methane yield during AcoD of multi-component substrates (dairy, chicken manure and wheat straw). The results showed that co-digestion performed better than individual digestion in terms of methane potential. Maximum methane productions were achieved with a dairy/chicken manure ratio of 40:60 and a C/N ratio of 27:1 (after optimization using response surface methodology). The results suggested therefore that better performance of AcoD can be fulfilled by optimizing feeding composition and C/N ratio.

Wang et al. (2013a) evaluated two statistical methods for optimizing feeding composition in AcoD systems. To this aim, a simplex-centroid mixture design (SCMD) and central composite design (CCD) were evaluated using methane potential as response variable. Each co-substrate (dairy manure, chicken manure, swine manure and rice straw) served as an independent variable in SCMD and CCD, involving two factors: the manure and C/N ratios together with the C/N ratio of the blend. Experiments demonstrated that co-digestion of three-component substrates resulted in higher methane potentials, as well as on better fitted models to predict the response based on selected variables. In response surface plots, SCMD showed the interactions among each component in the co-substrates and CCD presented the interaction between the ratio of manures and the C/N ratio.

SCMD and CCD were both suitable methods for optimizing feeding composition during anaerobic co-digestion.

Jiménez et al. (2014) optimised methanogenic activity using the response surface methodology during the AcoD of agriculture and industrial wastes. This optimisation accounted for microbial community performance, taking into account the effect of each substrate concentration and their interactive effects on specific methanogenic activity and microbial community diversity. The results showed a significant interaction among the substrates and an enhancement of the methane production and specific methanogenic activity. The optimization allowed identifying substrate interaction effects in a concentration range with a reduced number of experiments. The model validation proved to be useful for defining optimal combination of wastes in AcoD systems.

García-Gen et al. (2015) proposed a control strategy for optimising AcoD in terms of methane productivity, digestate quality and process stability. To this aim, a linear programming approach was adopted to calculate the feeding of multiple substrates for maximum methane productivity, taking into account restrictions based on experimental and heuristic knowledge. Alkalinity ratio measurements against reference values were used for quantitatively assessing process stability by using an empirical diagnosis function. A second empirical diagnosis function was defined to compare methane flow rate measurements against a reference value of maximum capacity. The quantitative change applied to the most active constraint of the substrate blend optimisation problem (leading to a new set-point of feeding substrates blend) was calculated by a variable-gain control function derived from the previously commented diagnosis functions. This closed-loop control architecture was successfully validated in a 1 m^3 hybrid Upflow Anaerobic Sludge Blanket-Anaerobic Filter (UASB-AF) reactor, treating blends of substrates (gelatine, glycerine and pig manure supernatant) at OLR values between 0.71 and 6.33 gCOD L^{-1} day^{-1}. The proposed controller was capable to increase methane productivity whilst recovering the system from transient acidifications.

3.4 Sulphide control

Different control strategies can be applied to minimize problems related to sulphide in the system (Cirne et al. 2008). The monitoring of sulphate in the influent cannot be considered as a realistic option since sulphate concentration in the influent cannot be predicted nor monitored. Final removal of sulphide (e.g., desulphuration of biogas) is based on the application of different physico-chemical or biological techniques sometimes requiring additional treatment units:

- Selective inhibition of SRB using compounds such as nitrite, antibiotics, or molybdate. However, these actions are not very effective when operating continuous AD processes and they also present a negative effect on MA.
- pH increase in order to move the H_2S/HS^- equilibrium towards less toxic HS^-.
- Sulphur precipitation using organic or inorganic compounds (mainly iron salts). The main drawbacks of this technique are the reagent cost, the increase in sludge production and possible pipes obstructions from precipitates.
- H_2S stripping by high stirring in the reactor, recycling the produced biogas after scrubber or other H_2S removal technologies.
- Oxidation of sulphide with oxygen or nitrate using chemical or biological processes. This process consists of introducing small amounts of these compounds without affecting process performance (van der Zee et al. 2007; Cirne et al. 2008; Fdz-Polanco et al. 2009a, b).

3.5 Control of anaerobic membrane bioreactors (AnMBR)

Several operating strategies to control membrane fouling in anaerobic or aerobic membrane reactors have been experimentally validated. For example, Jeison and van Lier (2006) developed an on-line cake-layer management protocol that monitored critical flux constantly and prevented excessive cake-layer from building up on the membrane surface; Smith et al. (2006) developed a control system to optimize back-flushing which reduced the water needed for back-flushing by up to 40 %; Vargas et al. (2008) established a control algorithm for fouling prevention which regulated back-flushing and Park et al. (2010) studied how membrane fouling could be reduced by successively increasing and decreasing membrane gas sparging intensities, and recorded the effectiveness in reducing membrane fouling.

Anaerobic membrane bioreactors (AnMBR) can be very efficiently used to treat urban wastewater but they require more sophisticated process control systems than for aerobic MBR systems or other conventional anaerobic systems—such as up-flow anaerobic sludge blanket (UASB); expanded granular sludge blanket (EGSB); or anaerobic filters (AF). For example, Robles et al. (2014) implemented a model-based supervisory controller to optimize filtration in an AnMBR demonstration plant. Energy savings of up to 25 % were achieved when using gas sparging to scour membranes and the downtime for physical cleaning was about 2.4 % of operating time. The operating cost of the AnMBR system after implementing the proposed supervisory controller was about €0.045/m^3, 53.3 % of which were energy costs. In another application, Robles et al. (2013, 2015) obtained similar results using a 2-layer control system measuring the treatment flow rate (controlling the HRT), the sludge wasting volume (controlling the SRT), the temperature, and the gas sparging intensity in the anaerobic reactor and controlling the permeate flow rate, the trans-membrane pressure (TMP), the sludge flow-rate recycled through the membrane tanks, and the gas sparging intensity in the membrane tanks.

4 What is next?

Many ideas and many perspectives arise from all the above details about current scientific and technical achievements.

4.1 Instrumentation

With respect to instrumentation, it is indeed believed that (1) more and more advanced sensors will be soon available (2) confidence index associated to the measurements will provide human operators with the ability to decide on the best actions based on the quality of the measurements (3) sensors network will allow the human operator to anticipate future problems, (4) software sensors and (5) use of large data base and all of this will improve by far the information content currently retrieved from AD plants. The simultaneous use of a sensor network (Steyer et al. 2004) and of numerical models will clearly help in extending and qualifying the available measurements.

As pointed out earlier in the paper, the analysis of individual VFA species has often been proposed as an important measurement parameter for the diagnosis, optimisation and control of anaerobic processes. Most of this information is today collected off-line and are mainly based on either GC or HPLC analysis and have been benchmarked comprehensively in Raposo et al. (2013). As off-line monitoring of VFAs is likely to have a significant lag in measuring VFA and inputting the data into a feedback control loop would have significant draw backs due to the time delay in analysis and inputting the data. There is a significant challenge to overcome in producing an instrument for on-line or at-line species specific VFA analysis that is relatively easy to operate at low capital and operational cost. There has been a significant amount of activity directed automating off-line techniques in particular GC headspace techniques (Boe et al. 2007; Boe et al. 2008) but there has been limited uptake for this method beyond the initial publications. An alternative approach has been the use of Near Infra Red Spectroscopy (NIRS) for acetate, propionate and TVFA analysis but the NIR analyser despite requiring relatively little maintenance was found to have a too high error of prediction for accurate quantification (Ward et al. 2011). An alternative approach to the traditional analytical techniques of GC, HPLC or IR spectroscopy may be to use biosensors as the measurement system. This offers the potential of a relatively low cost sensor system, with high specificity and sensitivity and no requirement for continuous supply of a chemical or gaseous mobile phase as required by GC or HPLC techniques. An approach based on microbial fuel cells (Kaur et al. 2013, 2014) and genetically engineered light emitting bacteria (Li and Yu 2015) have been proposed as possible solutions to develop a more effective on-line VFA instrument. A microbial fuel cell based biosensor was able to discriminate between acetate, propionate and butyrate, with a response time of 1–2 min with a sensitivity of 5 mg L^{-1} when cyclic voltammetry analysis was utilised (Kaur et al. 2013). The sensor linearity was limited to 5–40 mg L^{-1} but this could be addressed with appropriate sample dilution. An alternate biosensor approach using a genetically engineered *E. coli* based biosensor with light emitting response to propionate has been demonstrated with a linear response of 1–10 mM (Li and Yu 2015), however other VFAs such as acetate and butyrate are important species in anaerobic digestion require measurement. Despite a number of innovative approaches taken to measuring individual VFA species, an effective and low cost instrument for the on-line or at line measurement has yet to be identified.

In the first section dedicated to the instrumentation, the lack of "sensors" for monitoring biodegradability and bioaccessibility has been highlighted. As pointed out by the substrate evolution, agricultural and municipal solid wastes are more and more used. This kind of complex substrates need long HRT and the off-line option can be acceptable in order to drive their digestion or co-digestion.

Despite the fact that several tools are promising like NIRS for biodegradability prediction, this technique is, until now, applied on dried-frozen samples and the impact of drying samples on the BMP values obtain with not prepared sample has not been studied. As previously mentioned, NIRS technology has a great potential as sensor, and work has to be followed to develop a probe able to predict BMP value on raw samples. However, this technique does not give bioaccessibility or biodegradation rate parameters. In the case of co-digestion for example, these parameters are crucial. Other study does it in a faster way than BMP test (for example, Jimenez et al. 2014) but it needs advanced knowledge of the methodology used and advanced and expensive material (i.e., 3D fluorescence spectroscopy). Therefore, more efforts have to be done on how to transpose these promising but complex techniques into a cheap and practical "sensors". For example, research on multi-excitation wavelength fluorescence probes would be done, and, associated with an optimized chemical extraction protocol would be able to predict both biodegradability and bioaccessibility. These kinds of information would be very valuable in order to predict the optimal mixture to do during co-digestion for example.

As previously mentioned, spectroscopic on-line sensors are of particular interest to the AD industry and research as they allow the on-line monitoring of crucial process variables. Nevertheless, high prices and complex calibration routines hinder commercial success. Newly developed tunable Micro-Electronic-Mechanical-System (MEMS) based Fabry-Pérot interferometers for the UV/vis, NIR and MIR wavelength ranges provide a very promising solution. Not only are these spectrometers on a chip very small 5 × 10 cm but also relatively cheap, if manufactured in big numbers. Currently, two different system designs exist. Neumann et al. (2010) introduced a tunable MEMS interferometer for the middle- and long-infrared range using a pyro-detector. The different wavelengths can be generated by two bragg reflectors whose distance can be changed by a spring suspension. Although, the presented performance results are good, the spring suspension is considered to be a weakness as it makes the spectrometer sensitive to vibration and wear. Therefore, the Technical Research Centre of Finland (VTT) developed an interferometer design with piezo-effect based tuning of the gap between the reflectors (Antila et al. 2014; Mäkynen et al. 2014). In general, these MEMS systems allow for completely new probe designs where the spectrometer is directly integrated into the probe so that the fibre length can be reduced significantly, increasing the S/N ratio. Thus, not only the sensitivity of a sensor is increased but also the size of the whole sensor system is reduced. This particularly important for MIR sensors where a short fibre length is crucial to guarantee a high S/N ratio. Malinen et al. (2014) gives a broad overview of the possibilities in various applications. In high quantities, prices for MEMS spectrometers are expected to drop to 70-100€ per piece, which makes spectroscopic sensors attractive for the use in AD plants.

Confidence indexes are information about the way measurements are obtained. One important lesson from applying ICA in AD plants is that some sensor technologies are more useful than other ones. Indeed, if all on-line sensors provide numerical values of the measured variables, some (e.g., spectrometer or titrimeter) also provide information on how the measurements have been obtained (Steyer et al. 2006). This information can then be used as a confidence index on the measurement and is of great help to decide – in a closed loop context—if a control law can rely or not on the obtained measurements. In order to guarantee a safe operation of the plant, the controller can indeed be turned off in case of sensor fouling or any other dysfunctionning in the instrument.

However, this increase in complexity in the management of the sensor data and the automation of the process may involve dedicated highly qualified operators for permanently recalibrating and adapting the complex implemented algorithms. Indeed, most of the monitoring, diagnosis or control advanced strategies which are described on Table ESM (cf. attachment to the manuscript) have been tested (when they have been experimented) on short time periods (generally less than a few weeks), with a precalibrated set of parameters and initial conditions. These additional degrees of freedom, which are rarely clearly stated, must be managed on the long term for operational perspectives. Better accounting for such degrees of freedom, automating these aspects to reach robust autoadaptive algorithms, or allowing a remote expert to manage them (Bernard et al. 2005a, b) is thus a challenge for the future years.

4.2 Models and virtual sensors

Even if modeling AD has been an active research topic these last two decades, improving the models supporting monitoring and control strategies is also very challenging. Due to the increasing complexity of the substrates, hydrolysis was considered as the limiting step introducing the notion of bioaccessibility. Based on the degradation kinetics of the sludge, new variables appeared by taking into account the bioaccessibility of the substrate. A better knowledge of the sludge composition indeed leads to more realistic although more complex models. However, despite the techniques described the literature until now, input variables of ADM1 are still difficult to characterize. Advanced analytical techniques could provide a higher degree of information on the composition of any given substrate. Promising new tools can be used for direct measurement, such as NIRS, 3D-EEM SPF and LIF probes in order to describe the biodegradability of a waste. However, with the biodegradability, the bioaccessibility is a key concept of the model input variables characterization. Some studies proposed bioaccessibility assessment specific to sewage sludge. Further investigations need to be performed in order to find a relevant and rapid tool for organic matter characterization of more solid wastes in order to obtain reliable parameters for the biological processes models. ADM1 is sensitive to the substrate composition, and a methodology providing characterization rules based on substrate type using either upstream knowledge, chemical analysis (for simple substrates), or biochemical testing would greatly improve the predictability potential of the models (Batstone 2013), and then their further efficiency in monitoring and control strategies.

A more accurate description of the physicochemical models, and especially of the precipitation related to calcium and phosphorus (Batstone et al. 2012) is a difficult yet necessary step to better under the cycle of phosphorous. Even if it may strongly increase the model complexity, considering sulfur reduction and oxidation processes are also challenges for the future. Also, the spatial distribution of the chemicals and biomasses within the reactor should now be accounted for and integrated in the models. These points should be seen in a larger context than AD, and a plant wide approach (see e.g., Olsson et al. 2014) must prevail. For example, physicochemical models must describe phosphate speciation and release under aerobic and anaerobic conditions, while micropollutants must be tracked along the full treatment plant.

Soft sensing or virtual sensing is the use of models to predict process parameters that are expensive or difficult to measure from more accessible process measurements. They are an effective method of providing in-line estimates of quantities that are difficult to measure on-line, and as such offer the possibility of providing enhanced monitoring of processes, both in terms of providing additional process information and acting as a reference for sensor fault detection. They have previously been demonstrated for estimating parameters such alkalinity, chemical oxygen demand, inorganic carbon, and volatile fatty acids in waste water treatment plants (Bernard 2011) and more recently for total alkalinity in biogas plants (Ward et al. 2011). However, development and updating of soft sensor models requires expert knowledge due to the complex modeling techniques required and the need for tailored training data, putting them beyond the research of most small scale biogas plant operators. Newly developed powerful Machine Learning methods facilitate soft sensor development because of their ability to learn vastly complex and nonlinear relationships (Gaida et al. 2012). Further research in this area is necessary to tap the full potential of the existing methods with regard to AD processes.

4.3 Control

With respect to both observer and control design, one may also expect the development of high power computation capacity will fundamentally change our way of thinking. Modern control techniques usually necessitate the use of a limited order model to be able to guarantee stability and performance robustness. Techniques based on particulate filters [Cf. for instance Goffaux and Van de Wouwer (2005) and Benyahia et al. (2012) for applications to chemostat models] coupled with the use of nonlinear optimal controllers present the advantage of being able to use complex model while dealing with uncertainty. Of course, the price to pay is a relatively less degree of guarantee but the higher the on-line computer capabilities, the higher the state space to be investigated and the lower the probability to push the process towards a dangerous functioning zone. Another promising route concerns the use of innovative passive control approaches in which control objectives are considered at the initial conception step of the process. In terms of performances, it is for instance well known that series of reactors perform better than single processes. However, this design may penalize both investment costs and the stability of the process since reducing the size of the first reactor. The introduction of alternative configurations of the different reactors and the judicious choice for their respective volumes may lead to a more robust global system with respect to specific uncertainty and disturbances (if compared to a single tank reactor), cf. for instance the work by Rapaport et al. (2014) on the stabilization of chemostats with substrate-inhibited kinetics.

It is usually expected that a controller using a more complex model would lead to better performances. Assuming the on-line computation capability is available, it may be true. But the use of very simple models from which a control may "really" be optimal with respect to a given performance index, from a mathematical viewpoint, may be helpful to think of new control strategies. For instance, the work by Sbarciog et al. (2010) allowed us to propose a new control strategy able to guarantee sub-optimal performances while preserving the stability of the whole process (Rodriguez et al. 2013).

Microbial management of bioprocesses is another emerging topic with a great potential. This is particularly true for AD which involves a huge biodiversity (Carballa et al. 2015). Thanks to the development of molecular analytical tools (denaturing gradient gel electrophoresis, single-strand conformation polymorphism…), the anaerobic microbiome has been more and more characterized (Vanwonterghem et al. 2014, Sundberg et al. 2013). Considering the biodiversity can give raise to a new paradigm for the control and optimization of AD. Until now, the principal objective of control was to stabilize the digester. Nonetheless, a stable process tends to reduce the biodiversity through the section of the fittest species in the imposed environment. Although this selection process could increase the steady-state performance, it could seriously alter the resilience of the process (Ramirez et al. 2009). Dynamical feeding has been proposed in order to select a microbiome with a high ability to adapt to disturbances (De Vrieze et al. 2013). Bioaugmentation have been also applied, in particular in response to stress (e.g., Schauer-Gimenez et al. 2010; Tale et al. 2011). Concerning the model-based control laws, most of them are designed assuming one population for one function. Recently, Mairet and Bernard (2014) have proposed to evaluate the performances of such control laws when several species are present. Using the control law proposed by Mailleret et al. (2004) as an example, they have shown that a slow-growing species can lead to reactor shutdown. This framework can be used to design robust control laws which better tame biodiversity. Rapaport and Harmand (2002) also proposed a "biocontrol" strategy using biotic microbial ecosystem capabilities to select certain species. Although attractive, these approaches remain studied in simulations only. The control of the microbiome involved in AD is an exciting challenge for the future, but the lack of on-line instrumentation for biodiversity monitoring can limit process implementation. Recently, on-line flow cytometers have been proposed for AD (Koch et al. 2014) and can open new directions for closed-loop microbial control strategies.

Recently, novel potential actuators emerged to control methanogenic pathways (Liu et al. 2013; Lin et al. 2013). Indeed, methanogenic pathways (i.e., acetoclastic or hydrogenotrophic) have been analyzed using stable carbon isotope signature. This analysis is made on the biogas phase and thanks to an isoprime mass spectrometer linked with a gas chromatography, a carbon fractionation can be performed. This information is very valuable because it points out the contribution of the different methanogenic pathways producing methane and carbon dioxide. For example, Liu et al. (2013) made cartography of the methanogens type depending on ammonium and acetate high concentrations. In the same way, Lin et al. (2013) showed the impact of the addition of bicarbonate on the methanogenic biodiversity. This kind of information would be very valuable in order to drive a digester in case of acid or/and ammonia inhibition, without loose energetic performance.

The balance between the synergistic production and consumption of VFA intermediates in the AD with respect to process stability is important and has been outlines in Sect. 3.1. Disagregation of the trophic groups in the AD process by physically separating them into an acidogenic stage reactor and a methanogenic stage reactor is not a new idea. Two stage AD, often with the intention of improving hydrolytic processes, has been studied by many researcher over the last 40 years e.g., Ghosh et al. (1975). However, increased scope for control actuation may be available by such stage separation especially with the ability to monitor and manage microbial populations more effectively in recent years. Furthermore, the stages can be integrated with each other and other processes to improve gas yields as reported by Massanet-Nicolau et al. (2013). Guwy et al. (2011) described how the integration of multi stage bioprocesses can be used to extract or utilize the products. The extraction of VFAs for example may simultaneously deliver valuable chemicals and controlled supply of substrate for methanogenesis to a subsequent stage. This VFA extraction may be achieved by conventional electrodialysis, as proposed by Jones et al. (2015) in an acidogenic stage also generating hydrogen. VFAs are also an appropriate substrate for bioelectrochemical systems as has been demonstrated by many researchers and reviewed by Pant et al. (2010). The application of multivariable control strategies as described in this paper may deliver optimal system performance, although control of each of the stages or sub-processes may be independently controllable under a system level supervisory regime.

The capacity of ADs to utilize additional CO_2 was demonstrated by several authors, which could provide a potential solution for on-site sequestration of CO_2 streams while enhancing methane production by CO_2 sparging. CO_2 could then become an efficient actuator to improve AD performances. Few studies have indeed considered the potential of CO_2 biological conversion in anaerobic processes, reporting benefits both in terms of carbon uptake and renewable energy production (Salomoni and Petazzoni 2006; Salomoni et al. 2011). Interestingly, microorganisms operating under CO_2 saturated conditions continue to synthesize CH_4. Alimahmoodi and Mulligan (2008) stated a 69–86 % CO_2 uptake when dissolving this gas in the influent of an upflow anaerobic sludge blanket (UASB) reactor. Francioso et al. (2010) and Salomoni et al. (2011) further confirmed the potential of CO_2 biological conversion in two phase anaerobic digestion (TPAD), and observed 25 % methane (CH_4) yield enhancement when sparging CO_2 into the first stage. Moreover, the net production of CO_2 in CO_2-recirculating AD units can be reduced by a factor of 4. Fernández et al. (2014) addressed the reduction of CO_2 emissions and enhancement of biogas production associated with CO_2 enrichment of anaerobic digesters (ADs). The benefits of CO_2 enrichment were examined by injecting CO_2 at 0, 0.3, 0.6 and 0.9 M fractions into batch ADs treating food waste or sewage sludge. Daily specific methane (CH_4) production increased 11–16 % for food waste and 96–138 % for sewage sludge. Potential CO_2 reductions of 8–34 % for sewage sludge and 3–11 % for food waste were estimated. Mohd Yasin et al. (2015) used CO_2 as the substrate to generate methane by enriched methanogens after anaerobic enrichment of waste activated sludge (WAS) and they demonstrated that methanogens from WAS have significant potential for converting the greenhouse gas CO_2 into the fuel methane. Moreover, methane production was increased 70 fold by active methanogens in the enriched methanogens culture after 3 days in the presence of H_2 and CO_2.

Indeed, the addition of H_2 into an anaerobic digestion has been performed in several studies (Luo et al. 2012; Luo and Angelidaki 2013; Wang et al. 2013b; Díaz et al. 2015) in order to remove CO_2 from biogas while methane production increased, through the hydrogenotrophic pathway. For example, Luo et al. (2012) showed that increasing both hydrogen partial pressure and mixing intensity would give 22 % of methane production. One main barrier highlighted was the gas–liquid mass transfer of H_2 because of the low solubility of this gas.

5 Conclusions and perspectives

Over the years, knowledge on anaerobic digestion has increased and several instruments are now available to monitor efficiently the AD processes. Global parameters for organic matter characterization can indeed be used and biodegradability, bioavailability and bioaccessibility of complex solid substrates can be assessed. Modelling, especially through the development and consolidation of the ADM1 model, has successfully proven its ability to translate the biological steps occurring in the AD. Since its creation, many improvements have been carried out, and ADM1 has been tailored to a broad variety of substrates. But there are still progresses to be accomplished to better manage the influent composition, and further represent physicochemical processes such as precipitation. There is still a gap between these more and more accurate models, but also involving higher degrees of freedoms, and simpler models which support most of the monitoring, diagnosis and control algorithms. Bridging this gap, combining these theoretical approaches with information provided by innovative sensors, and reducing expert needs to run these algorithms will probably significantly improve the attractiveness of the approach together with its efficiency.

These developments will also contribute to improve emerging processes such as thermophilic and ultra-high rate processes (Ge et al. 2011), or supporting co-digestion strategies (Mata-Alvarez et al. 2011). Modelling, monitoring and control are also expected in the objective of recovering nutrients (Mehta and Batstone 2013) and for tracking micropollutants, trace organics, pathogens and recalcitrant (Fountoulakis et al. 2008).

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References

Aceves-Lara CA, Latrille E, Steyer JP (2010) Optimal control of hydrogen production in a continuous anaerobic fermentation bioreactor. Int J Hydrogen Energ 35:10710–10718

Aguado D, Montoya T, Ferrer J, Seco A (2006) Relating ions concentration variations to conductivity variations in a sequencing batch reactor operated for enhanced biological phosphorus removal. Environ Modell Softw 21:845–851

Aguilar-Garnica E, Dochain D, Alcaraz-González V, González-Álvarez V (2009) A multivariable control scheme in a two-stage anaerobic digestion system described by partial differential equations. J Process Contr 19:1324–1332

Ahring BK, Angelidaki I, Johansen K (1992) Anaerobic treatment of manure together with industrial waste. Water Sci Technol 25:311–318

Ajeej A, Thanikal JV, Narayanan CM, Senthil Kumar R (2015) An overview of bio augmentation of methane by anaerobic co-digestion of municipal sludge along with microalgae and waste paper. Renew Sustain Energy Rev 50:270–276

Alcaraz-González V, González-Álvarez V (2007) Selected topics in dynamics and control of chemical and biological processes. Springer, Berlin

Alcaraz-González V, Harmand J, Rapaport A, Steyer JP, González-Álvarez V, Pelayo-Ortiz C (2005a) Robust interval-based regulation for anaerobic digestion processes. Water Sci Technol 52:449–456

Alcaraz-González V, Salazar-Peña R, González-Alvarez V, Gouzé JL, Steyer JP (2005b) A tunable multivariable nonlinear robust observer for biological systems. C R Biol 328:317–325

Alferes J, Irizar I (2010) Combination of extremum-seeking algorithms with effective hydraulic handling of equalization tanks to control anaerobic digesters. Water Sci Technol 61:2825–2834

Alferes J, García-Heras JL, Roca E, García C, Irizar I (2008) Integration of equalisation tanks within control strategies for anaerobic reactors. Validation based on ADM1 simulations. Water Sci Technol 57:747–752

Alimahmoodi M, Mulligan CN (2008) Anaerobic bioconversion of carbon dioxide to biogas in an upflow anaerobic sludge blanket reactor. J Air Waste Manage Assoc 58:95–103

Alvarez JA, Otero L, Lema JM (2010) A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. Bioresour Technol 101:1153–1158

Alvarez-Ramirez J, Meraz M, Monroy O, Velasco A (2002) Feedback control design for an anaerobic digestion process. J Chem Technol Biotechnol 77:725–734

Anderson GK, Yang G (1992) Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration. Water Environ Res 64:53–59

Andrews JF, Graef SP (1971) Dynamic modelling and simulation of the AD process. Advances in chemistry series no. 105, Anaerobic Biological Treatment Processes. American Chemical Society, Washington, DC, p 126

Andrews JF, Pearson EA (1965) Kinetics and characteristics of volatile acid production in anaerobic fermentation processes. Air Water Pollut 9:439–461

Angelidaki I, Sanders W (2004) Assessment of the anaerobic biodegradability of macropllutants. Rev Environ Sci Biotechnol 3:117–129

Antila J, Tuohiniemi M, Rissanen A, Kantojärvi U, Lahti M, Viherkanto K, Kaarre M, Malinen J (2014) MEMS- and MOEMS-based near-infrared spectrometers. Encycl Anal Chem 1–36. doi:10.1002/9780470027318.a9376

Antoniades CD, Christofides P (2001) Integrating nonlinear output feedback control and optimal actuator/sensor placement for transport-reaction processes. Chem Eng Sci 56:4517–4535

APHA (2005) American Public Health Association/American Water Works Association/Water Environmental Federation, Standard methods for the Examination of Water and Wastewater, 21st edn. Washington, DC, USA

Appels L, Baeyens J, Degrève J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Prog Energ Combust 34:755–781

Appels L, Lauwers J, Gins G, Degreve J, Van Impe J, Dewil R (2011) Parameter identification and modeling of the biochemical methane potential of waste activated sludge. Environ Sci Technol 45:4173–4178

Aquino SF, Chernicharo CAL, Soares H, Takemoto SY, Vazoller RF (2008) Methodologies for determining the bioavailability and biodegradability of sludges. Environ Technol 29:855–862

Astals S, Esteban-Gutiérrez M, Fernández-Arévalo T, Aymerich E, García-Heras JL, Mata-Alvarez J (2013a) Anaerobic digestion of seven different sewage sludges: a biodegradability and modelling study. Water Res 47:6033–6043

Astals S, Nolla-Ardèvol V, Mata-Alvarez J (2013b) Thermophilic co-digestion of pig manure and crude glycerol: process performance and digestate stability. J Biotechnol 166:97–104

Babary JP, Julien S, Nihtilä MT et al (1999) New boundary conditions and adaptive control of fixed-bed bioreactors. Chem Eng Process Process Intensif 38:35–44

Barat R, Serralta J, Ruano MV, Jiménez E, Ribes J, Seco A, Ferrer J (2012) Biological nutrient removal model No 2 (BNRM2): a general model for wastewater treatment plants. Water Sci Technol 67:1481–1489

Bastin G, Dochain D (1990) On-line estimation and adaptive control of bioreactors. Elsevier Science, Amsterdam

Batstone DJ (2013) Modelling and control in anaerobic digestion: achievements and challenges. 13th IWA World Congress on Anaerobic Digestion (AD 13), pp 1–6

Batstone DJ, Keller J, Angelidaki I et al (2002) Anaerobic digestion model No. 1. (ADM1). IWA Scientific and Technical Report No. 13. IWA

Batstone DJ, Tait S, Starrenburg D (2009) Estimation of hydrolysis parameters in full-scale anaerobic digesters. Biotechnol Bioeng 102:1513–1520

Batstone DJ, Amerlinck Y, Ekama G et al (2012) Towards a generalized physicochemical framework. Water Sci Technol 66:1147–1161

Baumann WT, Rugh WJ (1986) Feedback control of nonlinear systems by extended linearization. IEEE Trans Automat Contr AC-31:40–46

Benyahia B, Campillo F, Cherki B, Harmand J (2012) Particle filtring for the chemostat. In: MED'12, Barcelone, Spain

Bernard O (2011) Hurdles and challenges for modelling and control of microalgae for CO2 mitigation and biofuel production. J Process Control 21:1378–1389

Bernard O, Gouzé JL (2004) Closed loop observers bundle for uncertain biotechnological models. J Process Control 14:765–774

Bernard O, Hadj-Sadok Z, Dochain D et al (2001a) Dynamical model development and parameter identification for an anaerobic wastewater treatment process. Biotechnol Bioeng 75:424–438

Bernard O, Polit M, Hadj-Sadok Z, Pengov M, Dochain D, Estaben M, Labat P (2001b) Advanced monitoring and control of anaerobic wastewater treatment plants: software sensors and controllers for an anaerobic digester. Water Sci Technol 43:175–182

Bernard O, Chachuat B, Hélias A, Rodriguez J (2005a) Can we assess the model complexity for a bioprocess? Theory and example of the anaerobic digestion process. Water Sci Technol 53:85–92

Bernard O, Chachuat B, Hélias A, Le Dantec B, Sialve B, Steyer JP, Lavigne JF (2005b) An integrated system to remote monitor and control anaerobic wastewater treatment plants through the internet. Water Sci Technol 52:457–464

Björnsson L, Hörnsten EG, Mattiasson B (2001a) Utilization of a palladium–metal oxide semiconductor (Pd-MOS) sensor for on-line monitoring of dissolved hydrogen in anaerobic digestion. Biotechnol Bioeng 73:35–43

Björnsson L, Murto M, Jantsch TG, Mattiasson B (2001b) Evaluation of new methods for the monitoring of alkalinity, dissolved hydrogen and the microbial community in anaerobic digestion. Water Res 35:2833–2840

Boe K (2006) Online monitoring and control of the biogas process. Technical University of Denmark

Boe K, Batstone D, Angelidaki I (2007) An innovative online VFA monitoring system for the anerobic process, based on headspace gas chromatography. Biotechnol Bioeng 96:712–721

Boe K, Steyer JP, Angelidaki I (2008) Monitoring and control of the biogas process based on propionate concentration using online VFA measurement. Water Sci Technol 57:661–766

Boe K, Batstone DJ, Steyer JP, Angelidaki I (2010) State indicators for monitoring the anaerobic digestion process. Water Res 44:5973–5980

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brinkmann K, Blaschke L, Polle A (2002) Comparison of different methods for lignin determination as a basis for calibration of near-infrared reflectance spectroscopy and implications of lignoproteins. J Chem Ecol 28:2483–2501

Buendía IM, Fernández FJ, Villaseñor J, Rodríguez L (2008) Biodegradability of meat industry wastes under anaerobic and aerobic conditions. Water Res 42:3767–3774

Buffiere P, Loisel D, Bernet N, Delgenes JP (2006) Towards new indicators for the prediction of solid waste anaerobic digestion properties. Water Sci Technol 53:233–241

Cao Y, Pawlowski A (2012) Sewage sludge-to-energy approaches based on anaerobic digestion and pyrolysis: brief overview and energy efficiency assessment. Renew Sust Energ Rev 16:1657–1665

Carballa M, Regueiro L, Lema JM (2015) Microbial management of anaerobic digestion: exploiting the microbiome-functionality nexus. Curr Opin Biotechnol 33:103–111

Carlos-Hernandez S, Beteau JF, Sanchez EN (2007) Intelligent control strategy for an anaerobic fluidized bed reactor. In: Michel P (ed) Computer applications in biotechnology, vol 1. Cancun, Mexico, pp 73–78

Carlos-Hernandez S, Sanchez EN, Bueno JA (2010) Neurofuzzy control strategy for an abattoir wastewater treatment process. In: Banga JR, Bogaerts P, Van Impe J, Dochain D, Smets I (eds) 11th International symposium on computer applications in biotechnology. Leuven, Belgium, pp 84–89

Chandler JA, Jewell WJ, Gossett JM (1980) Predicting methane fermentation biodegradability. Biotechnol Bioeng Symp 10:93–107

Chen YH (1990) Adaptive robust observers for non-linear uncertain systems. Int J Syst Sci 21:803–814

Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: a review. Bioresour Technol 99:4044–4064

Chynoweth DP, Turick CE, Owens JM, Jerger DE, Peck MW (1993) Biochemical methane potential of biomass and waste feedstocks. Biomass Bioenerg 5:95–111

Cirne DG, van der Zee FP, Fernandez-Polanco M, Fernandez-Polanco F (2008) Control of sulphide during anaerobic treatment of S-containing wastewaters by adding limited amounts of oxygen or nitrate. Rev Environ Sci Biotechnol 7:93–105

Colombié S, Latrille E, Sablayrolles JM (2007) Online estimation of assimilable nitrogen by electrical conductivity measurement during alcoholic fermentation in enological conditions. J Biosci Bioeng 103:229–235

Cord-Ruwisch R, Mercz TI, Hoh CY, Strong GE (1997) Dissolved hydrogen concentration as an on-line control parameter for the automated operation and optimization of anaerobic digesters. Biotechnol Bioeng 56:626–634

Cossu R, Raga R (2008) Test methods for assessing the biological stability of biodegradable waste. Waste Manage 28:381–388

Cresson R, Pommier S, Béline F et al (2014) Etude interlaboratoires pour l'harmonisation des protocoles de mesure du potentiel bio-méthanogène des matrices solides hétérogènes—Final report **(in French)** ADEME

Dalmau J, Comas J, Rodríguez-Roda I, Pagilla K, Steyer JP (2010) Model development and simulation for predicting risk of foaming in anaerobic digestion systems. Bioresour Technol 101:4306–4314

Davidsson A, Gruvberger C, Christensen TH, Hansen TL, Jansen J (2007) Methane yield in source-sorted organic fraction of municipal solid waste. Waste Manage 27:406–414

De Baere L (2000) Anaerobic digestion of solid waste: state-of-the-art. Water Sci Technol 41:283–290

De Baere L (2008) Partial stream digestion of residual municipal solid waste. Water Sci Technol 57:1073–1077

De Gracia M, Grau P, Huete E et al (2009) New generic mathematical model for WWTP sludge digesters operating under aerobic and anaerobic conditions: model building and experimental verification. Water Res 43:4626–4642

De Vrieze J, Verstraete W, Boon N (2013) Repeated pulse feeding induces functional stability in anaerobic digestion. Microb Biotechnol 6:414–424

Delattre C, Dochain D, Winkin J (2004) Observability analysis of nonlinear tubular (bio)reactor models: a case study. J Process Control 14:661–669

Di Pinto AC, Limoni N, Passino R, Rozzi A, Tomei MC (1990) Instrumentation, control and automation of water and wastewater treatment and transport systems. In: Proceedings of the 5th IAWPRC workshop, pp 51–58

Díaz I, Pérez C, Alfaro N, Fdz-Polanco F (2015) A feasibility study on the bioconversion of CO2 and H2 to biomethane by gas sparging through polymeric membranes. Bioresour Technol 185:246–253

Dochain D (2003) State and parameter estimation in chemical and biochemical processes: a tutorial. J Process Control 13:801–818

Dochain D, Tali-Maamar N, Babary JP (1997) On modelling, monitoring and control of fixed bed bioreactors. Comput Chem Eng 21:1255–1266

Dochain D, Perrier M, Guay M (2011) Extremum seeking control and its application to process and reaction systems: a survey. Math Comput Simulat 82:369–380

Donoso-Bravo A, Garcia G, Pérez-Elvira S, Fernandez-Polanco F (2011) Initial rates technique as a procedure to predict the anaerobic digester operation. Biochem Eng J 53(3):275–280

Doublet J, Boulanger A, Ponthieux A, Laroche C, Poitrenaud M, Cacho Rivero JA (2013) Predicting the biochemical methane potential of wide range of organic substrates by near infrared spectroscopy. Bioresour Technol 128:252–258

Dreywood R (1946) Qualitative test for carbohydrate material. Industrial & Engineering Chemistry Analytical Edition. Am Chem Soc 18:499

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Ekama GA, Sotemann SW, Wentzel MC (2007) Biodegradability of activated sludge organics under anaerobic conditions. Water Res 41:244–252

Ellison WJ, Pedarros-Caubet F, Caubet R (2007) Automatic and rapid measurement of microbial suspension growth parameters: application to the evaluation of effector agents. J Rapid Meth Aut Mic 15:369–410

Fang HHP (2012) Bioenergy production from waste and wastewater in China. In: Technical proceedings of the 2012 NSTI nanotechnology conference and expo, NSTI-nanotech 2012, pp 381–383

Fannin KF, Chynoweth DP, Isaacson R (1987) Start-up, operation, stability, and control. Anaerob Dig Biomass 171–196

Fdz-Polanco M, Díaz I, Pérez SI, Lopes AC, Fdz-Polanco F (2009a) Hydrogen sulphide removal in the anaerobic digestion of sludge by micro-aerobic processes: pilot plant experience. Water Sci Technol 60:3045–3050

Fdz-Polanco M, Pérez-Elvira SI, Díaz I, García L, Torío R, Acevedo AF (2009b) Eliminación de H_2S en digestión anaerobia de lodos por procesos microaerofílicos: experiencia en planta piloto. Tecnol del Agua 29:58–64

Feitkenhauer H, von Sachs J, Meyer U (2002) On-line titration of volatile fatty acids for the process control of anaerobic digestion plants. Water Res 36:212–218

Fernández YB, Soares A, Villa R, Vale P, Cartmell E (2014) Carbon capture and biogas enhancement by carbon dioxide enrichment of anaerobic digesters treating sewage sludge or food waste. Bioresour Technol 159:1–7

Fountoulakis MS, Stamatelatou K, Lyberatos G (2008) The effect of pharmaceuticals on the kinetics of methanogenesis and acetogenesis. Bioresour Technol 99:7083–7090

Francioso O, Rodriguez-Estrada MT, Montecchio D, Salomoni C, Caputo A, Palenzona D (2010) Chemical characterization of municipal wastewater sludges produced by two-phase anaerobic digestion for biogas production. J Hazard Mater 175:740–746

Frigon JC, Roy C, Guiot SR (2012) Anaerobic co-digestion of dairy manure with mulched switchgrass for improvement of the methane yield. Bioprocess Biosyst Eng 35:341–349

Frings CS, Dunn RT (1970) A colorimetric method for determination of total serum lipids based on the sulfo-phosphovanillin reaction. Am J Clin Pathol 53:89–91

Frølund B, Palmgren R, Keiding K, Nielsen PH (1996) Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Res 30:1749–1758

Gaida D, Wolf C, Meyer C, Stuhlsatz A, Lippel J, Bäck T, Bongards M, McLoone S (2012) State estimation for anaerobic digesters using the ADM1. Water Sci Technol 66:1088–1095

Ganesh R, Torrijos M, Sousbie P et al (2013) Anaerobic co-digestion of solid waste: effect of increasing organic loading rates and characterization of the solubilised organic matter. Bioresource Technol 130:559–569

García-Diéguez C, Molina F, Roca E (2011) Multi-objective cascade controller for an anaerobic digester. Process Biochem 46:900–909

García-Gen (2015) Modelling, optimisation and control of anaerobic co-digestion processes (2015), Ph.D. Thesis, Universidad de Santiago de Compostela, Departamento de Ingeniería Química

García-Gen S, Sousbie P, Rangaraj G et al (2015) Kinetic modelling of anaerobic hydrolysis of solid wastes, including disintegration processes. Waste Manag 35:96–104

Gauthier JP, Kupka IAK (1994) Observability and observers for nonlinear systems. SIAM J Control Optim 32:975–994

Gauthier JP, Hammouri H, Othman S (1992) A simple observer for nonlinear systems applications to bioreactors. Autom Control IEEE Trans 37:875–880

Ge H, Jensen PD, Batstone DJ (2011) Increased temperature in the thermophilic stage in temperature phased anaerobic digestion (TPAD) improves degradability of waste activated sludge. J Hazard Mater 187:355–361

Gendron S, Perrier M, Barrett J, Legault N (1993) Adaptive control of brightness: the model weighting approach. Annual meeting—technical section, Canadian Pulp and Paper Association, Preprints. Publ by Canadian Pulp & Paper Assoc

Ghosh S, Conrad JR, Klass DL (1975) Anaerobic acidogenesis of waste activated sludge, WPCF 47

Goffaux G, Van de Wouwer A (2005) Bioprocess state estimation: some classical and less classical approaches. Springer, Berlin

Gornall AG, Bardawill CJ, David MM (1949) Determination of serum proteins by means of the biuret reaction. J Biochem Chem 177:751–766

Gouzé JL, Rapaport A, Hadj-Sadok MZ (2000) Interval observers for uncertain biological systems. Ecol Model 133:45–56

Grau P, de Gracia M, Vanrolleghem PA, Ayesa E (2007) A new plant-wide modelling methodology for WWTPs. Water Res 41:4357–4372

Gregersen KH (2003) Økonomien i biogasfællesanlæg, Udvikling og status medio (2002) Report no. 150. Institute of Food and Resource Economic, Rolighedsvej 25, DK 1958, Frederiksberg C, Denmark

Grepmeier M (2002) Experimentelle Untersuchungen an einer zweistufigen fuzzy-geregelten anaeroben Abwasserreinigungsanlage mit neuartigem Festbettmaterial. TU Munich

Guay M, Dochain D, Perrier M (2004) Adaptive extremum seeking control of continuous stirred tank bioreactors with unknown growth kinetics. Automatica 40:881–888

Gunaseelan VN (2007) Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napiergrass on chemical composition. Bioresour Technol 98:1270–1277

Gunaseelan VN (2009) Predicting ultimate methane yields of Jatropha curcus and Morus indica from their chemical composition. Bioresour Technol 100:3426–3429

Guwy AJ, Hawkes FR, Wilcox SJ, Hawkes DL (1997) Neural network and on-off control of bicarbonate alkalinity in a fluidised-bed anaerobic digester. Water Res 31:2019–2025

Guwy AJ, Dinsdale RM, Kim JR et al (2011) Fermentative biohydrogen production systems integration. Bioresour Technol 102:8534–8542

Hao OJ (2003) Sulphate-reducing bacteria. In: Mara D, Horan N (eds) Handbook of water and wastewater microbiology. Academic Press Inc, London, pp 459–468

Harremoës P, Capodaglio AG, Hellström BG, Henze M, Jensen KN, Lynggaard-Jensen A, Otterpohl R, Søeberg H (1993) Wastewater treatment plants under transient loading – performance, modelling and control. Water Sci Technol 27(12):71–115

Hawkes FR, Guwy AJ, Rozzi AG, Hawkes DL (1993) A new instrument for on-line measurement of bicarbonate alkalinity. Water Res 27:167–170

Hawkes FR, Guwy AJ, Hawkes DL, Rozzi AG (1994) On-line monitoring of anaerobic digestion: application of a device for continuous measurement of bicarbonate alkalinity. Water Sci Technol 30:1–10

He XS, Xi BD, Wei ZM, Jiang YH, Yang Y, Cao JL, Liu HL (2011) Fluorescence excitation-emission matrix spectroscopy with regional integration analysis for characterizing composition and transformation of dissolved organic matter in landfill leachates. J Hazard Mater 190:293–299

Henneberg W, Stohmann F (1860) Beiträge zur Begründer einer rationellen. Fütterung Der Wiederkäer I & II.Braunschweig

Henson MA, Seborg DE (1997) Adaptive input-output linearization of a pH neutralization process. Int J Adapt Control Signal Process 11:171–200

Hobson PN (1985) A model of anaerobic bacterial degradation of solid substrates in a batch digester. Agric Wastes 14:255–274

Hoffmann F, Schmidt M, Rinas U (2000) Simple technique for simultaneous on-line estimation of biomass and acetate from base consumption and conductivity measurements in high-cell density cultures of Escherichia coli. Biotechnol Bioeng 70:358–361

Holm-Nielsen JB, Lomborg CJ, Oleskowicz-Popiel P, Esbensen KH (2008) On-line near infrared monitoring of glycerol-boosted anaerobic digestion processes: evaluation of process analytical technologies. Biotechnol Bioeng 99:302–313

Holubar P, Zani L, Hager M, Fröschl W, Radak Z, Braun R (2002) Advanced controlling of anaerobic digestion by means of hierarchical neural networks. Water Res 36:2582–2588

Holubar P, Zani L, Hager M, Fröschl W, Radak Z, Braun R (2003) Start-up and recovery of a biogas-reactor using a hierarchical neural network-based control tool. J Chem Technol Biot 78:847–854

Huang M, Li Y, Gu G (2010) Chemical composition of organic matters in domestic wastewater. Desalination 262:36–42

Huete E, de Gracia M, Ayesa E, Garcia-Heras JL (2006) ADM1-based methodology for the characterisation of the influent sludge in anaerobic reactors. Water Sci Technol 54:157–166

Hunt LR, Su R (1983) Linear approximations of nonlinear systems. In: Proceedings of the IEEE conference on decision and control. IEEE, pp 122–125

Huntington R (1998) Twenty years development of ICA in a water utility. Water Sci Technol 37:27–34

Ignatova MN, Lyubenova VN, García MR, Vilas C, Alonso AA (2008) Indirect adaptive linearizing control of a class of bioprocesses—estimator tuning procedure. J Process Control 18:27–35

Imhoff K (1938) Sedimentation and digestion in Germany, in modern sewage disposal. Lancaster Press, Lancaster, PA, USA

Isidori A (1989) Nonlinear control systems: an introduction, 2nd edn. Springer, New York

ISO 11734 (1995) Evaluation of the "ultimate" anaerobic biodegradability of organic compounds in digested sludge—method by measurement of the biogas

Jacobi HF, Moschner CR, Hartung E (2009) Use of near infrared spectroscopy in monitoring of volatile fatty acids in anaerobic digestion. Water Sci Technol 60:339–346

Jacobi HF, Ohl S, Hartung E (2012) NIRS-aided monitoring and prediction of biogas yields from maize silage at a full-scale biogas plant applying lumped kinetics. Bioresour Technol 103:162–172

Jáuregui-Medina EA, Alcaraz-González V, Méndez-Acosta HO, González-Alvarez V (2009) Observer-based input estimation in continuous anaerobic wastewater treatment processes. Water Sci Technol 60:805–812

Jeison D, van Lier JB (2006) On-line cake-layer management by trans-membrane pressure steady state assessment in Anaerobic Membrane Bioreactors for wastewater treatment. Biochem Eng J 29:204–209

Jensen PD, Hardin MT, Clarke WP (2009) Effect of biomass concentration and inoculum source on the rate of anaerobic cellulose solubilization. Bioresour Technol 100:5219–5225

Jimenez J, Vedrenne F, Denis C, Mottet A, Déléris S, Steyer JP, Cacho Rivero JA (2013) A statistical comparison of protein and carbohydrate characterisation methodology applied on sewage sludge samples. Water Res 47:1751–1762

Jimenez J, Gonidec E, Cacho Rivero JA, Latrille E, Vedrenne F, Steyer JP (2014) Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: towards ADM1 variables characterization. Water Res 50:359–372

Jiménez J, Guardia-Puebla Y, Romero-Romero O, Cisneros-Ortiz ME, Guerra G, Morgan-Sagastume JM, Noyola A (2014) Methanogenic activity optimization using the response surface methodology, during the anaerobic co-digestion of agriculture and industrial wastes. Microbial community diversity. Biomass Bioenerg 71:84–97

Jones RJ, Massanet-Nicolau J, Guwy AJ, Premier GC, Dinsdale RM, Reilly M (2015) Removal and recovery of inhibitory volatile fatty acids from mixed acid fermentations by conventional electrodialysis. Bioresour Technol 189:279–284

Kaur A, Kim JR, Dinsdale RM, Guwy AJ (2013) Microbial fuel cell type biosensor for volatile fatty acid with an acclimated bacterial community. Biosens Bioelectron 47:50–55

Kaur A, Ibrahim S, Pickett C, Michie I, Kim JR, Dinsdale RM, Guwy AJ, Premier GC (2014) Anode modification to improve the performance of a microbial fuel cell volatile fatty acid biosensor. Sensor Actuat B Chem 47:50–55

Kayhanian M (1995) Biodegradability of the organic fraction of municipal solid waste in a high-solids anaerobic digester. Waste Manage Res 13:123–136

Kjeldahl J (1883) A new method for the determination of nitrogen in organic matter. Z Anal Chem 22:366

Kleerebezem R, van Loosdrecht MCM (2006) Waste characterization for implementation in ADM1. Water Sci Technol 54:167–174

Koch C, Müller S, Harms H, Harnisch F (2014) Microbiomes in bioenergy production: from analysis to management. Curr Opin Biotechnol 27:65–72

Krapf LC, Heuwinkel H, Schmidhalter U, Gronauer A (2013) The potential for online monitoring of short-term process dynamics in anaerobic digestion using near-infrared spectroscopy. Biomass Bioenerg 48:224–230

Kurtz MJ, Henson MA (1997) Input-output linearizing control of constrained nonlinear processes. J Process Contr 7:3–17

Labatut RA, Angenent LT, Scott NR (2011) Biochemical methane potential and biodegradability of complex organic substrates. Bioresour Technol 102:2255–2264

Lara-Cisneros G, Aguilar-López R, Femat R (2015) On the dynamic optimization of methane production in anaerobic digestion via extremum-seeking control approach. Comput Chem Eng 75:49–59

Lauwers J, Appels L, Thompson IP, Degrève J, Van Impe JF, Dewil R (2013) Mathematical modelling of anaerobic digestion of biomass and waste: power and limitations. Prog Energ Combust 39:383–402

Lesteur M, Bellon-Maurel V, Gonzalez C, Latrille E, Roger JM, Junqua G, Steyer JP (2010) Alternative methods for determining anaerobic biodegradability: a review. Process Biochem 45:431–440

Lesteur M, Latrille E, Maurel VB, Roger JM, Gonzalez C, Junqua G, Steyer JP (2011) First step towards a fast analytical method for the determination of Biochemical Methane Potential of solid wastes by near infrared spectroscopy. Bioresour Technol 102:2280–2288

Li YF, Yu Z (2015) Construction and evaluation of a genetic construct for specific detection and measurement of propionate by whole-cell bacteria. Biotechnol Bioeng 112:280–287

Lin Y, Lü F, Shao L, He P (2013) Influence of bicarbonate buffer on the methanogenetic pathway during thermophilic anaerobic digestion. Bioresour Technol 137:245–253

Liu J, Olsson G, Mattiasson B (2004a) Control of an anaerobic reactor towards maximum biogas production. Water Sci Technol 50:189–198

Liu J, Olsson G, Mattiasson B (2004b) Monitoring and control of an anaerobic upflow fixed-bed reactor for high-loading-rate operation and rejection of disturbances. Biotechnol Bioeng 87:43–53

Liu J, Olsson G, Mattiasson B (2006) Extremum-seeking with variable gain control for intensifying biogas production in anaerobic fermentation. Water Sci Technol 53:35–44

Liu X, Li R, Ji M, Han L (2013) Hydrogen and methane production by co-digestion of waste activated sludge and food waste in the two-stage fermentation process: substrate conversion and energy yield. Bioresour Technol 146:317–323

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Luo G, Angelidaki I (2013) Co-digestion of manure and whey for in situ biogas upgrading by the addition of H2: process performance and microbial insights. Appl Microbiol Biotechnol 97:1373–1381

Luo G, Johansson S, Boe K, Xie L, Zhou Q, Angelidaki I (2012) Simultaneous hydrogen utilization and in situ biogas upgrading in an anaerobic reactor. Biotechnol Bioeng 109:1088–1094

Madsen M, Holm-Nielsen JB, Esbensen KH (2011) Monitoring of anaerobic digestion processes: a review perspective. Renew Sust Energ Rev 15:3141–3155

Mailleret L, Bernard O, Steyer JP (2004) Nonlinear adaptive control for bioreactors with unknown kinetics. Automatica 40:1379–1385

Mairet F, Bernard O (2014) Robustness of closed-loop control to biodiversity: a didactic example. In: Proceedings of the 19th IFAC world congress. Cape Town, South Africa

Mäkynen JH, Tuohiniemi M, Näsilä A, Mannila R, Antila JE (2014) MEMS Fabry-Perot interferometer-based spectrometer demonstrator for 7.5 μm to 9.5 μm wavelength range. MOEMS and miniaturized systems XIII. SPIE Proc 8977:1–8

Malinen J, Rissanen A, Saari H, Karioja P, Karppinen M, Aalto T, Tukkiniemi K (2014) Advances in miniature spectrometer and sensor development. Next-generation spectroscopic technologies VII. SPIE proceedings (9101)

Marcos NI, Guay M, Dochain D (2004a) Output feedback adaptive extremum seeking control of a continuous stirred tank bioreactor with Monod's kinetics. J Process Control 14:807–818

Marcos NI, Guay M, Dochain D, Zhang T (2004b) Adaptive extremum-seeking control of a continuous stirred tank bioreactor with Haldane's Kinetics. J Process Control 14:317–328

Marsili-Libelli S, Beni S (1996) Shock load modelling in the anaerobic digestion process. Ecol Modell 84:215–232

Massanet-Nicolau J, Dinsdale R, Guwy A, Shipley G (2013) Use of real time gas production data for more accurate comparison of continuous single-stage and two-stage fermentation. Bioresour Technol 129:561–567

Mata-Alvarez J, Macé S, Llabres P (2000) Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour Technol 74:3–16

Mata-Alvarez J, Dosta J, Macé S, Astals S (2011) Codigestion of solid wastes: a review of its uses and perspectives including modelling. Crit Rev Biotechnol 31:99–111

Mata-Alvarez J, Dosta J, Macé S, Romero-Güiza MC, Fonoll X, Peces M, Astals S (2014) A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renew Sust Energ Rev 36:412–427

Mathiot S, Escoffier Y, Ehlinger F, Couderc JP, Leyris JP, Moletta R (1992) Control parameter variations in an anaerobic fluidised bed reactor subjected to organic shockloads. Water Sci Technol 25:93–101

McCartney DM, Oleszkiewicz JA (1991) Sulfide inhibition of anaerobic degradation of lactate and acetate. Water Res 25:203–209

McCartney DM, Oleszkiewicz JA (1993) Competition between methanogens and sulfate reducers: effect of COD:sulfate ratio and acclimation. Water Environ Res 65:655–664

McCarty PL (1964) Anaerobic waste treatment fundamentals. Public Works 95:91–99

McMahon K (2001) Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions—II: microbial population dynamics. Water Res 35:1817–1827

Mehta CM, Batstone DJ (2013) Nutrient solubilization and its availability following anaerobic digestion. Water Sci Technol 67:756–763

Méndez-Acosta HO, Femat R, Campos-Delgado DU (2004) Improving the performance on the chemical oxygen demand regulation in anaerobic digestion. Ind Eng Chem Res 43:95–104

Méndez-Acosta HO, Campos-Delgado DU, Femat R, González-Alvarez V (2005) A robust feedforward/feedback control for an anaerobic digester. Comput Chem Eng 29:1613–1623

Méndez-Acosta HO, Palacios-Ruiz B, Alcaraz-González V, Steyer JP, González-Álvarez V, Latrille E (2008) Robust control of volatile fatty acids in anaerobic digestion processes. Ind Eng Chem Res 47:7715–7720

Méndez-Acosta HO, Palacios-Ruiz B, Alcaraz-González V, González-Álvarez V, García-Sandoval JP (2010) A robust control scheme to improve the stability of anaerobic digestion processes. J Process Control 20:375–383

Méndez-Acosta HO, García-Sandoval JP, González-Álvarez V, Alcaraz-González V, Jáuregui-Jáuregui JA (2011) Regulation of the organic pollution level in anaerobic digesters by using off-line COD measurements. Bioresour Technol 102:7666–7672

Misawa EA, Hedrick JK (1989) Nonlinear observers—a state-of-the-art survey. J Dyn Syst Meas Control Trans ASME 111:344–352

Mohd Yasin NH, Maeda T, Hu A, Yu CP, Wood TK (2015) CO2 sequestration by methanogens in activated sludge for methane production. Appl Energy 142:426–434

Moisan M, Bernard O, Gouzé JL (2009) Near optimal interval observers bundle for uncertain bioreactors. Automatica 45:291–295

Mosey FE (1983) Mathematical modelling of the anaerobic digestion process: regulatory mechanisms for the formation of short-chain volatile acids from glucose. Water Sci Technol 15:209–232

Motte JC, Escudié R, Beaufils N et al (2014) Morphological structures of wheat straw strongly impacts its anaerobic digestion. Ind Crops Prod 52:695–701

Mottet A, François E, Latrille E, Steyer JP, Déléris S, Vedrenne F, Carrère H (2010) Estimating anaerobic biodegradability indicators for waste activated sludge. Chem Eng J 160:488–496

Mottet A, Ramirez I, Carrère H et al (2013) New fractionation for a better bioaccessibility description of particulate organic matter in a modified ADM1 model. Chem Eng J 228:871–881

Mu SJ, Zeng Y, Tartakovsky B, Wu P (2007) Simulation and control of an upflow anaerobic sludge blanket (UASB) reactor using an ADM1-based distributed parameter model. Ind Eng Chem Res 46:1519–1526

Murnleitner E, Becker TM, Delgado A (2002) State detection and control of overloads in the anaerobic wastewater treatment using fuzzy logic. Water Res 36:201–211

Neave SL, Buswell AM (1930) The anaerobic oxidation of fatty acids. J Am Chem Soc 52:3308–3314

Neumann N, Ebermann M, Gittler E, Meinig M, Kurth S, Hiller K (2010) Uncooled IR sensors with tunable MEMS Fabry-P. IEEE sensors conference, pp 2383–2387

Nghiem LD, Manassa P, Dawson M, Fitzgerald SK (2014) Oxidation reduction potential as a parameter to regulate micro-oxygen injection into anaerobic digester for reducing hydrogen sulphide concentration in biogas. Bioresour Technol 173:443–447

Nielfa A, Cano R, Vinot M et al (2015) Anaerobic digestion modeling of the main components of organic fraction of municipal solid waste. Process Saf Environ Prot 94:180–187

O'Flaherty V, Mahony T, O'Kennedy R, Colleran E (1998) Effect of pH on growth kinetics and sulphide toxicity thresholds of a range of methanogenic, syntrophic and sulphate-reducing bacteria. Process Biochem 33:555–569

Olsson G, Newell R (1998) Reviewing, assessing and speculating. Water Sci Technol 37:397–401

Olsson G, Nielsen MK, Yuan Z, Lynggaard-Jensen A, Steyer JP (2005) Instrumentation, control and automation in wastewater systems. IWA Publishing, London

Olsson G, Carlsson B, Comas J et al (2014) Instrumentation, control and automation in wastewater—from London 1973 to Narbonne 2013. Water Sci Technol 69:1373–1385

Owen WF, Stuckey DC, Healy J, Young LY, McCarty PL (1979) Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res 13:485–492

Pant D, Van Bogaert G, Diels L, Vanbroekhoven K (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 101:1533–1543

Park C, Novak JT (2007) Characterization of activated sludge exocellular polymers using several cation-associated extraction methods. Water Res 41:1679–1688

Park C, Novak JT, Helm RF, Ahn YO, Esen A (2008) Evaluation of the extracellular proteins in full-scale activated sludges. Water Res 42:3879–3889

Park HD, Lee YH, Kim HB et al (2010) Reduction of membrane fouling by simultaneous upward and downward air sparging in a pilot-scale submerged membrane bioreactor treating municipal wastewater. Desalination 251:75–82

Pauss A, Nyns E-J (1993) Past, present and future trends in anaerobic digestion applications. Biomass Bioenerg 4:263–270

Perrier M, de Azevedo SF, Ferreira EC, Dochain D (2000) Tuning of observer-based estimators: theory and application to the on-line estimation of kinetic parameters. Control Eng Pract 8:377–388

Pind PF, Angelidaki I, Ahring BK, Stamatelatou K, Lyberatos G (2003) Monitoring and control of anaerobic reactors. Adv Biochem Eng Biot 82:135–182

Pratt S, Yuan Z, Gapes D et al (2003) Development of a novel titration and off-gas analysis (TOGA) sensor for study of biological processes in wastewater treatment systems. Biotechnol Bioeng 81:482–495

Pratt S, Liew D, Batstone DJ, Werker G, Morgan-Sagastume F, Lant P (2012) Inhibition by fatty acids during fermentation of pre-treated waste activated sludge. J Biotechnol 159:38–43

Provenzano MR, Malerba AD, Pezzolla D, Gigliotti G (2014) Chemical and spectroscopic characterization of organic matter during the anaerobic digestion and successive composting of pig slurry. Waste Manag 34:653–660

Pullammanappallil P, Harmon J, Chynoweth DP, Lyberatos G, Svoronos SA (1991) Avoiding digester imbalance through real-time expert system control of dilution rate. Appl Biochem Biotech 28–29:33–42

Pullammanappallil PC, Svoronos SA, Chynoweth DP, Lyberatos G (1998) Expert system for control of anaerobic digesters. Biotechnol Bioeng 58:13–22

Puñal A, Palazzotto L, Bouvier JC, Conte T, Steyer JP (2003) Automatic control of volatile fatty acids in anaerobic digestion using a fuzzy logic based approach. Water Sci Technol 48:103–110

Purser BJJ, Thai SM, Fritz T et al (2014) An improved titration model reducing over estimation of total volatile fatty acids in anaerobic digestion of energy crop, animal slurry and food waste. Water Res 61:162–170

Ramirez I, Volcke EIP, Rajinikanth R, Steyer JP (2009) Modeling microbial diversity in anaerobic digestion through an extended ADM1 model. Water Res 43:2787–2800

Rapaport A, Dochain D (2005) Interval observers for biochemical processes with uncertain kinetics and inputs. Math Biosci 193:235–253

Rapaport A, Harmand J (2002) Robust regulation of a class of partially observed nonlinear continuous bioreactors. J Process Contr 12:291–302

Rapaport A, Haidar I, Harmand J (2014) Global dynamics of the buffered chemostat for a general class of response functions. J Math Biol 71:69–98

Raposo F, De la Rubia MA, Fernandez-Cegri V, Borja R (2011) Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. Renew Sust Energ Rev 16:861–877

Raposo F, Borja R, Cacho JA et al (2013) Review—First international comparative study of volatile fatty acids in aqueous samples by chromatographic techniques: evaluating sources of error. Trends Anal Chem 51:127–144

Ras M, Girbal-Neuhauser E, Paul E, Sperandio M, Lefebvre D (2008) Protein extraction from activated sludge: an analytical approach. Water Res 42:1867–1878

Raunkjær K, Hvitved-Jacobsen T, Nielsen PH (1994) Measurement of pools of protein, carbohydrate and lipid in domestic wastewater. Water Res 28:251–262

Reilly M, Dinsdale R, Guwy A (2015) Enhanced biomethane potential from wheat straw by low temperature alkaline calcium hydroxide pre-treatment. Bioresour Technol 189:258–265

Renard P, Dochain D, Bastin G, Naveau H, Nyns EJ (1988) Adaptive control of anaerobic digestion processes—a pilot-scale application. Biotechnol Bioeng 31:287–294

Reynolds DM, Ahmad SR (1997) Rapid and direct determination of wastewater BOD values using a fluorescence technique. Water Res 31:2012–2018

Ripley LE, Boyle WC, Converse JC (1985) Improved alkalimetric monitoring for anaerobic digestion of poultry manure. In: Proceedings of the industrial waste conference, pp 141–149

Robles A, Ruano MV, Ribes J, Ferrer J (2013) Advanced control system for optimal filtration in submerged anaerobic MBRs (SAnMBRs). J Membrane Sci 430:330–341

Robles A, Ruano MV, Ribes J, Seco A, Ferrer J (2014) Model-based automatic tuning of a filtration control system for submerged anaerobic membrane bioreactors (AnMBR). J Membrane Sci 465:14–26

Robles A, Durán F, Ruano MV, Ribes J, Rosado A, Seco A, Ferrer J (2015) Instrumentation, control, and automation for submerged anaerobic membrane bioreactors. Environ Technol 36:1795–1806

Rodriguez CER, Latrille E, Harmand J, Alcaraz González V, González Álvarez V, Steyer JP (2013) A switching control strategy based on alkalinity to optimize biogas outflow in the startup of an anaerobic digester, IWA ICA2013, September 18–20. Narbonne, France

Rosén C, Jeppsson U (2006) Aspects on ADM1 Implementation within the BSM2 Framework. Lund University

Ruiz G, Castellano M, González W et al (2005) European symposium on computer-aided process engineering-15, 38th european symposium of the working party on computer aided process engineering. Elsevier, New York

Ryhiner G, Dunn IJ, Heinzle E, Rohani S (1992) Adaptive online optimal control of bioreactors: application to anaerobic degradation. J Biotechnol 22:89–105

Ryhiner GB, Heinzle E, Dunn IJ (1993) Modeling and simulation of anaerobic wastewater treatment and its application to control design: case whey. Biotechnol Progr 9:332–343

Salomoni C, Petazzoni E (2006) Capture de co2 et utilisation dans la digestion de matiere organique en vue de la production de methane. Patent WO 2006108532:A1

Salomoni C, Caputo A, Bonoli M et al (2011) Enhanced methane production in a two-phase anaerobic digestion plant, after CO2 capture and addition to organic wastes. Bioresour Technol 102:6443–6448

Sarraguça MC, Paulo A, Alves MM, Dias AMA, Lopes JA, Ferreira EC (2009) Quantitative monitoring of an activated sludge reactor using on-line UV-visible and near-infrared spectroscopy. Anal BioAnal Chem 395:1159–1166

Sbarciog M, Loccufier M, Noldus E (2010) Determination of appropriate operating strategies for anaerobic digestion systems. Biochem Eng J 51:180–188

Sbarciog M, Moreno JA, Vande Wouwer A (2012) A biogas-based switching control policy for anaerobic digestion systems. In: Vinay K (ed) Advanced control of chemical processes vol 8. Furama Riverfront, Singapore, pp 603–608

Scaglia B, Confalonieri R, D'Imporzano G, Adani F (2010) Estimating biogas production of biologically treated municipal solid waste. Bioresour Technol 101:945–952

Schauer-Gimenez AE, Zitomer DH, Maki JS, Struble CA (2010) Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. Water Res 44:3555–3564

Shanmugam P, Horan NJ (2009) Simple and rapid methods to evaluate methane potential and biomass yield for a range of mixed solid wastes. Bioresour Technol 100:471–474

Silvestre G, Bonmatí A, Fernández B (2015) Optimisation of sewage sludge anaerobic digestion through co-digestion with OFMSW: effect of collection system and particle size. Waste Manag 43:137–143

Simeonov I, Queinnec I (2006) Linearizing control of the anaerobic digestion with addition of acetate (control of the anaerobic digestion). Control Eng Pract 14:799–810

Smith PK, Krohn RI, Hermanson GT et al (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85

Smith PJ, Vigneswaran S, Ngo HH, Ben-Aim R, Nguyen H (2006) A new approach to backwash initiation in membrane systems. J Membrane Sci 278:381–389

Spanjers H, van Lier JB (2006) Instrumentation in anaerobic treatment—research and practice. Water Sci Technol 53:63–76

Spanjers H, Bouvier JC, Steenweg P, Bisschops I, van Gils W, Versprille B (2006) Implementation of in-line infrared monitor in full-scale anaerobic digestion process. Water Sci Technol 53:55–61

Speece RE (2008) Anaerobic biotechnology and odor/corrosion control for municipalities and industries. Archae Press, Nashville, TN, USA

Steyer JP, Rolland D, Bouvier JC, Moletta R (1997) Hybrid fuzzy neural network for diagnosis—application to the anaerobic treatment of wine distillery wastewater in a fluidized bed reactor. Water Sci Technol 36:209–217

Steyer JP, Buffière P, Rolland D, Moletta R (1999) Advanced control of anaerobic digestion processes through disturbances monitoring. Water Res 33:2059–2068

Steyer JP, Bouvier JC, Conte T, Gras P, Harmand J, Delgenes JP (2002) On-line measurements of COD, TOC, VFA, total and partial alkalinity in anaerobic digestion processes using infra-red spectrometry. Water Sci Technol 45:133–138

Steyer JP, Lardon L, Bernard O (2004) Sensors network diagnosis in anaerobic digestion processes using evidence theory. Water Sci Technol 50:21–29

Steyer JP, Bernard O, Batstone DJ, Angelidaki I (2006) Lessons learnt from 15 years of ICA in anaerobic digesters. Water Sci Technol 53:25–33

Strömberg S, Nistor M, Liu J (2015) Early prediction of biochemical methane potential through statistical and kinetic modelling of initial gas production. Bioresour Technol 176:233–241

Sundberg C, Al-Soud WA, Larsson M (2013) 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol Ecol 85:612–626

Szarka N, Scholwin F, Trommler M et al (2013) A novel role for bioenergy: a flexible, demand-oriented power supply. Energy 61:18–26

Tafdrup S (1994) Centralized biogas plants combine agricultural and environmental benefits with energy production. Water Sci Technol 30:133–141

Tale VP, Maki JS, Struble CA, Zitomer DH (2011) Methanogen community structure-activity relationship and bioaugmentation of overloaded anaerobic digesters. Water Res 45:5249–5256

Tartakovsky B, Lishman LA, Legge RL (1996) Application of multi-wavelength fluorometry for monitoring wastewater treatment process dynamics. Water Res 30:2941–2948

Theilliol D, Ponsart JC, Harmand J et al (2003) On-line estimation of unmeasured inputs for anaerobic wastewater treatment processes. Control Eng Pract 11:1007–1019

Triolo JM, Sommer SG, Møller HB, Weisbjerg MR, Jiang XY (2011) A new algorithm to characterize biodegradability of biomass during anaerobic digestion: influence of lignin concentration on methane production potential. Bioresour Technol 102:9395–9402

Val Del Rio MA, Morales N, Isanta E, Mosquera-Corral A, Campos JL, Steyer JP, Carrère H (2011) Thermal pretreatment of aerobic granules, impact on anaerobic biodegradability. Water Res 45:6011–6020

Van der Zee FP, Villaverde S, García PA, Fdz-Polanco F (2007) Sulfide removal by moderate oxygenation of anaerobic sludge environments. Bioresour Technol 98:518–524

Van Ginkel S, Logan BE (2005) Inhibition of biohydrogen production by undissociated acetic and butyric acids. Environ Sci Technol 39:9351–9356

van Lier JB (2008) High-rate anaerobic wastewater treatment: diversifying from end-of-the-pipe treatment to resource-oriented conversion techniques. Water Sci Technol 57:1137–1148

Van Soest PJ (1963) Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J Assoc Off Anal Chem 46:829–835

Vanwonterghem I, Jensen PD, Ho DP et al (2014) Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. Curr Opin Biotechnol 27:55–64

Vargas A, Moreno-Andrade I, Buitrón G (2008) Controlled backwashing in a membrane sequencing batch reactor used for toxic wastewater treatment. J Membrane Sci 320:185–190

Varley J, Brown AK, Boyd JWR, Dodd PW, Gallagher S (2004) Dynamic multi-point measurement of foam behaviour for a continuous fermentation over a range of key process variables. Biochem Eng J 20:61–72

Vavilin VA, Rytov SV, Lokshina LY (1997) A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. Bioresour Technol 56:229–237

Verbruggen HB, Bruijn PM (1997) Fuzzy control and conventional control: what is (and can be) the real contribution of Fuzzy Systems? Fuzzy Set Syst 90:151–160

Von Sachs J, Meyer U, Rys P, Feitkenhauer H (2003) New approach to control the methanogenic reactor of a two-phase anaerobic digestion system. Water Res 37:973–982

Waewsak C, Nopharatana A, Chaiprasert P (2010) Neural-fuzzy control system application for monitoring process response and control of anaerobic hybrid reactor in wastewater treatment and biogas production. J Environ Sci 22:1883–1890

Wan S, Xi B, Xia X, Li M, Lv D, Wang L, Song C (2012) Using fluorescence excitation-emission matrix spectroscopy to monitor the conversion of organic matter during anaerobic co-digestion of cattle dung and duck manure. Bioresour Technol 123:439–444

Wang X, Yang G, Feng Y, Ren G, Han X (2012) Optimizing feeding composition and carbon-nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour Technol 120:78–83

Wang X, Yang G, Li F, Feng Y, Ren G, Han X (2013a) Evaluation of two statistical methods for optimizing the feeding composition in anaerobic co-digestion: mixture design and central composite design. Bioresour Technol 131:172–178

Wang W, Xie L, Luo G et al (2013b) Performance and microbial community analysis of the anaerobic reactor with coke oven gas biomethanation and in situ biogas upgrading. Bioresour Technol 146:234–239

Ward AJ, Bruni E, Lykkegaard MK, Feilberg A, Adamsen APS, Jensen AP, Poulsen AK (2011) Real time monitoring of a biogas digester with gas chromatography, near-infrared spectroscopy, and membrane-inlet mass spectrometry. Bioresour Technol 102:4098–4103

Ward AJ, Lewis DM, Green FB (2014) Anaerobic digestion of algae biomass: a review. Algal Res 5:204–214

Wijekoon KC, Visvanathan C, Abeynayaka A (2011) Effect of organic loading rate on VFA production, organic matter removal and microbial activity of a two-stage thermophilic anaerobic membrane bioreactor. Bioresour Technol 102:5353–5360

Wolf C, Gaida D, Stuhlsatz A, Ludwig T, McLoone S, Bongards M (2013) Predicting organic acid concentration from UV/vis spectrometry measurements—a comparison of machine learning techniques. T I Meas Control 35:5–15

Wolf C, Gaida D, Bongards M (2014) Online monitoring of AD processes using a fully automated, low maintenance middle-infrared (MIR) measurement system. Conf Proc Int Sci Conf Biogas Sci 2014:24–25

Xiong Y, Mehrdad S (2003) Unknown disturbance inputs estimation based on a state functional observer design. Automatica 39:1389–1398

Yasui H, Sugimoto M, Komatsu K, Goel R, Li YY, Noike T (2006) An approach for substrate mapping between ASM and ADM1 for sludge digestion. Water Sci Technol 54:83–92

Yasui H, Goel R, Li YY, Noike T (2008) Modified ADM1 structure for modelling municipal primary sludge hydrolysis. Water Res 42:249–259

Zadeh LA (1965) Fuzzy sets. Inf Control 8:338–353

Zhang ML, Sheng GP, Mu Y, Li WH, Yu HQ, Harada H, Li YY (2009) Rapid and accurate determination of VFAs and ethanol in the effluent of an anaerobic H2-producing bioreactor using near-infrared spectroscopy. Water Res 43:1823–1830

Zhou H, Li H, Wang F (2012) Anaerobic digestion of different organic wastes for biogas production and its operational control performed by the modified ADM1. J Environ Sci Health A Toxic Hazard Subst Environ Eng 47:84–92

Zumbusch PV, Meyer-Jens T, Brunner G, Märkl H (1994) On-line monitoring of organic substances with high-pressure liquid chromatography (HPLC) during the anaerobic fermentation of waste-water. Appl Microbiol Biotechnol 42:140–146

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Prediction of anaerobic biodegradability and bioaccessibility of municipal sludge by coupling sequential extractions with fluorescence spectroscopy: Towards ADM1 variables characterization

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ABSTRACT

Advanced dynamic anaerobic digestion models, such as ADM1, require both detailed organic matter characterisation and intimate knowledge of the involved metabolic pathways. In the current study, a methodology for municipal sludge characterization is investigated to describe two key parameters: biodegradability and bioaccessibility of organic matter. The methodology is based on coupling sequential chemical extractions with 3D fluorescence spectroscopy. The use of increasingly strong solvents reveals different levels of organic matter accessibility and the spectroscopy measurement leads to a detailed characterisation of the organic matter. The results obtained from testing 52 municipal sludge samples (primary, secondary, digested and thermally treated) showed a successful correlation with sludge biodegradability and bioaccessibility. The two parameters, traditionally obtained through the biochemical methane potential (BMP) lab tests, are now obtain in only 5 days compared to the 30–60 days usually required. Experimental data, obtained from two different laboratory scale reactors, were used to validate the ADM1 model. The proposed approach showed a strong application potential for reactor design and advanced control of anaerobic digestion processes.

1. Introduction

In the current context, where anaerobic digestion has become a key process for organic matter treatment and energetic valorization, precise control and prediction of process performance is a must-be. For that purpose, advanced mathematical models have to be implemented. Ten years ago, the International Water Association (IWA) specialist group on anaerobic digestion developed the Anaerobic Digestion Model N° 1 (ADM1) (Batstone et al., 2002). The major elements of the model were biological pathways through the identification of limiting step and detailed organic matter characterization. Indeed, according to Angelidaki and Sanders (2004) and Buffiere et al. (2006), the methane yields depend on the nature

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Nomenclature

AD	Anaerobic Digestion
ADM1	Anaerobic Digestion Model N° 1
AMPTS	Automated Methane Potential Test System
BD	Anaerobic BioDegradability %
BMP	Biochemical Methane Potential NmlCH$_4$ gCOD^{-1}
COD	Chemical Oxygen Demand gO$_2$ L^{-1} or gCOD L^{-1}
DOM	Dissolved Organic Matter
DOM_fluo	Fluorescent DOM
EPS	Extracellular Polymeric Substances
fxc$_{r/s}$_xpr, fxc$_{r/s}$_xch, fxc$_{r/s}$_xli	Protein, carbohydrates, lipids compound fraction in X$_{RC}$ or X$_{SC}$ variables of ADM1%
FRI	Fluorescence Regionalization Integration
HSL	Humic Substances Like
IWA	International Water Association
LPF	Liquid Phase Fluorescence
Km_(Xbio_X$_{PR}$, Xbio_X$_{CH}$, Xbio_X$_{LI}$)	Growth rate of hydrolytic biomass (protein, carbohydrates, lipids) d^{-1}
Kdec_(Xbio_X$_{PR}$, Xbio_X$_{CH}$, Xbio_X$_{LI}$)	Decay rate of hydrolytic biomass (protein, carbohydrates, lipids)
K$_S$_X_ac	Half saturation constant of acetoclastic methanogen biomass kg m^{-3}
$P_f(i)$	Fluorescence percentage of a zone *i* %
PLS	Partial Least Square
R^{2}Y	Cumulated variance for Y variable/Correlation coefficient
RMSE	Root Mean Square Error
RMSEP	Root Mean Square Error of Prediction
RSF	Relative Sensitive Function
RE-EPS	Readily Extractible EPS
Q^2	Percent of variation of Y predicted by model in cross-validation
S_cat	Cations content variable in ADM1 Kmol.m^{-3}
S-EPS	Soluble EPS
S_IC	Inorganic carbon content variable in ADM1 Kmol.m^{-3}
SI	Primary Sludge
SII	Secondary Sludge
SD	Digested Sludge
STT	Thermally Treated Sludge
VFA	Volatile Fatty Acids
VS	Volatile Solids content gVS L^{-1}
WWTP	Waste Water Treatment Plant
X$_{RC}$	Readily biodegradable particulate COD kgCOD m^{-3}
X$_{SC}$	Slowly biodegradable particulate COD kgCOD m^{-3}
3D-EEM	3 Dimension Excitation Emission Matrix (3D-EEM) fluorescence spectroscopy

of substrate characterization. Besides, a key-point for a successful description of a bioprocess using a mathematical model is a good influent characterisation (Huete et al., 2006). From 1969 to 2002, researchers made important efforts to better understand and represent anaerobic digestion as mentioned by a review from Jimenez et al. (2013b). The historical evolution showed a “complexification” of the models, searching to detail more the metabolic pathways, and closer to the reality. The increasing substrate complexity led to a more detailed model input, taking into account the main biochemical families: lipids, proteins and carbohydrates. However, Kleerebezem and van Loosdrecht (2006) admitted that identification of individual substrate concentrations from ADM1 requires specific and not easily available analytical techniques. Many authors have proposed experimental protocol or methodologies in order to characterize ADM1 input variables.

Kleerebezem and van Loosdrecht (2006), Zaher et al. (2009) and Huete et al. (2006) proposed the techniques which consist on lumping variables with practical analysis. However, there are many assumptions on the carbon and nitrogen inert content, on biochemical fractionation and on the non-biodegradable variables. Another technique consists on mapping activated sludge models variables with the ADM1 variables (Copp et al., 2003; Jones et al., 2008; Nopens et al., 2009; Ekama et al., 2007). The main drawbacks of this method are the knowledge of the whole wastewater treatment plant, the assumptions made about non-biodegradable fraction (i.e. non-biodegradable fraction in activated sludge is the same than in anaerobic digestion) and about biochemical fractions mapping.

The last technique found in the literature is the methane production curve identification (Yasui et al., 2006, 2008; Girault et al., 2012; Mottet et al., 2013). However, using low ratios of substrates on biomass allowed decreasing batch test duration (i.e. 4–10 days) but it could bring underestimations possible of entire biodegradable fractions and an overestimation of non-biodegradable fraction. In the case of the model developed by Yasui et al. (2008), fractionation has to be adapted for each type of substrate. Besides of a fine characterization of substrates, the limiting step of anaerobic digestion has to be well represented. For municipal sludge, a particulate and complex substrate, hydrolysis of macromolecules has been identified as the limiting step (Vavilin et al., 2008; Yasui et al., 2008; Ramirez et al., 2009). However, the classical ADM1 does not take into account the surface limitation and enzymatic colonization occurring during the hydrolysis of sludge (Vavilin et al., 2008; Mottet et al. 2013). In terms of organic matter characterisation of complex substrates, three concepts are of the most relevance: the bioavailability, the bioaccessibility and the biodegradability. Various definitions of bioavailability are used across many disciplines. Aquino et al. (2008) defined bioavailability as the direct access to the molecule to be degraded by a microorganism. Molecules with a weight below 1000 Da can pass through the cell wall. Due to the complex organisation of sludge, bioaccessibility is mainly defined for the particles by their possible access to the molecule depending on the digestion time, hydrolytic activity and the pretreatment applied to the sludge (a molecule bioaccessible could become bioavailable with a sufficient HRT). And finally, the biodegradable fraction is the organic matter bioavailable consumed by the biomass.

Both were traditionally obtained from the laboratory batch test used to determine the biochemical methane potential (BMP). Although reliable, it is a time consuming and tedious methodology. Many authors wanted to predict BMP value using characterization information such as biochemical fractionation (Chandler et al., 1980; Gunaseelan, 2009; Mottet et al., 2010), aerobic tests (Cossu and Raga, 2008; Scaglia et al., 2010), elemental analysis (Davidsson et al., 2007; Shanmugam and Horan, 2009) or near infrared spectroscopy (Lesteur et al., 2011). But these techniques are sometimes not applied for sludge or not validated enough by data and they do not take into account the bioaccessibility aspect. The main conclusion, withdrawn from the mentioned above, is the lack of a rapid and pertinent tool to determine in municipal sludge both anaerobic biodegradability and bioaccessibility for hydrolysis prediction and dynamic models implementation.

In the recent years, a new promising technology – the three dimensional excitation emission matrix (3D-EEM) fluorescence spectroscopy – has been widely used for qualitative characterization of Extracellular Polymeric Substances (EPS) extracted from sludge (Esparza-Soto and Westerhoff, 2001; Sheng et al., 2011; Li et al., 2008; Henderson et al., 2009; He et al., 2013; Muller et al., 2013). Coupled with sequential extractions of municipal sludge, the technique also revealed information on bioaccessibility (Wang et al., 2010). Results were encouraging to establish the link between complexity, sludge stabilisation degree and accessibility (i.e.: the complexity of the EPS found in dissolved matter is different compared to the bound EPS). Concerning anaerobic digestion, Wan et al. (2012) showed the potential of the fluorescence spectroscopy to be linked with anaerobic biodegradation of cattle and duck manure. They compared digestion and co-digestion of both substrates. Through the 3D-EEM analysis of dissolved organic matter sampled through the digestion time, authors identified molecules remaining after digestion. Thanks to different fluorescence intensity peak ratios (protein-like on fulvic acid-like, and protein-like on humic acid-like), they showed that fulvic acid-like and humic acid-like remained stable during both separated digestion and co-digestion whereas the aromatic proteins tyrosine-like decreased, suggesting hydrolysis of these molecules into non-fluorescent structures.

Based on these observations, this study focuses on developing a performing and practical methodology to characterize anaerobic digestion model input variables linked with the biodegradable and bioaccessible fractions of municipal sludge.

2. Material and methods

2.1. Sludge samples

Fifty two municipal sludge samples were recovered through a large measurement campaign in wastewater treatment plants in Europe. They were of different nature: 6 samples are primary sludge, 25 are secondary sludge, 15 are anaerobic sludge and 8 are thermally treated secondary sludge. Nomenclature were created as following:

- "S" for sludge and "R" for refusal (from screeners for example)
- "I", "II", "D" or TT temperature for respectively primary, secondary, digested and thermal treatment
- Alphabetical letter from the wastewater treatment plant name

2.2. Volatile Fatty Acids

Volatile Fatty Acid (VFA) concentrations were measured by gas chromatography (7890A Agilent), from acetate to heptanoate.

2.3. Biochemical methane potential test

Biochemical Methane Potential (BMP) measurement tests were performed according to Angelidaki and Sanders (2004) in the Automated Methane Potential Test System (AMPTS) from BIOPROCESS CONTROL®. BMP was calculated by normalizing the cumulated production of volume methaneV_{CH4} with the COD mass of the substrate introduced COD_0 (equation (1)). From the Buswell formula and the organic matter oxidation reaction, the theoretical BMP is 350 $NmL_{CH_4}\cdot gCOD^{-1}$ (Angelidaki and Sanders, 2004). Biodegradability BD could be calculated from BMP (equation (2)).

$$\mathrm{BMP}\left(\mathrm{Nml}_{\mathrm{CH_4}}\cdot g^{-1}_{\mathrm{COD_0}}\right)=\frac{V_{\mathrm{CH_4}}}{\mathrm{COD_0}} \tag{1}$$

$$\mathrm{BD}(\%)=\frac{\mathrm{BMP}}{350}\times 100 \tag{2}$$

Bioaccessibility, represented in this study by the readily X_{RC} and slowly X_{SC} biodegradable fractions of the modified model ADM1 from Mottet et al. (2013), was obtained from methane production curve interpretation. The Figure A1 from the Appendix A showed the variables identification. Indeed, from experimental data, sludge biodegradation is composed of two phases. The readily hydrolysable fraction X_{RC} is biodegraded in the first phase while the slowly hydrolysable fraction X_{SC} is biodegraded in the second phase. Then, reporting the time when X_{RC} is completely degraded to the cumulated production curve, the ratio cumulated V_{CH4} (X_{RC}) on V_{CH4} (final) corresponds to the fraction X_{RC} of biodegradable COD. To normalize this value, this ratio is multiplied by BD (Equation (3)). The remaining biodegradable COD composed of slowly biodegradable organic matter through the variable X_{SC} is then deduced from BD and X_{RC} (Equation (4)).

$$X_{RC}(\%\mathrm{COD})=\frac{V_{\mathrm{CH_4}}(X_{RC})}{V_{\mathrm{CH_4}}(\mathrm{final})}\times \mathrm{BD}(\%) \tag{3}$$

$$X_{SC}(\%\mathrm{COD})=\mathrm{BD}(\%)-X_{RC}(\%\mathrm{COD}) \tag{4}$$

2.4. Chemical sequential extractions

Based on the sludge floc definition, the applied sequential extraction correlates bioavailability of sludge organic matter to its chemical accessibility. The performed protocol was based on Muller et al. (2013) but optimised to reduce the number of sequential extractions to 4 instead of 20. This

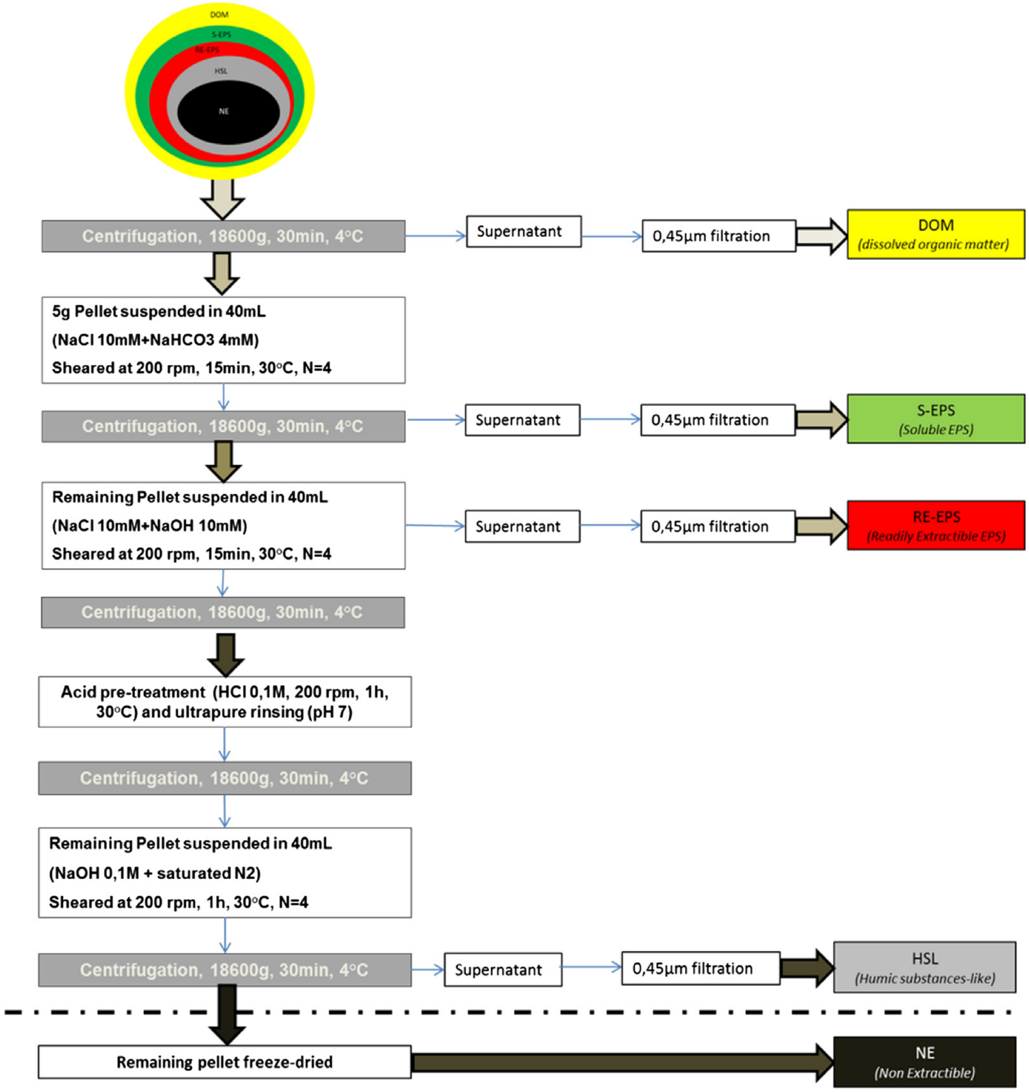

Fig. 1 – Sequential extraction protocol.

optimisation allowed reaching the sludge fractionation in only 5 days instead of 15 or more days initially needed. The obtained fractions were Dissolved Organic Matter (DOM) obtained by centrifugation and filtration at 0.45 µm, soluble EPS (S-EPS) obtained by washing the remaining pellet with a neutral saline solution, readily bound EPS (RE-EPS) obtained by saline basic extraction and Humic Substances Like (HSL) obtained by a strong basic extraction. The extraction procedure is schematized by Fig. 1.

2.5. *Fluorescence spectroscopy analysis*

Fluorescence is a light emission provided by the molecule excitation, usually by absorption of a photon, followed by a spontaneous emission. The fluorescence spectrometer used was a Perkin Elmer LS55 in liquid phase fluorescence mode (LPF). Excitation wavelengths varied from 200 to 600 nm with increments of 10 nm. The slit width of emission and excitation monochromators was fixed at 10 nm. Scanning monochromator speed was about 1200 nm s^{-1}. Fluorescence values were recorded every 0.5 nm between 200 and 600 nm. Based on Chen et al. (2003) and He et al. (2013), spectra were decomposed on seven zones (zone I to VII) corresponding on each molecule families-like fluorescence. The Figure B1 from the Appendix B shows an example of spectra decomposed into 7 fluorescences zones. The proportion of fluorescence of a zone "i" P_f (i) was calculated from the fluorescence zone volume (Equations (5) and (6)). Combined with chemical extractions, the methodology was named 3 Dimension Excitation Emission Matrix in Liquid Phase Fluorescence (EEM LPF).

$$V_f(i)(\mathrm{U.A./mg.COD.L}=1)=\frac{V_{\mathrm{image}J(i)}}{\mathrm{COD}_{\mathrm{sample}}}\times 1/\frac{S(i)}{\sum_{i=1}^{7}S(i)} \tag{5}$$

$$P_f(i)(\%)=\frac{V_f(i)}{\sum_{i=1}^{7}V_f(i)}\times 100 \tag{6}$$

where

V_f (i) (U.A. mg COD L^{-1}) is the fluorescence zone volume of a zone "i"

$V_{\mathrm{image\ J}}$ (i) (U.A.) is the raw volume obtained in IMAGE J (U.A)

$\mathrm{COD}_{\mathrm{sample}}$(mg L^{-1}) is the COD concentration of the sample analyzed

S(i) (nm^2)is the area of a zone i
P_f(i) (%) is the fluorescence proportion of a zone i

2.6. Laboratory scale reactors

Two laboratory scale reactors (named P1 and P2) fed with mix sludge (i.e. primary and secondary sludge mix) coming from full scale plants were used for anaerobic digestion modelling. Mixed sludge was provided once a week by the wastewater treatment plant. Lab scale reactors consisted of 4 L glass reactors cylinder round-bottom-shaped stirred by an impeller with seascape (rotor) blades. Temperature of 35 °C was maintained in the reactors through a double wall fed by a water bath. Biogas flow-rate was measured by a milligascounter Ritter MGC-1V3.0 and its composition was measured by a Bluesense system. Hydraulic retention time was 18 days and the organic load was 0.13 gCOD $gVS_{reactor}^{-1}$ d^{-1} for both reactors. The reactor P1 was the "reference" reactor since it was fed by the same sludge during all the collection data. The reactor P2 was the "disturbance" reactor. During the first 42 days of operation, P1 and P2 were fed by the same sludge and operating at the same conditions in order to reach the steady-state and to calibrate the model: it was the "reference" period. After that, P2 was fed by the same sludge than P1 but with changes in the organic matter. Indeed, the most accessible fractions were removed (DOM, S-EPS and RE-EPS): it was the "disturbance" period. The aim of this experiment was to validate the model with organic matter bioaccessibility disturbations. Feed sludge stored at 4 °C was changed once a week. Detailed characterization was made once a week and classical data collection (COD, pH, VFA, gas flowrate, methane flowrate) was performed each day.

2.7. Anaerobic digestion modelling

The dynamic model of anaerobic digestion used was the Mottet et al. (2013) implemented in the WEST software (DHI®). It is based on the classical ADM1 but it considers two major modifications to consider the bioaccessibility concept and the hydrolysis as the limiting step:

- The organic complex substrate was replaced by two substrates X_{RC} and X_{SC} for respectively readily hydrolysable COD and slowly hydrolysable COD.
- The disintegration and hydrolysis processes were not modelled by a first-order equation but by a Contois model in order to take into account the surface limitations and enzymatic colonization by enzymatic biomass.

Stoichiometric parameters ($fxc_{r/s}$_xpr, $fxc_{r/s}$_xch, $fxc_{r/s}$_xli) was defined as dynamic variables and were calculated from the biochemical measurement according with Mottet et al. (2013) and biochemical protocols recommended by Jimenez et al. (2013a) for carbohydrates, proteins and lipids. The not biodegradable fractions ($fxc_{r/s}$_xi, $fxc_{r/s}$_si) were calculated thanks to the BMP test and were assumed to be the same for X_{RC} and X_{SC}. The X_{RC} and X_{SC} variables, such as X_I fraction was measured by the new technology developed in this paper.

2.8. Partial least square regression

In order to find correlations between BD, X_{RC} and indicators from SE-3D-LPF, partial least square (PLS) regressions were performed. The software used SIMCA from UMETRICS.

Partial least squares regression is an extension of the multiple linear regressions. In its simplest form, a linear model specifies the (linear) relationship between a dependent (response) variable Y, and a set of predictor variables, the X's.

The parameters from PLS models used to assess the model robustness are the following:

- Correlation coefficient R^2
- Root Mean Square Error (RMSE), used as an accuracy measurement of differences between values predicted and model values observed.
- RMSEP is the RMSE for the prediction of validation samples (not included in calibration PLS model)
- Q^2: percent of variation of Y predicted by model according to cross-validation. This parameter indicates how well the model predicts the data. A large Q^2 (>0.5) indicates good predictivity. Moreover, it is a compromise between mean square error and R^2. Q^2 represents also the criteria of component number choice. When cumulated Q^2 reach its maximum value, the corresponding component is chosen.

3. Results and discussion

3.1. Extracted fractions and fluorescence footprint

Fractionation obtained by chemical extractions was performed for all the sludge samples. As primary, secondary, digested and thermally treated sludge were used, analysis of the repartition of the fractions was performed in order to check the variability and profile differences of each sludge. Median of all the sludge samples was about 41% of total COD and half of the sludge samples were extracted with percentages between 38 and 52% of total COD. This extraction yield is not very high (i.e. about 50% of COD was not extracted). However, some preliminary tests (personal data) showed that in average 77% of the extracted matter is biodegraded under anaerobic conditions. Besides 30 days' BMP tests of non-extracted fractions revealed low biodegradability values (16–23%). Therefore, the extracted organic matter has been considered enough representative of the biodegradable part of the sludge. Concerning the extracted fractions, primary, secondary and anaerobic digested sludge have similar profiles. They are mainly composed of HSL and RE-EPS representative of the less chemically accessible fractions. HSL compartment, the least accessible, was the main extracted part of the sludge (from median values of 25–30% of COD). RE-EPS fraction was extracted with a median value between 5 and 8% of COD. DOM and S-EPS, the more accessible fractions, were less extracted with medians respectively of 3–8% of COD and 1–5% of COD. Whereas fractionation did not lead to discriminant profile for primary, secondary and anaerobic sludge, thermally treated sludge had a specific profile in agreement with the thermal solubilization occurring in the pre-treatment. DOM and S-EPS

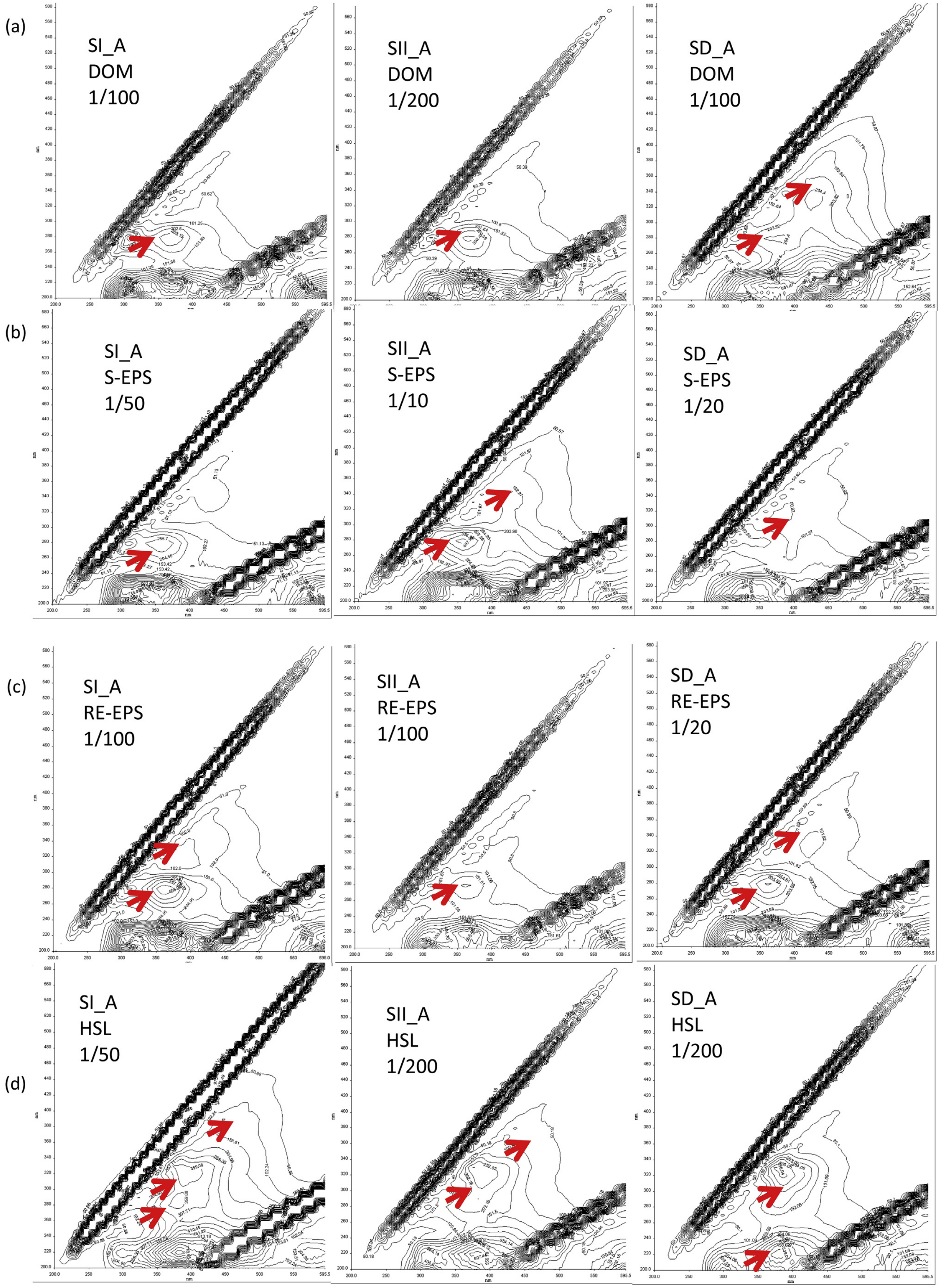
(a)
SI_A DOM 1/100
SII_A DOM 1/200
SD_A DOM 1/100
(b)
SI_A S-EPS 1/50
SII_A S-EPS 1/10
SD_A S-EPS 1/20
(c)
SI_A RE-EPS 1/100
SII_A RE-EPS 1/100
SD_A RE-EPS 1/20
(d)
SI_A HSL 1/50
SII_A HSL 1/200
SD_A HSL 1/200
nm
200.0
250
300
350
400
450
500
550
595.5

Table 1 – PLS regression performances parameters.

Y-variable	PLS regressions	Number of components	Calibration				Validation	
			R^2X	R^2	Q^2	RMSE	R^2	RMSEP
BD	28 variables + VFA	5	0.877	0.802	0.687	7.6%	Not selected	
	28 variables	5	0.877	0.827	0.694	7.1%	0.8546	8.6%
X_{RC}	28 variables + VFA	6	0.909	0.882	0.723	5.5%	0.8958	6.4%
	28 variables	6	0.912	0.863	0.631	5.9%	Not selected	

were highly extracted with respectively 30% and 8% of COD and HSL such as RE-EPS were less extracted (respectively 5% and 18% of COD). From these observations, one conclusion was that the complexity of the molecules was not explained through the only extracted fractions despite of a good simulation of accessibility compartments. Consequently, how to characterize the biodegradable part of each fraction? A possible answer was inspired from the literature review. Indeed, some authors used 2D fluorescence technique for 5 days' biological oxygen demand assessment (Reynolds and Ahmad, 1997; Henderson et al., 2009). More recently, Muller et al. (2011) showed the potential of such a tool when applied on solid wastes through solid phase fluorescence measurement, revealing complexity. Spectroscopy fluorescence seemed to be a promising tool for complexity characterization.

In order to go further in studying the ability of fluorescence to highlight complexity, spectra from fractions extracted from primary, secondary and anaerobic digested sludge sampled in the same wastewater treatment plant were studied. Evolution of fluorescence spectra for each fraction is highlighted by Fig. 2a–d. Spectra from fraction considered were presented for SI_A, SII_A and SD_A, by decreasing order of anaerobic biodegradability (respectively 51.1, 43.5 and 16.5%). The general trend showed that the less biodegradable was the total sludge, the more complex peaks in all the fractions. Indeed, the more the peaks were located on the right of the spectra, the more complex the organic matter. That means fluorescence had a real potential to be linked with biodegradability. Another general observation was that spectra obtained after fractionation of all sludge samples showed that complexity increased when accessibility decreased as in Wang et al. (2010) study. Indeed, from DOM spectra to HSL spectra, there was an evolution of the fluorescence peaks to the right. Concerning DOM fraction, fluorescence zone I, II and III (protein-like compounds) were the main peaks in the primary and secondary sludge whereas in digested sludge DOM had a poorest fluorescence zone III and a main peak in fluorescence zone VI. The fluorescence zone VI is usually associated to lignocellulose-like compound (Muller et al., 2011), humic acid-like (Chen et al., 2003) or melanoidin-like compound fluorescence, known to be recalcitrant and slowly biodegradable (Chandra et al., 2008). Similar observations were made for S-EPS. In RE-EPS fraction, primary sludge had a higher fluorescence zone III than secondary sludge. Protein-like compounds from fluorescence zone III would seem a biodegradability indicator (as Reynolds and Ahmad, 1997 work with BOD_5). This zone was still present in RE-EPS from digested sludge but peak in fluorescence zone VI showed that this fraction was complex too. Finally, HSL fractions fluorescence evolved too. The three spectra were composed of fluorescence zone IV and VI peaks showing that this slowly accessible fraction was the most complex too. Besides, a peak in fluorescence zone III was still apparent in primary sludge whereas it led to disappear in secondary sludge and was absent in digested sludge. Fluorescence zone VI was also higher in digested sludge than in primary and secondary sludge. Therefore, thanks to qualitative observation made from fluorescence spectra, total sludge biodegradability and chemical accessibility evolved in the

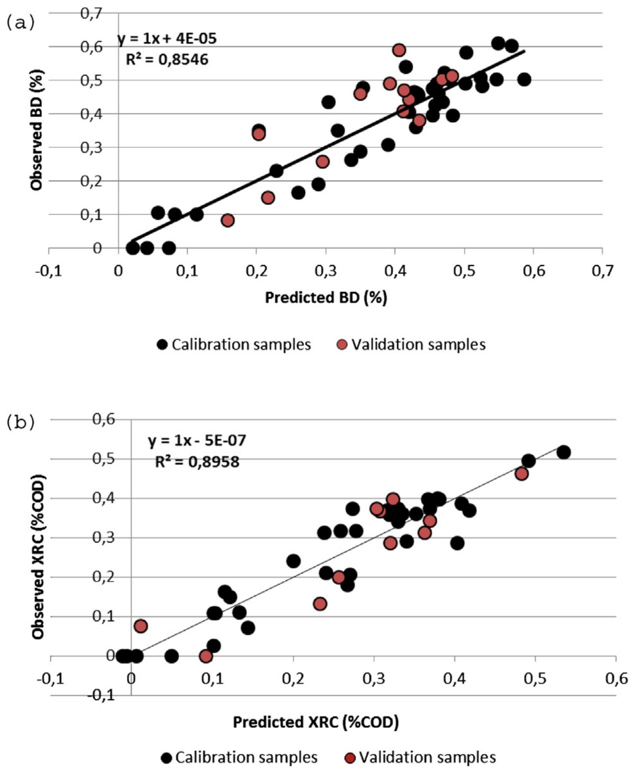

Fig. 3 – Observed versus predicted biodegradability (a) and X_{RC} (b).

Fig. 2 – 3D-LPF spectra obtained for extracted fractions of SI_A, SII_A and SD_A. (a) DOM 3D-LPF spectra. (b) S-EPS 3D-LPF spectra. (c) RE-EPS 3D-LPF spectra. (d) HSL 3D-LPF spectra.

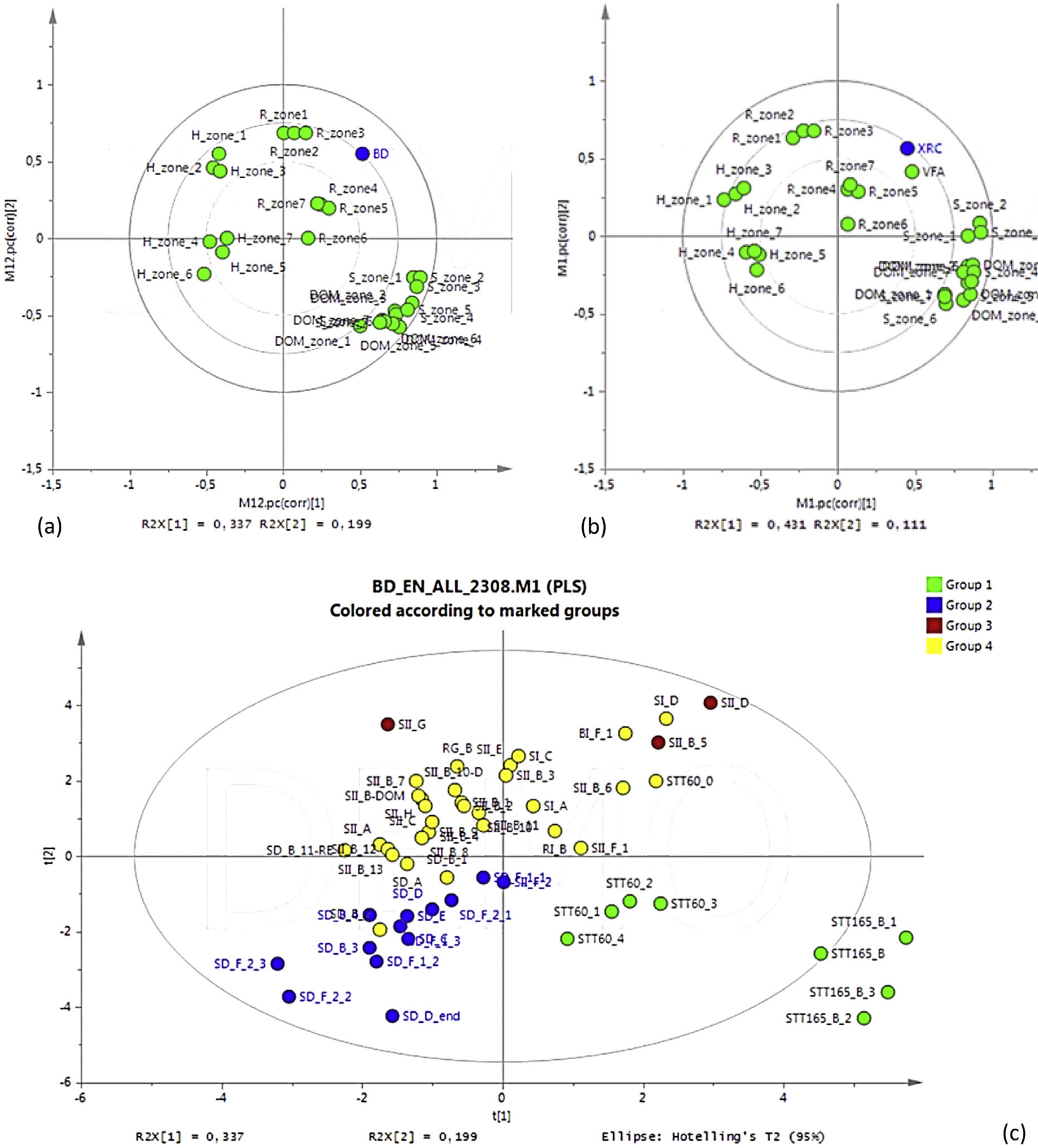

Fig. 4 – Correlation circles obtained for BD with X-variables (a) and the observations used (b) in the PLS regression and correlation circles obtained for X_{RC} with X-variables (c).

same way than the complexity. From the most biodegradable sludge to the less one, complex molecules fluorescence increased in each fraction.

3.2. *Indicator of biodegradability building*

The only consideration of organic matter fractions was not enough discriminant to predict both biodegradability BD and readily bioaccessible fraction X_{RC} in all type of sludge Indeed, as previously said, no discriminant fractionation appeared between digested and not digested sludge. Each fraction extracted could be biodegradable or not. A biodegradability indicator for each fraction was still missing. To solve this problem, we have shown that fluorescence spectroscopy could be correlated with biodegradability. One solution was to use this technique on each extracted fraction in order to reveal its complexity. The idea is to combine the accessibility feature brought by sequential extractions with the complexity aspect brought by the fluorescence measurement of each fraction. Moreover, by considering the whole information from these spectra, the volume of each zone associated to each fraction extracted was calculated. An indicator was then obtained from the analysis measurement of the 52 sludge studied. The fluorescence percentage of a zone i $P_f(i)$ was

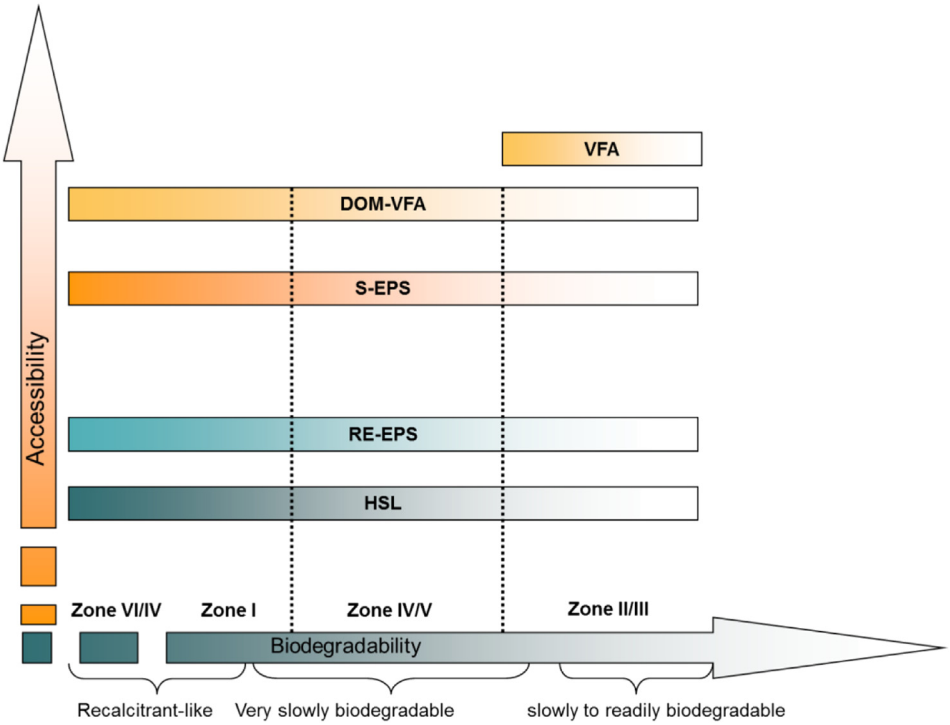

Fig. 5 – **Matrix representation of the indicators contribution to BD and X_{RC} prediction.**

Table 2 – **Steady-state values of state variables from ADM1 with calibrated values parameters.**

Calibration parameter	Unit	Default value (temperature °C)	Calibration value	Impact
Km (Xbio_X_{PR}, Xbio_X_{CH}, Xbio_X_{LI})	d^{-1}	10 (55 °C)	5 (35 °C)	Decrease the COD particulate concentration of hydrolytic biomass
kdec (Xbio_X_{PR}, Xbio_X_{CH}, Xbio_X_{LI})	d^{-1}	0.04 (55 °C)	0.2 (35 °C)	
K_S_X_ac	kg m^{-3}	0.15 (35 °C)	0.40 (35 °C)	VFA calibration (increase)
Constant k S_cat = S_IC + k	–	0.035 (Personal data)	0.025	pH calibration Ammonium

Table 3 – **Steady-state values of state variables from ADM1 with calibrated values parameters.**

Variable	Units	Model before calibration (pilot 2)	Model after calibration (pilot 2)	Experiment data steady-state (pilot 2)	Relative error (%)
pH	–	7.76	7.49	7.51	−0,3
COD particulate	kg m^{-3}	36.05	30.39	30.44	−1
COD total	kg m^{-3}	38.09	32.61	32.53	0
COD soluble	kg m^{-3}	2.04	2.23	2.08	8
S_ac	kg m^{-3}	0.006	0.12	0.016–0.316	27
S_pro	g L^{-1}	0.019	0.02	0.02	7
Q_BG	kg m^{-3}	2.93	2.58	2.50	3
%CH_4	%	0.68	0.64	0.64	0
S_INN	kmol m^{-3}	2310.00	1512.09	1331.00	14

multiplied by the fraction COD percentage called *Fract*. From the 4 fractions chemically extracted and the 7 fluorescence zones, there were 28 indicators for a sludge sample as explained by Equation (7).

$$\text{Fract}_{\text{zone i}} = \sum_{i=1}^{\text{VII}} P_f(i) \times \text{Fract}(\%\text{COD}) \qquad (7)$$

The DOM fraction from secondary, thermally treated and primary sludge contained a significant part of volatile fatty acids (VFA) (respectively 36.6, 23.0 and 67.6% of COD). However, VFA are not naturally fluorescent. Thus, for the DOM fraction, the equivalent COD from VFA was subtracted to have a correct estimation of the fluorescent COD. Moreover, VFA were included as an explicative variable on top of the 28 other ones.

3.3. *Biodegradability and bioaccessibility prediction*

To investigate the correlation between the explicative variables and biodegradability, two partial least square (PLS) regressions were performed for all sludge samples. In the first one, the explicative variables (*X*-variables) considered were the 28 indicators and the VFA percentage of the total COD. The explained variables (*Y*-variables) were the biodegradability BD and the readily bioaccessible variable X_{RC}. In the second one, *X*-variables were only represented by the 28 indicators. The quality of the model was represented by values obtained for R^2X (cumulated variance for the explicative variables), R^2 (correlation coefficient), Q^2 (prediction quality when upper than 0.5) and RMSE and RMSEP as presented in Table 1. The validation samples were sorted out according to the increasing biodegradability or bioaccessibility values. One sample out of four was used for validation.

The PLS regression showed that there was a good correlation between the *X* and *Y* variables. Concerning BD, the best model quality parameters were obtained without including VFA percentage in the regression models. On the opposite, for the prediction of X_{RC}, the best results were obtained when including VFA in the explicative variables. VFA variable was poorly correlated with BD whereas it was highly correlated with X_{RC}. Therefore, this variable was more important for bioaccessibility prediction than for biodegradability concerning municipal sludge. Indeed, VFA must play a higher role in accessibility mechanisms than in the biodegradability aspect. Quality of models such as their validation with sludge samples was shown by Fig. 3a and b for respectively BD and X_{RC}. Line of perfect fit ($y = x$) was closed to the repartition of observed *Y*-variable versus predicted *Y*-variable for both BD and X_{RC}. Both correlation coefficients were closed to 1 (0.8546 for BD and 0.8958 for X_{RC}) and mean errors of prediction were respectively 5.5 and 3.8% for BD and X_{RC}. Error prediction RMSEP was 8.6% for BD and 6.4% for X_{RC}. From these results, the methodology developed was able to predict with accuracy both biodegradability and bioaccessibility in a range of values between 0 and 60% of COD.

Correlation circles plot of the *X* variables obtained from the PLS study (Fig. 4a) showed that BD was correlated positively with RE-EPS variables and soluble variables (S-EPS and DOM) in the first component whereas it was correlated negatively with all zones from HSL. Then, in the second component, BD was negatively correlated with fluorescence zone IV to VI from HSL and with all the S-EPS and DOM fluorescence zones. These observations were in agreement with the sludge samples repartition in the second correlation circles plot (Fig. 4b). Indeed, thermally treated sludge (green-coloured) had a positive influence on biodegradability through the variables DOM

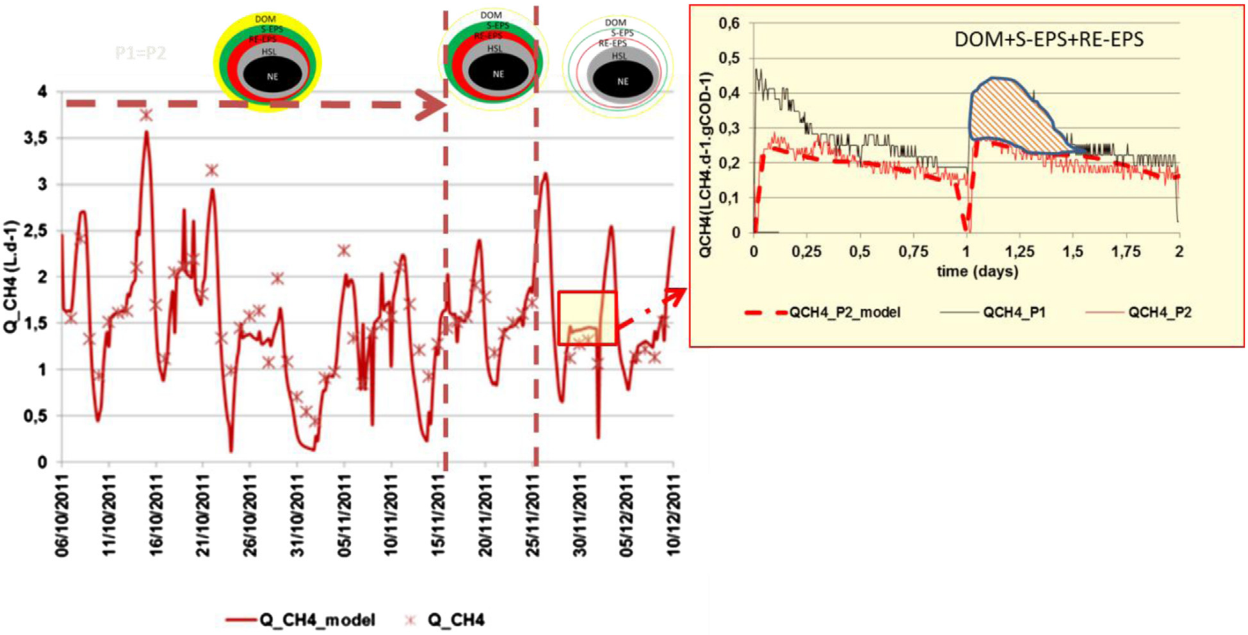

Fig. 6 – Results obtained on methane production by the two lab reactors dynamic modelling.

and SEPS for all fluorescence zones in the first component. The negative influence of these sludge samples observed in the second component was certainly due to the complex compounds present at high temperatures (fluorescence zones IV to VI). Anaerobic digested sludge (blue coloured) was in the same position than HSL from fluorescence zone IV to VI and had negative influence through both first components on BD, as expected. In a contrary way, primary sludge was all correlated positively with BD and had an influence through all zones of RE-EPS variables.

Concerning X_{RC} prediction, there was a coherent hierarchy of indicators in regards with X_{RC} meaning (Fig. 4c). VFA, the easiest accessible fraction, had the most positive impact on X_{RC}. Then, came fluorescence zones I to III from S-EPS easily biodegradable and accessible followed by all fluorescence zones from DOM and S-EPS (less easily biodegradable). Fluorescence zones I to III from RE-EPS were next correlated (biodegradable compounds in slowly accessible fraction). An intermediary was after found with fluorescence zones I to III from HSL (biodegradable compounds in very slowly accessible fraction). Finally, fluorescence zones IV to VII from HSL were correlated to the least biodegradable and bioaccessible compounds such as for BD prediction.

Based on this analysis, the positive coefficients were defined by the fractions from fluorescence zone II or III which are protein-like compounds fluorescence area. Fluorescence zones II and III from all fractions had a positive influence on biodegradability. Indeed, as mentioned by Henderson et al. (2009), several authors made correlations between protein fluorescence intensity from tryptophan peak and biodegradable oxygen demand concen tration.

Negative coefficients were defined by the HSL fraction through the fluorescence zones IV and VI (i.e. less accessible complex zones), the complex fluorescence zone VI from RE-EPS and the fluorescence zone I from DOM. It seemed curious because fluorescence zone I was a protein-like zone. In all fractions, zone I had a negative impact on BD whereas the two others fluorescence zones describing protein-like compounds were positive. However, zone I contained fluorescence from tyrosine-like compound. This amino acid was known to contain a phenol group, more or less hydrophobic. One assumption would be that the zone I fluorescence is related to proteins which are less biodegradable and/or not accessible. They could be contained into a hydrophobic complex structure. For example, some low polar proteins are embedded in cellular membranes, between the two-layers of phospholipids. When membrane cell is lysed, these molecules could be solubilized.

From the previous PLS interpretation, a matrix representation of indicators contribution to bioaccessibility and biodegradability is set up in Fig. 5.

This matrix was composed of two axes:

- bioaccessibility level depending on the fraction extracted considered,
- biodegradability level depending on the fluorescence zones found in the extracted fractions.

3.4. Validation of the methodology: ADM1 input variables characterization

The PLS equation models previously found to predict X_{RC}, X_{SC} and f_{xI} (i.e. 1-BD) were implemented in an external calculation tool in order to generate measurement of these state variables and parameter. The values obtained were added to the influent “specifier” of WEST software (DHI®). Using the modified model of Mottet et al. (2013), calibration and validation of two lab scale reactors were performed with few parameters change. Calibration step was first set up at steady-state using steady-state experimental values of P1 and P2 during the “reference” period. Indeed, Table 2 presents the parameters values used during the calibration. Default values of ADM1 are used for all the parameters. The only parameters values change was the growth rate kinetic of hydrolytic biomass parameters from the modified model as shown by Table 2. Indeed, the parameters first implemented came from a calibration of a batch test operating at 55 °C (Mottet et al., 2013). Consequently, they were modified during the calibration in order to simulate a slowlier hydrolysis at 37 °C and in order to properly predict the particulate COD in the digester. Results of this steady-state dataset simulation are presented in Table 3. The prediction of the main variables of ADM1 was satisfying, in regards with the low errors found between model and experimental data.

The “disturbance” dataset was used for validation step. During this period, the P1 reactor was fed by the same secondary sludge than previously. However, the second reactor (P2) was fed by the same sludge but deprived of some extracted fractions (DOM, S-EPS and RE-EPS). Results plotted in Fig. 6 showed that concerning the methane production, PLS predicted variables used to characterize the ADM1 input variables led to a satisfying fit of experimental data. Modelled methane production prediction for P2 fitted with experimental data such as its kinetic behaviour as shown by the zoom made in Fig. 6. Indeed, depriving the most accessible fractions of the sludge led to depriving the most readily biodegradable fractions. A superposition of the methane production curves of both pilots showed that the difference between them was due to the readily hydrolysable fraction biodegradation. This difference was explained by the removal of the most accessible fractions in the feed sludge. As explained by Yasui et al. (2006), we found the same repartition of degradable fractions with, on one hand the readily hydrolysable fraction and, on another hand, the slowly hydrolysable fraction. This means that the chemical accessibility fractionation defined in this study was well correlated to bioacce ssibility.

4. Conclusions

The 3D-SE-LPF methodology allowed to measure both the biodegradability and the readily/slowly bioaccessible sludge fractions X_{RC}/X_{SC}. Validation of the methodology was successfully performed through the modelling of two lab scale reactors. To determine optimal design of anaerobic digesters, the use of the PLS results and the ADM1 modified model led to an efficient methane production prediction and reactor performances. Moreover, PLS models could also be used for sensitive analysis to identify the recalcitrant molecules and their location in the sludge floc for further pre or post-treatments. Indeed, the fluorescence zones I and VI of the main fractions RE-EPS and HSL are the molecules to target in order to improve both bioaccessibility and biodegradability. Next step would be identifying which compounds are related with these molecules in order to propose some new pre or post-treatments. Further improvements would be made by decreasing the number of extraction for practical measurement. Indeed, to transform this methodology into an instrumentation tool for process control, the time of analysis would be crucial. Besides, building a fluorescence probe by selecting the most relevant excitation wavelength (for example 7 excitation wavelengths representing the main peaks observed to describe organic matter of sludge) would be a great improvement towards instrumentation purpose.

From the results obtained in this study, it is strongly believed that fluorescence spectroscopy will lead to main achievements in the near future for better knowledge of organic matter and for optimization of anaerobic digestion processes such as for make advanced treatment diagnostics.

Acknowledgements

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Appendix A. Experimental determination of X_{RC} and X_{SC} from bmp test curves

The Figure A1 presents the experimental methodology proposed to assess BD, the model variables X_{RC} and X_{SC}, based on Mottet et al. (2013) study.

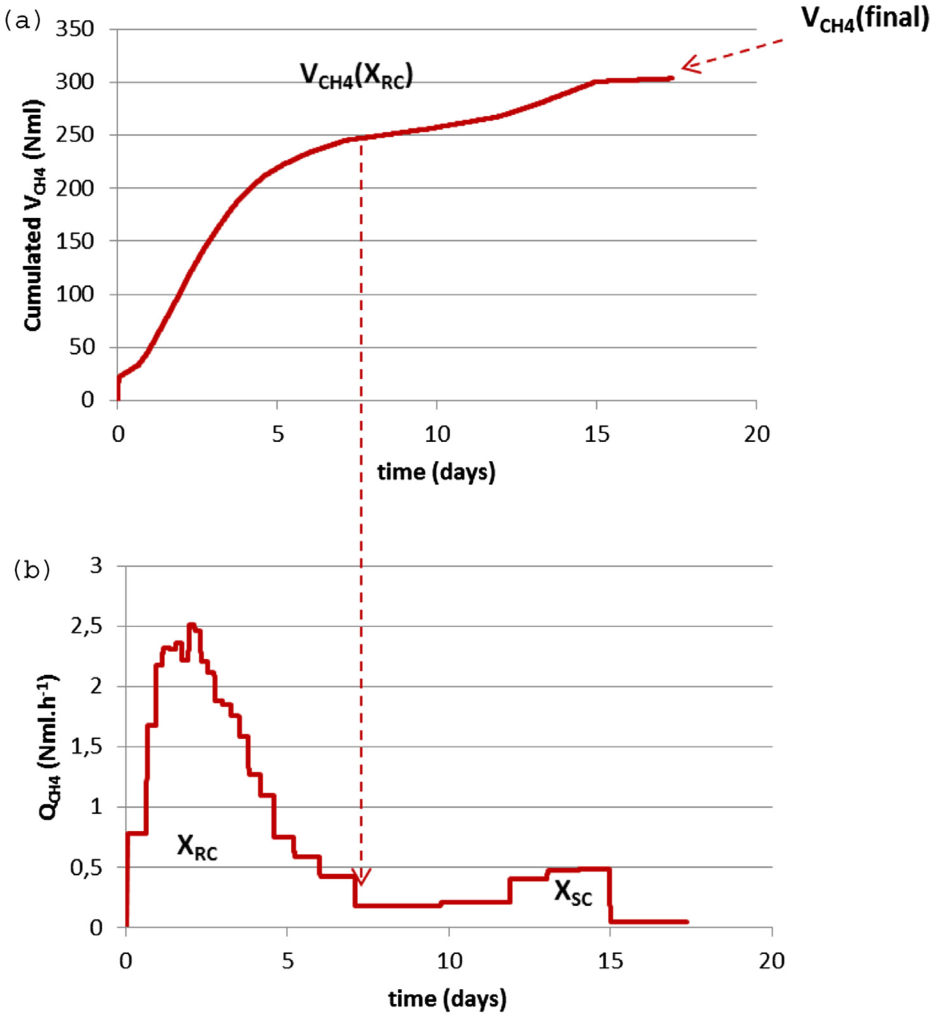

Figure A.1 – Cumulated methane production curve (a) and methane production rate curve (b) for the same sludge degradation with a ratio S/X = 1gCOD gVS^{-1}.

Appendix B. Definition of the fluorescence zones obtained by 3D fluorescence spectroscopy

The Figure B1 presents an example of 3D fluorescence spectra obtained. Based on Chen et al. (2003), a fluorescence regionalization could be proposed in order to identify and locate the fluorescent molecule families.

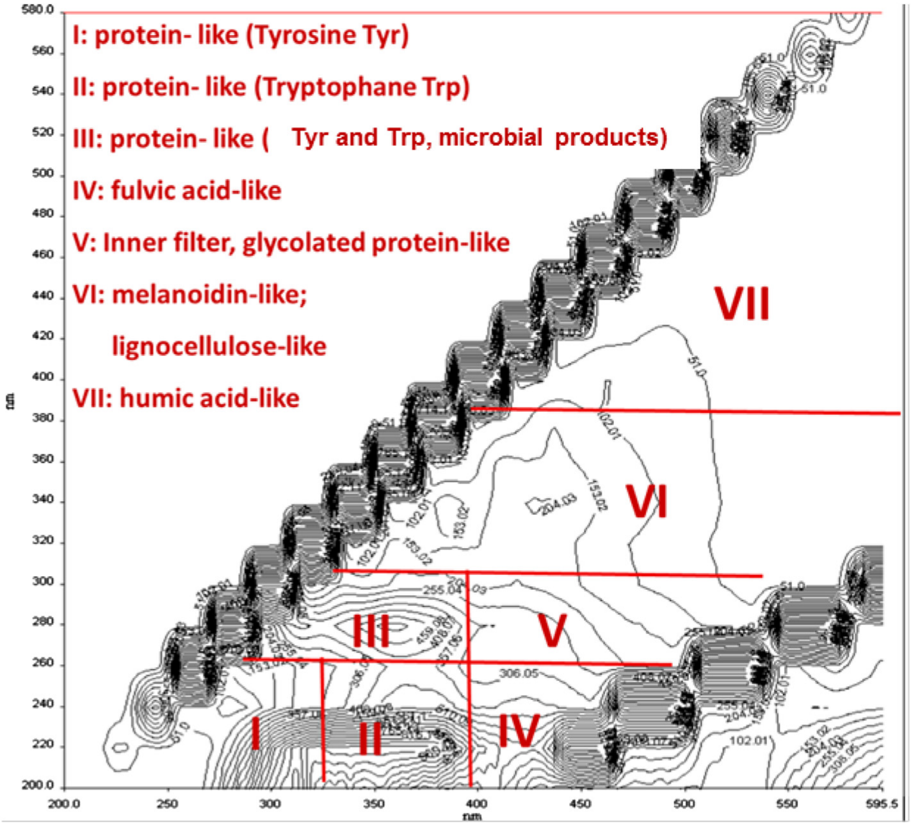

Figure B.1 – Fluorescence regionalization integration for spectra interpretation and quantification.

REFERENCES

Angelidaki, I., Sanders, W., 2004. Assessment of the anaerobic biodegradability of macropllutants. Rev. Environ. Sci. Bio/Technol. 3, 117–129.

Aquino, S.F., Chernicharo, C.A.L., Soares, H., Takemoto, S.Y., Vazoller, R.F., 2008. Methodologies for determining the bioavailability and biodegradability of sludges. Environ. Technol. 29 (8), 855–862.

Buffiere, P., Loisel, D., Bernet, N., Delgenes, J.P., 2006. Towards new indicators for the prediction of solid waste anaerobic digestion properties. Water Sci. Technol. 53 (8), 233–241.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A., 2002. Anaerobic Digestion Model No.1. (ADM1). IWA Scientific and Technical Report No. 13. IWA, ISBN 1-900222-78-7.

Chen, W., Westerhoff, P., Leenheer, J.A., Booksh, K., 2003. Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. Environ. Sci. Technol. 37 (24), 5701–5710.

Copp, J.B., Jeppsson, U., Osen, C., 2003. Towards an ASM1 – ADM1 state variable interface for plant-wide wastewater treatment modeling. In: Proceedings of the 76th Annual WEF Conference and Exposition (WEFTEC). Oct. 11–15, Los Angeles, USA.

Chandler, J.A., Jewell, W.J., Gossett, J.M., 1980. Predicting methane fermentation biodegradability. Biotechnol. Bioeng. Symp. 10 (10), 93–107.

Chandra, R., Bharagava, R.N., Rai, V., 2008. Melanoidins as major colorant in sugarcane molasses based distillery effluent and its degradation. Bioresour. Technol. 99 (11), 4648–4660.

Cossu, R., Raga, R., 2008. Test methods for assessing the biological stability of biodegradable waste. Waste Manage. 28 (2), 381–388.

Davidsson, A., Gruvberger, C., Christensen, T.H., Hansen, T.L., Jansen, J., 2007. Methane yield in source-sorted organic fraction of municipal solid waste. Waste Manage. 27 (3), 406–414.

Ekama, G.A., Sotteman, S.W., Wentzel, M.C., 2007. Biodegradability of activated sludge organics during anaerobic digestion. Water Res. 41 (1), 244–252.

Esparza-Soto, M., Westerhoff, P.K., 2001. Fluorescence spectroscopy and molecular weight distribution of extracellular polymers from full-scale activated sludge biomass. Water Sci. Technol. 43 (6), 87–95.

Girault, R., Bridoux, G., Nauleau, F., Poullain, C., Buffet, J., Steyer, J.-P., Sadowski, A.G., Béline, F., 2012. A waste characterisation procedure for ADM1 implementation based on degradation kinetics. Water Res.. http://dx.doi.org/10.1016/j.watres.2012.04.028.

Gunaseelan, V.N., 2009. Predicting ultimate methane yields of Jatropha curcus and Morus indica from their chemical composition. Bioresour. Technol. 100 (13), 3426–3429.

He, X.S., Xi, B.D., Wei, Z.M., Jiang, Y.H., Yang, Y., An, D., Cao, J.L., Liu, H.L., 2013. Fluorescence excitation-emission matrix spectroscopy with regional integration analysis for characterizing composition and transformation of dissolved organic matter in landfill leachates. J. Hazard. Mater. (in press).

Henderson, R.K., Baker, A., Murphy, K.R., Hambly, A., Stuetz, R.M., Khan, S.J., 2009. Fluorescence as a potential monitoring tool for recycled water systems: a review. Water Res. 43 (4), 863–881.

Huete, E., de Gracia, M., Ayesa, E., Garcia-Heras, J.L., 2006. ADM1-based methodology for the characterisation of the influent sludge in anaerobic reactors. Water Sci. Technol. 54 (4), 157–166.

Jimenez, J., Vedrenne, F., Denis, C., Mottet, A., Déléris, S., Steyer, J.-P., Cacho Rivero, J.A., 2013a. A statistical comparison of protein and carbohydrate characterization methodology applied on sewage sludge samples. Water Res. 47, 1751–1762.

Jimenez, J., Vedrenne, F., Steyer, J.-P., Cacho Rivero, J.A., 2013b. Better input variables for enhanced anaerobic digestion models: towards new methodologies for municipal sludge characterization. Water Res. (submitted for publication).

Jones, R., Parker, W., Khan, Z., Murthy, S., Rupke, M., 2008. Characterization of sludge for predicting anaerobic digester performance. Water Sci. Technol. 57 (5), 721–726.

Kleerebezem, R., van Loosdrecht, M.C.M., 2006. Waste characterization for implementation in ADM1. Water Sci. Technol. 54 (4), 167–174.

Li, W.H., Sheng, G.P., Liu, X.W., Yu, H.Q., 2008. Characterizing the extracellular and intracellular fluorescent products of activated sludge in a sequencing batch reactor. Water Res. 42 (12), 3173–3181.

Lesteur, M., Latrille, E., Maurel, V.B., Roger, J.M., Gonzalez, C., Junqua, G., Steyer, J.P., 2011. First step towards a fast analytical method for the determination of biochemical methane potential of solid wastes by near infrared spectroscopy. Bioresour. Technol. 102 (3), 2280–2288.

Mottet, A., François, E., Latrille, E., Steyer, J.P., Déléris, S., Vedrenne, F., Carrère, H., 2010. Estimating anaerobic biodegradability indicators for waste activated sludge. Chem. Eng. J. 160 (2), 488–496.

Mottet, A., Ramirez, I., Carrère, H., Déléris, S., Vedrenne, F., Jimenez, J., Steyer, J.P., 2013. New fractionation for a better bioaccessibility description of particulate organic matter in a modified ADM1 model. Chem. Eng. J. 228, 871–881.

Muller, M., Bastos Pereira Milori, D.M., Déléris, S., Steyer, J.P., Dudal, Y., 2011. Solid-phase fluorescence spectroscopy to characterize organic wastes. Waste Manage. 31, 1916–1923.

Muller, M., Antonini, M., Dudal, Y., Latrille, E., Vedrenne, F., Steyer, J.P., 2013. Combining Chemical Sequential Extractions with 3D Fluorescence Spectroscopy to Characterize Sludge Organic Matter (submitted for publication).

Nopens, I., Batstone, D.J., Copp, J.B., Jeppsson, U., Volcke, E., Alex, J., Vanrolleghem, P.A., 2009. An ASM/ADM model interface for dynamic plant-wide simulation. Water Res. 43 (7), 1913–1923.

Reynolds, D.M., Ahmad, S.R., 1997. Rapid and direct determination of wastewater BOD values using a fluorescence technique. Water Res. 31 (8), 2012–2018.

Ramirez, I., Mottet, A., Carrère, H., Déléris, S., Vedrenne, F., Steyer, J.P., 2009. Modified ADM1 disintegration/hydrolysis structures for modeling batch thermophilic anaerobic digestion of thermally pretreated waste activated sludge. Water Res. 43 (14), 3479–3492.

Shanmugam, P., Horan, N.J., 2009. Simple and rapid methods to evaluate methane potential and biomass yield for a range of mixed solid wastes. Bioresour. Technol. 100 (1), 471–474.

Scaglia, B., Confalonieri, R., D'Imporzano, G., Adani, F., 2010. Estimating biogas production of biologically treated municipal solid waste. Bioresour. Technol. 101 (3), 945–952.

Sheng, G.P., Yu, H.Q., 2006. Characterization of extracellular polymeric substances of aerobic and anaerobic sludge using three-dimensional excitation and emission matrix fluorescence spectroscopy. Water Research 40, 1233–1239.

Vavilin, V.A., Fernandez, B., Palatsi, J., Flotats, X., 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: an overview. Waste Manage. 28 (6), 939–951.

Wan, S., Xi, B., Xia, X., Li, M., lv, D., Wang, L., Song, C., 2012. Using fluorescence excitation emission matrix spectroscopy to monitor the conversion of organic matter during anaerobic co-digestion of cattle dung and duck manure. Bioresour. Technol. 123, 439–444.

Wang, Z., Tang, S., Zhu, Y., Wu, Z., Zhou, Q., Yang, D., 2010. Fluorescent dissolved organic matter variations in a submerged membrane bioreactor under different sludge retention times. J. Membr. Sci. 355 (1–2), 151–157.

Yasui, H., Sugimoto, M., Komatsu, K., Goel, R., Li, Y.Y., Noike, T., 2006. An approach for substrate mapping between ASM and ADM1 for sludge digestion. Water Sci. Technol. 54 (4), 83–92.

Yasui, H., Goel, R., Li, Y.Y., Noike, T., 2008. Modified ADM1 structure for modelling municipal primary sludge hydrolysis. Water Res. 42 (1–2), 249–259.

Zaher, U., Li, R., Jeppsson, U., Steyer, J.P., Chen, S., 2009. Giscod: general integrated solid waste co-digestion model. Water Res. 43 (10), 2717–2727.