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MODELISATION DE LA DIVERSITE MICROBIENNE DANS LES PROCEDES DE DIGESTION ANAEROBIE

JURY

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AVANT-PROPOS

Chère lectrice et cher lecteur,

ce manuscrit de thèse traite d'une approche de la modélisation de la diversité microbienne dans les procédés de digestion anaérobie, procédés de traitement de la pollution carbonée connus et reconnus depuis de nombreuses années mais qui reçoivent actuellement une attention particulière du fait de regain d'attention portée sur la production de bioénergie.

Les travaux présentés ici ont pour objectif de développer un modèle basé sur l'ADM1 (*Anaerobic Digestion Model #1*) de l'IWA (*International Water Association*) capable de tenir compte de la diversité microbienne en conditions normale (i.e., ne conduisant pas à un déséquilibre du système) et anormale de fonctionnement (i.e., présence d'un inhibiteur). Le modèle ADM1 modifié permet d'analyser et d'évaluer la relation entre les performances du procédé et la composition des communautés microbiennes, c'est-à-dire les mécanismes entre la diversité des espèces et les propriétés épuratoires du procédé biologique (parmi autres la résilience et la résistance du système).

La diversité microbienne est en effet devenue aujourd'hui un paramètre majeur pour la conduite d'un procédé. Ce faisant, le modèle développé apporte un grand nombre de perspectives en termes de modélisation et d'estimation des paramètres, mais également en termes de régulation et contrôle du procédé.

Du fait de sa présentation particulière (*i.e.,* rédaction en anglais et recueil d'articles scientifiques), ce manuscrit peut vous surprendre mais je me permets d'espérer que vous aurez autant de plaisir à le lire que j'ai eu à le construire et à le rédiger. Excepté le premier, chaque chapitre est précédé d'un résumé (en anglais et en français) qui souligne les principaux résultats obtenus. Dans un souci d'homogénéité, la présentation de chaque article est modifiée par rapport à leurs publications dans les revues et conférences. Si la lecture des introductions peut paraître parfois redondante, voire rébarbative, de par leur répétition, je ne peux qu'espérer que le cœur des différentes études vous permettra d'apprécier les multiples facettes que j'ai pu aborder au cours de ces trois années.

Le premier chapitre concerne une revue bibliographique de certains points importants qui ne sont pas détaillés au sein des articles que j'ai pu publier ou soumettre à ce jour. Il était toutefois important de les préciser avant de rentrer plus avant dans le contenu scientifique de cette thèse.

Le deuxième chapitre aborde les premières étapes de l'introduction de notions de diversité microbienne au sein d'un modèle complexe tel que l'ADM1. Là encore, deux articles sont proposés, le premier sur la description de l'idée de base de ces travaux (*i.e.,* l'introduction d'un terme stochastique dans les équations décrivant les dynamiques de croissance des microorganismes, de consommation des substrats et de production des intermédiaires réactionnels), le second sur l'application directe de ce type de modélisation pour le démarrage de digesteurs hybrides (*i.e.,* combinant un lit de boues avec un procédé à lit fixe) :

- I Ramirez, J P Steyer: *"Modeling microbial diversity in anaerobic digestion"*, Water Science & Technology, vol, 57, n° 2, pp. 265-270 (2008).
- R. Rajinikanth, I. Ramirez, J. P. Steyer, R. Escudie, M. Torrijos, I. Mehrotra and P. Kumar : *"Experimental and modeling investigations of a hybrid upflow anaerobic sludge-filter bed (UASFB) reactor"*, Water Science & Technology, vol. 58 , n°1, pp. 109–117, (2008).

Le chapitre III décrit les implications que ce genre de modèle peut avoir en Ecologie Microbienne. En particulier, les liens entre diversité de l'écosystème et performance épuratoires du procédé sont abordés ainsi que la stabilité et la structure des communautés face à des toxiques et l'adaptation des populations microbiennes lors de changements de composition de la pollution à traiter. Trois articles sont proposés :

- I. Ramirez, E.I.P. Volcke, J-Ph. Steyer: *"Modeling and Monitoring of Microbial Diversity in Ecosystems - Application to Biological Wastewater Treatment",* Conférence orale lors de l'IFAC World Congress 2008, Seoul, Corée (6 pages sur CDROM).
- I. Ramirez, E.I.P. Volcke,R. Rajinikanth, J-Ph. Steyer: *"Modelling microbial diversity in anaerobic digestion through an extended ADM1 model"*, publié dans la revue Water Research (2009) - http://dx.doi.org/10.1016/j.watres.2009.03.034.

- I. Ramirez, A. Mottet, H. Carrère, S. Déléris, F. Vedrenne, J-Ph. Steyer: *"Relationship between microbial community structure and batch thermophilic anaerobic digestion performance of thermally pretreated WAS : A modeling Approach"*, article en préparation.

Les différents chapitres sont par ailleurs complétés par une discussion finale des résultats et une tentative de dégager des pistes de recherche pour l'avenir.

Finalement, ce manuscrit de thèse est accompagné de trois annexes. Les deux premières abordent un bilan des potentialités de la digestion anaérobie à des fins de dépollution et de production de bioénergie (méthane et/ou hydrogène). Elles sont constituées de deux articles rédigés de concert avec les différents membres de l'équipe à laquelle j'ai appartenu:

- C.A. Aceves-Lara, E. Trably, J.R. Bastidas-Oyenadel, I. Ramirez, E. Latrille, J-Ph. Steyer. (2008) *"Production de bioénergies à partir de déchets: Exemples du biométhane et du biohydrogène",* Journal de la Société de Biologie. 202(3) : 177-189.
- J-Ph. Steyer, E. Latrille, C.A. Aceves, I. Ramirez, A. Elias, J. Hess, O. Bernard, H. Bangsø Nielsen, K. Boe, I. Angelidaki: *"Optimizing Biogas Production from Anaerobic Digestion",* Presentation invitée à l'International Workshop on "Energy Savings Through Better Design, Control, & Optimization - A Compilation of US and International Experiences", WEFTEC Conference, 25-28 Octobre 2006, Dallas, Texas, USA, 13 pages sur CDROM.

Au sein du annexe III, un essai de modélisation de la digestion anaérobie des boues de stations d'épuration est mené. Il ressort que les étapes de désintégration et d'hydrolyse doivent être plus finement modélisées que l'existant disponible dans la littérature actuelle. Cet article est accepté dans la revue *Water Research* et concerne un travail mené en très étroite collaboration avec la société Véolia Environnement :

- Ramirez, A. Mottet, S. Déléris, F. Vedrenne, H. Carrère and J-Ph. Steyer: *"Modified ADM1 disintegration/hydrolysis structures for modeling batch thermophilic anaerobic digestion of thermally pretreated waste activated sludge",* accepté pour publication, Water Research 2009.

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Nomenclature and Description of Parameters and Variables

Stoichiometric coefficients

Symbol	Description	Units
$\nu_{i,j}$	Rate coefficients for component i on process j	Nominally kg COD.m^{-3}
$f_{product,substrate}$	Yield (catabolism only) of product on substrate	kg COD.kg COD^{-1}

Equilibrium coefficients and constants

Symbol	Description	Units
Hgas	Gas law constant (equal to K_H^{-1})	bar.M^{-1} (bar.m^3 ·kmol^{-1})
Ka	Acid acid-base equilibria coefficient	M (kmole.m^{-3})
KH	Henry's law coefficient	M bar^{-1} (kmole.m^{-3}.bar^{-1})
pKa	$\log_{10}$[Ka]	
R	Gas law constant (8.314×10^{-2})	bar.M^{-1}.K^{-1} (bar.m^3.kmole^{-1}.K^{-1})

Kinetic parameters and rates

Symbol	Description	Units
$k_{A/Bi}$	Acid base kinetic parameter	M^{-1}.d^{-1}
k_{dec}	First order decay rate	d^{-1}
$I_{inhibitor,\ process}$	Inhibition function (see K_I)	
$k_{process}$	First order parameter (for disintegration and hydrolysis)	d^{-1}
$k_{La,i}$	Gas-liquid transfer coefficient of gas i	d^{-1}
Di	Diffusivity of gas i	m^2.s^{-1}
$K_{I,\ inhibit,\ substrate}$	50% Inhibitory concentration	kg COD.m^{-3}
$k_{m,\ process}$	Monod maximum specific uptake rate (μ_{max}/Y)	kg COD_S.kg COD_X^{-1}.d^{-1}
$K_{S,process}$	Half saturation value	kg COD_S.m^{-3}
ρ_j	kinetic rate of process j	kg COD_S.m^{-3}.d^{-1}
$Y_{substrate}$	Yield of biomass on substrate	kg COD_X.kg COD_S^{-1}
μ_{max}	Monod maximum specific growth rate	d^{-1}

Algebraic variables

Symbol	Description	Units
pH	$-\log_{10}[H^+]$	
$p_{gas,i}$	Pressure of gas i	bar
p_{gas}	Total gas pressure	bar
Si	Soluble component i	kg COD.m^{-3}
$t_{res,X}$	Extended retention of solids	D
T	Temperature	K
V	Volume	m^3
Xi	Particulate component i	kg COD.m^{-3}

Dynamic State Variables

Name	**I**[1]	**Description**	**Units**[2]
Xc	13	Composites	
X_{csh}	43	Slowly hydrolyzed Composites	
X_{crh}	48	Readily hydrolyzed Composites	
Xch	14	Carbohydrates	
Xpr	15	Proteins	
Xli	16	Lipids	
X_I	24	Particulate inerts	
S_I	12	Soluble inerts	
Ssu	1	Monosaccharides	
Saa	2	Amino acids3	
Sfa	3	Total LCFA4	
Sva	4	Total valerate	
Sbu	5	Total butyrate	
Spro	6	Total propionate	
Sac	7	Total acetate	
Sh_2	8	Hydrogen	
Sch_4	9	Methane	
S_{IC}	10	Inorganic carbon	**M**
S_{IN}	11	Inorganic nitrogen	**M**
X_{su} .....X_{h2}	17-23	ADM1 Biomass	
X_{Xxc}, X_{Xch}, X_{Xpr}, X_{Xli}	43-46	Modified ADM1 Biomass	
X_{Xxc1}, X_{Xxc2}, X_{Xxc3}…… X_{Xxc10}	101-110	ADM1_10 Composite Biomass	
X_{Xch1}, X_{Xch2}, X_{Xch3}…… X_{Xch10}	111-120	ADM1_10 Carbohidrate Biomass	
X_{Xpr1}, X_{Xpr2}, X_{Xpr3}…… .X_{Xpr10}	121-130	ADM1_10 Protein Biomass	
X_{Xli1}, X_{Xli2}, X_{Xli3}…… ...X_{Xli10}	131-140	ADM1_10 Lipid Biomass	
X_{su1} ,X_{su2}, X_{su3}……X_{su10}	17-26	ADM1_10 Sugar Biomass	
X_{aa1} ,X_{aa2}, X_{aa3}……X_{aa10}	27-36	ADM1_10 Amino acids Biomass	
X_{fa1} ,X_{fa2}, X_{fa3}……X_{fa10}	37-46	ADM1_10 LCFA Biomass	
X_{c41} ,X_{c42}, X_{c43}……X_{c410}	47-56	ADM1_10 Valerate Biomass	
X_{c41} ,X_{c42}, X_{c43}……X_{c410}	47-56	ADM1_10 Butyrate Biomass	
X_{pro1} ,X_{pro2}, X_{pro3}……X_{pro10}	57-66	ADM1_10 Propionate Biomass	
X_{ac1} ,X_{ac2}, X_{ac3}……X_{ac10}	67-76	ADM1_10 Acetate Biomass	
X_{h21} ,X_{h22}, X_{h23}……X_{h210}	76-86	ADM1_10 Hydrogen Biomass	
Scat		Cations	**M**
San		Anions	**M**

1. See process kinetics and stoichiometry matrix in Appendix A,B chapter III and STR No 13.
2. Unless otherwise stated, kg COD.m^{-3}

Chapitre I

Revue Bibliographique

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RESUME

La première partie de ce chapitre est consacrée à la présentation des concepts de diversité et d'écosystèmes et à leurs interactions dans un système biologique défini. Depuis une vingtaine d'années, l'utilisation de nouveaux outils moléculaires ne nécessitant pas de mise en culture a en effet permis de caractériser la diversité phylogénétique des communautés microbiennes et d'étudier leurs dynamiques dans des procédés biologiques. L'apport de ces nouvelles connaissances sur l'interaction entre l'activité et la diversité des microbes permet à la Microbiologie d'être désormais une discipline prédictive et non plus purement descriptive, dans laquelle les principes d'écologie microbienne sont intégrés et peuvent être optimisés. Les écosystèmes impliqués dans un bioprocédé et dans un système naturel possèdent par ailleurs des similitudes de fonctionnement et il est possible d'utiliser les premiers afin de mieux comprendre l'écologie des micro-organismes dans leur globalité. Par exemple, la stabilité d'un réacteur de méthanisation contenant des groupes trophiques définis semble dépendante de la diversité des groupes fonctionnels de chaque niveau trophique ainsi que de la complémentarité entre chaque groupe fonctionnel. En plus de l'utilisation d'un bioprocédé dans le but d'étudier des questions d'écologie microbienne, la synergie entre la Microbiologie et le Génie des Procédés est nécessaire afin de mieux comprendre les conditions opératoires, les processus et les interactions existantes et ainsi permettre à l'ingénierie écologique des bioréacteurs de devenir une réalité.

Diverses études récentes ont montré que le fonctionnement d'un bioprocédé, système fermé et maîtrisé, reflétait certaines des interactions et processus se déroulant au sein d'un système naturel. Ainsi, il a été démontré que la stabilité était corrélée à la redondance fonctionnelle des communautés microbiennes et non à la diversité des populations en tant que telle. Les processus et les interactions qui assurent une stabilité fonctionnelle résultent en effet habituellement d'une grande redondance fonctionnelle et d'une importante complémentarité des niches fonctionnelles. Les biofilms et les granules sont un parfait exemple d'une structure de micro-organismes compacte et possédant les principales caractéristiques permettant d'assurer une stabilité fonctionnelle. Bien que la stabilité fonctionnelle soit fortement dépendante du rôle des populations fonctionnelles majeures, il est également très important d'être capable de caractériser les groupements fonctionnels et les interactions des espèces mineures, et ce afin de mieux contrôler la stabilité sur une longue durée de fonctionnement ou après de fortes perturbations du système. Au final, nous sommes convaincus qu'une meilleure compréhension de la redondance fonctionnelle et des interactions entre les populations fonctionnelles devrait permettre une approche plus rationnelle de l'ingénierie écologique.

Dans cette optique, des stratégies de contrôle appropriées permettraient d'éviter des problèmes d'instabilité dans les digesteurs anaérobies. Cependant, de telles stratégies d'exploitation nécessitent généralement le développement de modèles mathématiques spécifiques. La seconde partie de ce chapitre sera ainsi consacrée à l'état de l'art sur la modélisation de la digestion anaérobie, ce qui permettra d'identifier les développements majeurs nécessitant des approfondissements scientifiques.

SUMMARY

After discussing some concepts about diversity and ecosystems functioning, the relationships between them are assessed in the first section of this chapter. New high-throughput culture-independent molecular tools allow the scientific community to characterize and understand the microbial communities underpinning environmental biotechnology processes in unprecedented ways. By creatively leveraging these new data sources, microbial ecology has the potential for a transition from a purely descriptive to a predictive framework, in which ecological principles are integrated and exploited into engineered systems that are biologically optimized for the desired objective. As engineered systems are often more manageable than large-scale ecosystems, and because parallels between engineered environments and other ecosystems exist, we will show in this first section that the former can be used to elucidate some unresolved ecological issues. For example, the process stability of methanogenic bioreactors containing well-defined trophic groups appears to depend on the diversity of the functional groups within each trophic level as well as on how these functional groups complement each other. In addition, in order to use engineered systems to study general ecological questions, we will suggest that microbial ecologists and environmental engineers need to investigate conditions, processes and interactions in engineered environments in order to make the ecological engineering of bioreactor design and operation more practicable.

Preliminary findings suggest that complex bioreactor designs in many ways mirror the interactions and processes of large-scale ecosystems. It has been demonstrated that stability is better correlated not to population diversity per se, but to functional redundancy. Processes and interactions that promote functional stability usually result from greater functional redundancy and functional niche complementation. Biofilms and granules incorporate the characteristics of functional stability within highly compact structures. Although functional stability is highly dependent on the role of functionally important populations, it is also crucial to be able to characterize so-called minor species (*i.e.,* their functional groupings and interactions) in order to better understand stability over long time periods and after a wide range of perturbations. An improved understanding of functional redundancy and the interactions between functional compartments of a bioreactor should lead to more rational ecological engineering approaches.

Because unstability problems in anaerobic digesters may be avoided through appropriate control strategies and because such strategies require in general the development of appropriate mathematical models, which adequately represent the key processes that take place, the second section of this chapter will review the current state of the art in anaerobic digestion modeling, and we will identify the key areas that require further research endeavors.

I.1 INTEGRATING ECOLOGY IN BIOTECHNOLOGY

Ecology is the study of the distribution and abundance of organisms and their biotic and abiotic interactions in an environmental setting. Biotechnological processes often rely on microbial organisms contained within an engineered environment designed to allow some level of operating control. Ecological engineering is a term used to describe the process of designing and operating bioreactors and other engineered systems to foster the development of specific microbial communities that can accommodate the desired functional processes (Sloan et al., 2006). As pointed out by Grady and Filipe (2000), environmental engineers have been practicing ecological engineering, either consciously or unconsciously, ever since the first bioreactor was built. It requires an understanding of ecological principles, the physiological requirements of the desired population(s), and the spatial juxtaposition of various populations.

The advent of numerous molecular biological tools, which preclude the need for cultivation, now allows us to better integrate the information gained from (microbial) ecological research within the design and optimization of bioreactors. It us also recognized that parallels between large-scale ecosystems and engineered environments can lead to a better integration of concepts and theories in community and ecosystem ecology as well as to improvements in bioreactor design (McMahon et al., 2007). In addition, the need to apply theoretical ecology can be emphasized to understand, to model, and to manage environmental biotechnology systems. The basic principles of ecology developed during a century of studies focused on how macroscale organisms interact with their environment and fellow community members should be mined out by environmental biotechnologists for useful conceptual frameworks.

Interactions between microbial ecologists and environmental engineers, combined with the availability of new methods to characterize community structure, offer exciting opportunities to integrate the concepts and theories of community and ecosystem ecology into a unified picture. This should lead to better ways to describe and predict how any kind of stability develop, how it is maintained, and how an engineered system can recover from unstable periods. Furthermore, we have learned much about the links between community diversity, composition and process performance in environmental biotechnology systems by applying the powerful molecular tools developed by microbiologists. However, there is still a great

need to develop new curricula and concerted research efforts to better integrate the knowledge and tools of molecular microbiology and engineering (Daims et al., 2006). We propose, as have done others (see for example Briones et al., 2003 ; Graham and Smith, 2004 ; Rittmann et al., 2006), that students and practitioners of environmental biotechnology should also embrace the principles of Ecology, with a capital 'E'.

Although a thorough discussion of an appropriate definition of species is beyond the scope of the current work, we feel that it is important to provide the ''species concept'' used throughout this manuscript. In our model, species are defined as groups of like individuals that share a common set of kinetic and stoichiometric characteristics. This may or may not correspond to species as defined by 16S ribosomal DNA sequence comparisons (McGaig et al., 1999) nor species as defined by operational taxonomic units (OTU) based on molecular fingerprinting assays (Marsh et al., 1998).

Now, a clear discussion of the effects of *biodiversity* on *ecosystem functioning* requires clear definitions of these two terms. These definitions are detailed in the following sections.

I.1.1 *BIODIVERSITY*

As underlined by Purvis et al. (2000), the concept of diversity is slippery! Some biologists simply say, "I know it when I see it," others dismiss it as a 'non-concept'.

I.1.1.1 The Meaning of Biological Diversity

Our definition of diversity comes from biological diversity Conference (Rio, 1992) where the following definition was adopted: "Variability of all living organisms, including, among others, terrestrial and marine ecosystems and other aquatic ecosystems, and the ecological complexes of which they are part; that includes diversity within the species (genetic diversity) and between species (specific diversity) as well as the ecosystems one (ecosystem diversity)". The biodiversity is indeed generally thought in three stages: genetic, species and ecosystem diversity. These levels are connected between them but they are sufficiently different so that each of them can be separately studied. Whatever theoretical or experimental, the majority of the studies are interested in the specific level (the species one) because it is the most accessible stage at the conceptual level but also at practical one. In the following sections, we

will calculate the specific diversity (measured by its diversity indices) in anaerobic reactors under constant or dynamic inputs.

I.1.1.2 The Measurement of Diversity

To detect the changes which affect the biodiversity, it is necessary to be able to measure it. At first sight, biological diversity seems to be an obvious concept, easy to understand. However, when we look closer, it appears that it is not so easy to quantify it. Moreover, to try to express it by a single number would be a vain attempt: only one measurement cannot give us a value of all its components. In fact, three methods have been developed to measure diversity.

I.1.1.2.1 Species richness represents the number of species in an area, or the number of alleles (the variants of same gene, which determines for example the eyes's color, brown or blue) that a species has for same locus (the same place) or, the number of functional groups (group of species which are equivalent from the point of view of the function) or the taxonomic groups with a higher rank than the species rank which is present in the ecosystem. Even though species richness is a incomplete measurement, this quantitative method makes it possible to measure diversity (number of identified individuals). The doubts that it causes are related mainly with the difficulty of bringing at the same level, measurements taken at different scales.

I.1.1.2.2 Species accumulation curves. A simple species accumulation curve can be drawn by plotting the number of species vs the number of individuals as samples are collected and analysed. The species accumulation curve starts by climbing rapidly, then flattens out. If enough samples are collected so that all species present have been picked up, it would level off. As an illustration the species accumulation curve for anaerobic digestors are depicted in Figure I.1 together with other ecosystems and it was obtained from analysis of the microbial community structure in a fluidized bed reactor fed with vinasses (Godon et al., 1997). After PCR amplification, three 16S rRNA clone libraries of bacteria, archaea and eucarya populations were established. Community structure was determined by phylogenetic analysis of 556 partial rDNA sequences. 556 clones (i.e., 460 bacteria and 96 archaea) were grouped into 139 OTUs (Operational taxonomic units): 133 for bacteria and 6 for archaea.

Recent estimates of the number of species in different ecosystems have gained much attention because the ecosystems evolution over millions of years predicts that the composition of microbial communities should be much greater than the published estimates based on conventional molecular techniques. The highest estimate suggests that the number may be so large that it is impractical to test by amplification and sequencing of the highly conserved16S rRNA gene from DNA sequences. To provide a broader context, the reader can evaluate the substantial amount of information accumulated on bacterial diversity in a variedad of environment. See, for example in aquatic systems (Kemp and Aller, 2004 ; Sogin, et al. 2006 ; Huber, et al. 2007) and in soils (Roesch, et al. 2007), among other ecosystems.

Nowadays, there are computer programs that constructs collector\'s and species accumulation curves for sampling intensity, richness estimators, and diversity indices by using the OTU composition data. As an example, DOTUR (Defining Operational Taxonomic Units and estimating species Richness) is a computer program that takes a distance matrix describing the genetic distance between DNA sequence data and assigns sequences to operational taxonomic units (OTUs) using either the furthest, average, or nearest neighbor algorithms for all possible distances that can be described using the distance matrix (Schloss and Handelsman, 2005).

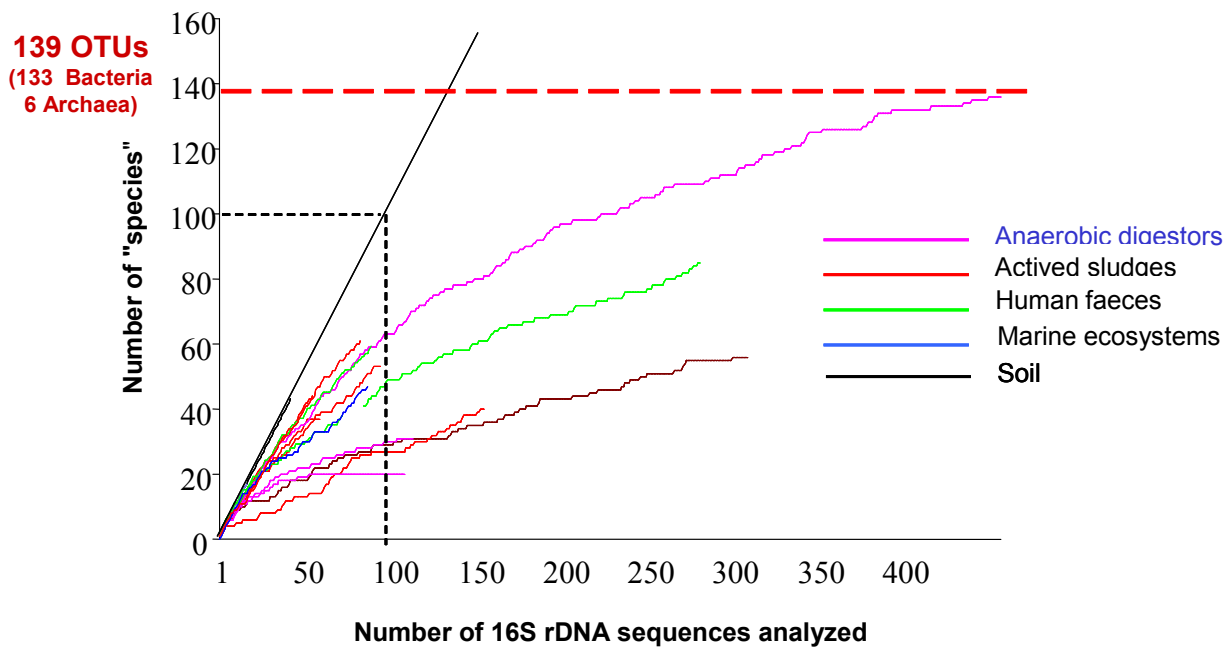

Figure I.1 Species acccumulation curves for different anaerobic ecosystems (taken from Godon et al., 1997)

I.1.1.2.3 Diversity indices. The diversity indices are numerous but only some of them (i.e. the Simpson's diversity and Shannon's diversity indices) are commonly used in microbial ecology.

The general Hill's diversity (Hill, 1973) is:

$$N_\alpha = \frac{1}{\sqrt[\alpha-1]{\sum_{i=1}^{N} p_i^\alpha}}$$

This index involves the proportional abundances (p_i) of each species, i.e. for each species (i), the number of individuals (abundance) or concentration (biomass) of that species in the sample (n_i) is divided by the total number of individuals or total concentration in the sample ($N = \Sigma ni$). As an example, For $\alpha = 2$, the p_i's are squared, added together, and the reciprocal is calculated. Squaring the p_i's means that the common species have greater weight than rare ones: a species with 50 % in the sample has $p_i^2 = 0.25$, but for a species with 1 %, it is only 0.0001. The main Hill's diversity numbers of interest are:

- N_0 = species richness (all species, rare or common, count equally),
- $N_1 = e^H$, where H is Shannon's diversity index,
- N_2 = 1/ Simpson's index (without the small sample correction),
- N_{inf} = 1/ Berger-Parker index.

These numbers decrease steadily as α increases: N_0 is the largest while N_{inf} the smallest one.
To summarize, one can say that:

- Hill's numbers are related to well-known indices,
- Rare species have decreasing weight from N_0 to N_{inf},
- Missing out rare species has less effect on the index and reflects the relative ecological importance of common species,
- N_2 or 1/Simpson's index seems a reasonable compromise.

I.1.1.2.4 Evenness. In principle, diversity is a combination of richness and evenness. In practice however, evenness measures are defined in terms of diversity. If I is an index of diversity, the corresponding evenness index, E, is defined as:

$$E = \frac{I}{I_{\max}}$$

where I_{max} is the value I would take if the abundances in the sample were all equal. Unfortunately, I_{max} is usually highly sensitive to the number of species in the sample. We have managed to devise a diversity index which is not overly sensitive to the number of rare species captured in our sample, but the trade-off is an evenness index which is more sensitive to missing rare species.

Populations with large numbers of species and even distributions of individuals have higher diversity than other populations with either fewer species or disproportionate populations of each species. As a consequence, the diversity indices – and therefore the evenness indices – cannot be compared directly one to each other and only their respective changes for the different communities can be compared.

I.1.2 *ECOSYSTEM FUNCTIONING*

Ecosystem functioning is another broad term that encompasses a variety of phenomena, including **ecosystem properties** (i.e. pools of organic matter and fluxes), **ecosystem goods** (i.e. properties that have direct market value, such as plant and animal breeding, genes for gene products in biotechnology, biogas produced) and **ecosystem services** (i.e. properties of ecosystems that either directly or indirectly benefit human endeavors such as, for example, regulating climate, cleaning air and water, maintaining atmospheric composition, storing and cycling of nutrients, etc. Christensen et al. 1996; Daily 1997). When discussing effects of biodiversity on ecosystem functioning, it is important to specify which components of biodiversity are affecting which components of functioning. As an example in Chapter 5, we will assesses the effect of specific diversity on both, biogas production rate (ecosystem good) and VFAs concentration (ecosystem property).

Ecosystem stability is often divided into three aspects: (i) **persistence**, i.e., the tendency of a system to exist in the same state through time; (ii) **resistance**, i.e., the capability of a system to remain unchanged in the face of external pressures such as disturbances; (iii) **resilience**, i.e., the ability of a system to return to its original or equilibrium state after it has been displaced from it by external pressures. In addition, temporal variability is often used as an inverse measure of resistance (Grim, at al., 1992; Neubert and Caswell, 1997). As response variables, community composition (i.e. identities of species, abundances) is most often used

but other community characteristics such as biomass production or nutrient pool sizes can also be of interest.

Each of these ecosystem stability properties can be associated with the population level (e.g., population resistance) or the functional one (e.g., functional resistance). It is important to notice that the resilience of a compound variable does not need the resilience of each population (Tilman et al. 2002). For example for the variable carbon assimilation, a disturbance can to lead the extintion of a entire population level in benefit of other population level, that will assimilate an equivalent amount of carbon. In this case we have a resilience at functional level but not at population one. In the following sections, we will evaluate the resistance and the resilience, only in the functional level.

Finally, **sustainability** refers to the capacity for a given ecosystem service to persist at a given level for a long period of time. While sustainability has been widely discussed, very few experiments have addressed it directly, in part because of the complexities involved (Lubchenco et al. 1991, Valiela et al. 2000).

I.1.3 *BIODIVERSITY AND ECOSYSTEM FUNCTIONING*

There are nowadays experimental and observational studies available that analyze the influence of biodiversity on ecosystem stability (see for example Loreau *et al* 2002; Kinzig *et al* 2002 and related references). Mostly, these studies analyzed the effects of some external perturbation and they essentially support theory that species diversity has a positive effect on the resistance of biomass production. However, they not always adequately incorporated the influence of parallel and independant factors in the analyses (e.g.fertilization gradients in case of plant studies) and evidence for diversity effects on resilience is even weaker.

As a matter of fact, for a long time, the ecologists study the ecological function (i.e. the role) of different species but on the other hand, the study of the ecological function of the biodiversity is relatively recent. This field of research, extremely complex, is currently in full expansion.

An understanding of how changes in species richness and composition – and the biodiversity in general – influence ecosystem properties requires an understanding of the functional traits

of the species involved. By definition, **functional traits** are those that influence ecosystem properties or species' responses to environmental conditions. Species are often grouped together according to their functional traits to understand the general mechanisms or to make studies of complex systems more tractable. In other words, several species of the same ecosystem can be equivalent at the functional level, i.e., they have the same function, they play the same role, they occupy the similar ecological niches,…). Functional types (Aka functional groups: groups of species which are equivalent from the point of view of the function) are at first glance a relatively simple concept. A **functional type** is a set of species that have similar effects on a specific ecosystem process or similar responses to environmental conditions. Functional types are similar to the **guild** concept from animal community ecology (Root 1967; Simberloff and Dayan 1991; Wilson 1999), and to **niche** concepts (Leibold 1995). The total **suite** of functional traits in a community is one of the main determinants of ecosystem properties (Chapin et al. 1997, Chapin et al. 2000). However, even though functional types can be quite useful, the practice of defining them and quantifying functional diversity can be difficult. It is worth noting that two species can have the same functional traits but with different niches if they are in different places or they have a temporal shift in their activities.

Obviously, the different functional types contribute to ecosystem functional diversity, but the multiplicity of the species which are equivalent in the field of function also contributes to it. **Functional diversity** is the variety of answers that species of an ecosystem contribute to their environment changes or the variety of the responses that the ecosystem itself can contribute to these environment changes. In theory, if functional diversity is high, there is higher probability that certain species react well to environment changes, therefore ecosystem stability is high. On the other hand, if functional diversity is low, the overall community is likely to suffer from the environment changes. One might see this as an "insurance hypothesis": biodiversity buffers ecosystem processes against environmental changes because different species respond differently to such changes. This results in functional compensations among species and hence more stable community properties. However, the number of species alone may not be the best predictor of ecosystem properties and the relationship between species or taxonomic richness and functional diversity in natural ecosystems is still being explored (Hooper et al. 2002, Petchey 2002, Schmid et al. 2002, Tilman et al. 2002, Hooper et al. 2005, Bell et al. 2005, Scherer et al. 2005).

I.1.4 *EFFECTS OF DIVERSITY IN THE CONTEXT OF OTHER ECOSYSTEM FACTORS*

A number of human activities are sufficiently widespread so that their ecological effects have now reached global proportions. These ecological effects alter both the biotic community and abiotic controls. As can be seen in Figure I.2, external factors (such as climate, species invasions, reactor type, influent) in addition to abiotic controls (such as resourse availibity, pH, temperature and feeding regime) interact with functional traits of organisms to control the ecosystem properties (Chapin et al. 1997, 2000, 2002). The last half-century of ecosystem ecology research has yielded to large amounts of information about how organismal traits influence ecosystem properties in both terrestrial and aquatic ecosystems, and about trade-offs and linkages of these traits in individual organisms (se for example Aerts et al. 1990, Berendse and Elberse 1990, Chapin et al. 1993, Dıaz et al. 1999). However, ecosystem ecologists have traditionally focused on the functional traits of the most dominant organisms (i.e. those that are the most abundant or that have the greatest biomass within each trophic level) because they are the most obvious biotic factors regulating ecosystem properties. Of course, certain species, although relatively rare or of low total biomass, can also have large effects (see as an illustration the review of Keystone species in Power et al. 1996).

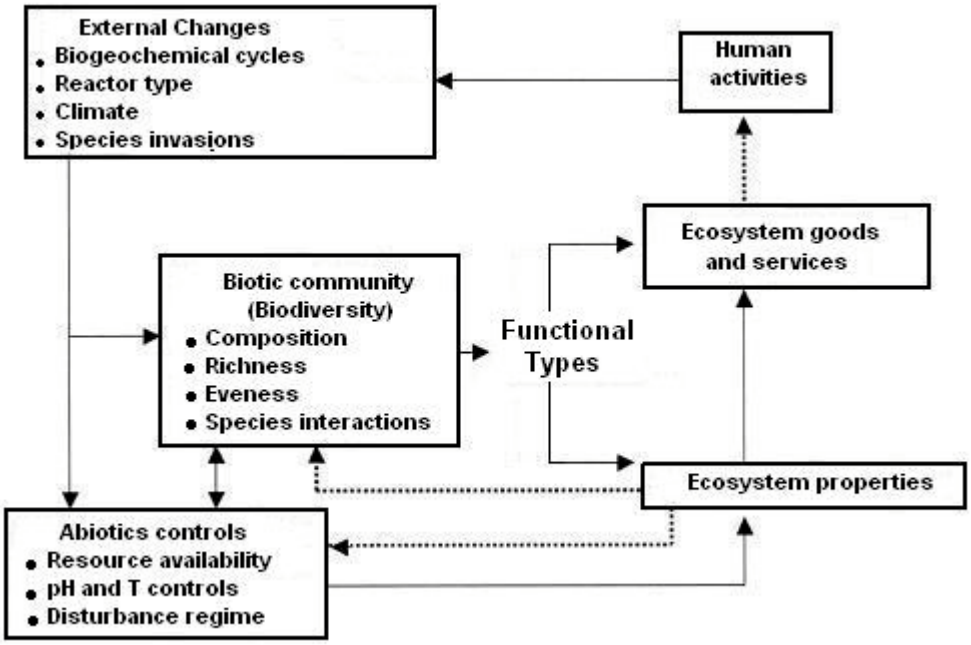

Figure I.2 Feedbacks between human activities, External changes, and biotic and abiotic controls on ecosystem (biorector) properties. This figure is modified from Chapin et al. (2000).

In fact, changes in biota can have even greater effects on ecosystem properties than changes in abiotic conditions (e.g., Chapin et al. 2000). Different types of environmental change are hypothesized to lead to different patterns of biodiversity modification for different types of species and ecosystems (Sala et al. 2000). For example, in experiments conducted on replicated continuously mixed methanogenic reactors where two different microbial communities (designated as the high-spirochaete and low-spirochaete) were maintained, the less stable community structure was correlated to the most stable function (Fernandez et al., 2000). In this case, the less stable community was the one that displayed greater temporal variation of bacterial populations in response to substrate (glucose) shock (i.e. environmental change). The high-spirochaete community responded to glucose perturbation by shifting the relative abundance of fermenting bacteria and then, by returning to structural characteristics close to those before the perturbation. By contrast, reactors that were dominated by streptococci prior to glucose perturbation (i.e. the low-spirochaete reactor set) showed minimal community changes in response to the substrate shock load. These results were correlated to the substrate processing structure that developed in each reactor type prior to the perturbation: substrate processing through parallel pathways was associated with a functionally more stable system, in contrast to serial processing of substrat (Hashsham et al., 2000). In other words, a system with more pathways towards methane production was functionally more stable than the one that relied on a series of interdependent metabolic events (*Cf.* Figure I.3).

An important outcome of these and other experiments is the realization that population diversity alone does not drive ecosystem stability. The positive relationship between the presence of multiple pathways towards a product (i.e. parallel processing of substrate) and functional stability parallels theoretical concepts in higher ecological organization (Peterson et al., 1998). Ecosystem stability is the outcome not of population diversity per se, but of functional redundancy, which is ensured by the presence of a reservoir of species able to perform the same ecological function.

This leads us to recognize that the diversity and the links within each key functional group of an ecosystem can lead to better ways to model diversity and function (Hulot et al., 2000), as well as to improve process stability (Watanabe et al., 2002). It is indeed nowadays clear that, even using molecular techniques, identification of every species in most environments is a daunting task (Curtis et al., 2002, Sogin et al. 2006, Huber et al. 2007). Nevertheless, much

progress has been made in linking identity to function among key microbial players in a variety of engineered systems (Wagner et al., 2002; Hofman et al., 2003). Armed with this knowledge, the discrepancy in performance sometimes noticed between similarly constructed bioreactors can be explained (Lee et al., 2002).

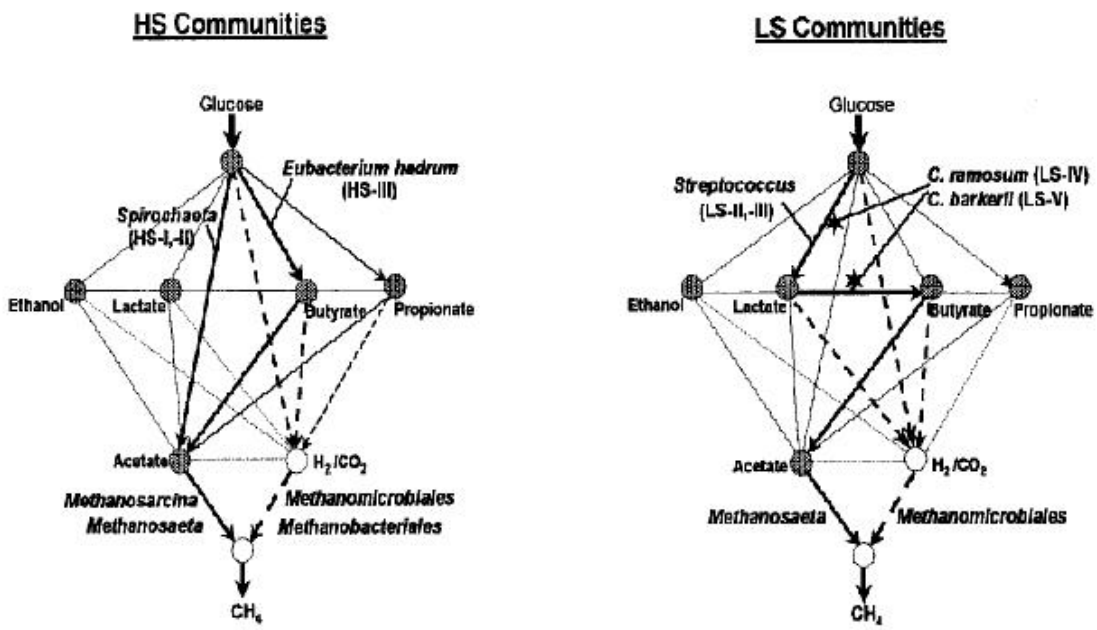

Figure I.3 Parallel and serial pathways towards methane production in high-spirochaete (HS) and low-spirochaete (LS) reactor sets (taken from Fernandez et al., 2000). The dotted lines and open circles indicate gas products. The thickness of each line represents the relative contribution of the pathway.

From these different elements available in the literature, it clearly appears that an important goal for research is to improve our understanding of the relative importance of the changes in different abiotic and biotic controls over specific ecosystem properties in different ecosystems. Success in answering these questions requires a very close coupling of recent theoretical and experimental approaches with the substantial information available from physiological, population, community and ecosystem ecology on which sets of traits influence species distributions, species interactions and particular aspects of ecosystem functioning. The model developed within this thesis and that will be later presented is one item in this direction. It has been indeed shown to be useful in assessing the influence of changes in different abiotic (such as feeding disturbances and presence of toxicans) and biotic controls (microbial community structure) over specific properties (such as pH changes, Volatile Fatty Acids accumulation, soluble COD removal and biogas production) in anaerobic reactors.

Some of the abiotic controls could also be considered as ecosystem properties of interest. ‘‘Modulators’’ are abiotic conditions that influence process rates (e.g., temperature and pH) but are not directly consumed in the process, in contrast to resources (Chapin et al. 2002). Various of aspects of the biotic community influence the range and proportion of species traits. These traits can further alter the abiotic controls, directly affect ecosystem properties, or directly affect ecosystem goods and services. Changes in ecosystem properties can further alter the biotic community either directly or via further alterations in abiotic controls. Feedbacks from altered goods and services can lead to modification of human activities, as evidenced in a variety of responses to environmental problems. From a very global point of view, a critical question is whether the rates and magnitudes of human changes will be sufficient to offset some of the original adverse ecological effects.

I.2 ANAEROBIC DIGESTION MODELS

As already pointed out, anaerobic digesters can exhibit significant unstability problems that can be avoided through appropriate control strategies. Such strategies require, in general, the development of appropriate mathematical models, which adequately represent the main biological processes that take place. This section reviews the current state of the art in anaerobic digestion modelling and identifies the key areas that require further research endeavors.

I.2.1 *SOME DEFINITIONS*

As will be discussed in details in Chapter II, **anaerobic digestion** is a multi-step biological process where the organic carbon is converted into its most oxidized (i.e. carbon dioxide CO_2) and most reduced (i.e. methane CH_4) states. The main product is indeed the biogas which is a mixture of CH_4 and CO_2, as well as trace gases such as hydrogen sulfide (H_2S) and hydrogen (H_2). The process is achieved as a result of the consecutive biochemical breackdown of polymers into methane and carbon dioxide in an environment in which a variety of microorganisms harmoniously grow and produce reduced end products. These microorganisms include fermentative bacteria (acidogens); hydrogen-producing, acetate-forming bacterias (acetogens) and methane-producing archaeas (methanogens).

I.2.2 *BRIEF HISTORY*

Most of the early anaerobic digestion models (see for example Graef and Andrews 1974; Hill and Barth 1977; Hill 1982; Kleinstreur and Powegha 1982; Mosey 1983; Bryers 1985; Moletta et al. 1986; Smith et al. 1988; Costello et al. 1991a, 1991b; Pullammanappallil et al. 1991) were capable of predicting digester failure, caused e.g. by a specific disturbance, either through a pH drop and/or the accumulation of volatile fatty acids. This is a commonly observed behaviour in digesters treating municipal sludge and/or high organic content industrial wastewaters. Later on, a model was developed (Angelidaki, 1992; Angelidaki et al. 1993) to describe anaerobic digestion of manure which exhibits a self-regulation of pH attributed to the generated ammonia. As other models, it considers hydrolysis, acidogenesis, acetogenesis and methanogenesis (*Cf.* Figure I.4) but it also accounts for free ammonia that inhibits methanogenesis. The maximum specific growth rate of the microorganisms and the degree of ionisation of ammonia are assumed to be dependent of temperature and pH. The pH self-regulation mechanism is as follows. Whenever free ammonia (high for high pH) inhibits methanogenesis, acetic acid is accumulated. This causes an inhibition to acetogenesis and a consequent accumulation of propionic and butyric acids, leading to inhibition of acidification.

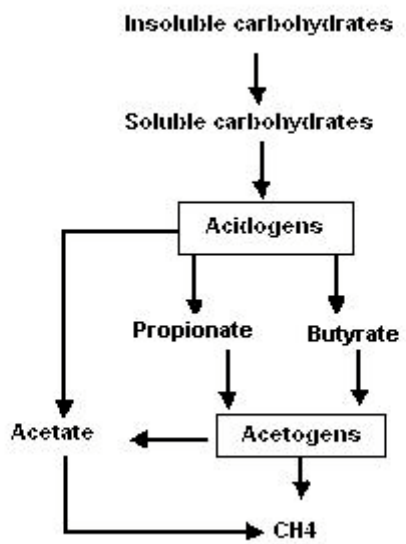

Figure I.4 The flow chart assumed in the Angelidaki et al (1993) model.

VFA accumulation reduces the pH, causing a decrease in the free ammonia concentration and the inhibition of methanogenesis. The process is thus self-regulatory unless the magnitude of the disturbance is larger than what the system can withstand. When this occurs, the pH drops

significantly, causing digester failure. A more complicated model that takes into account ammonia inhibition, lysis and hydrolysis of cell biomass, description of a physical-chemical system of pH-level, including the main buffer systems, can be also found in Siegriest et al. (1993).

However, all models described so far consider organic matter as a whole and do not account for the nature of the organic macromolecules in the feed composition. A modelling approach that takes the complex feed composition (i.e. breakdown of the particulate organic matter into carbohydrate, protein, VFAs and other organics) into account was proposed by Gavala et al., (1996). This model was capable of predicting adequately the COD and VFAs dependence on the operating conditions and is useful for designing codigestion processes of agroindustrial wastewaters (Lyberatos et al., 1997). This topic is presently intensively studied in the literature (Buffière et al., 2008). In parallel, significant research effort has been spent during the last years on the understanding of biofilm or granule formation in high-rate systems, such as fixed and fluidized beds and UASB reactors. Although the precise mechanism of biofilm and granule formation still remains unknown, their composition and the factors influencing their formation are understood to a great extent (see for example Cresson et al 2008 and related references). Some of them are even already included in mathematical models (see for example Batstone et al. 2005; Escudié et al. 2005; Batstone et al. 2006a; Mu et al. 2008) but additional research efforts still need to be spent on these aspects.

I.2.3 *THE IWA ANAEROBIC DIGESTION MODEL N° 1 (ADM1)*

Relatively recently, the International Water Association (IWA) task group for mathematical modelling of anaerobic digestion process developed a common model that can be used by researchers and practitioners (ADM1, Batstone *et al.*, 2002). This model has a structure similar to the IWA Activated Sludge Models (ASM) that have received wide acceptance over the last 15 years. The following sections provide a brief overview of the main aspects of the ADM1 model that will be further used in the framework of this study.

I.2.3.1 Model Description

The ADM1 model is a structured model that reflects the major processes that are involved in the conversion of complex organic substrates into CH_4 and CO_2 and inert byproducts. In Figure I.5, an overview of the substrates and conversion processes that are addressed in

ADM1 is presented. Extracellular solubilisation steps are divided into disintegation and hydrolysis, of wich the first is largely non-biological step and converts complex solids into inert substances, carbohydrates, proteins and lipids. The second step is an enzymatic hydrolysis of particulate carbohydrates, proteins and lipids into monosaccharides, amino acids and long chain fatty acids (LCFA) respectively. Disintegration is meanly included to describe degradation of composite particulate material with lumped characteristics (such as WAS), while the hydrolysis steps are used to describe well defined and relatively pure substrates (such as cellulose, starch and protein feeds). Monosaccharides and amino acids are fermented to produce VFAs (acidogenesis) and H_2. LCFA are oxidized anaerobically to produce acetate and H_2. Propionate, butyrate and valerate are converted into acetate (acetogenesis) and H_2. CH_4 is produced by both cleavage of acetate to CH_4 (aceticlastic methanogenesis) and reduction of CO_2 by H_2 to produce CH_4 (hydrogenotrophic methanogenesis). For more details about anaerobic digestion process biochemistry we suggest the reader addressing to annexe I.

To address these mechanisms and to reproduce the dynamic behaviour of soluble and particulate components, ADM1 includes 26 state variables. All organic matter and molecular hydrogen are described in terms of Chemical Oxygen Demand (COD). Nitrogen and inorganic carbon are described in terms of their molar concentrations. Soluble components (represented with a capital ‘‘S’’) are those that can pass through microbial cellular walls and include the monomers of complex polymers (sugars, amino acids, LCFAs), volatile fatty acids (propionate, butyrate, valerate, acetate), hydrogen, and methane.

In addition to the organic matter, the model also addresses inorganic carbon (carbon dioxide and bicarbonate) and nitrogenous compounds (ammonia and ammonium). All components that dissociate as a function of pH (i.e. VFAs and ammonia) have variables defined for both the protonated and non-protonated forms. Moreover, the model maintains a charge balance among ionic species and hence there are variables for inorganic anions and cations including the hydrogen ion. The model also tackles the dynamics of hydrogen ion concentration, and thereby the pH, by ensuring chemical neutrality in solution. Particulate components consist of either active biomass species or particulate substances that are incapable of directly passing through bacterial cell walls. In Figure I.5, particulate components are those with a capital ‘‘*X*’’. The microbial species that are considered in the model include sugar fermenters (X_{su}), amino acid fermenters (X_{aa}), LCFA oxidizers (X_{fa}), butyrate and valerate oxidizers (X_{c4}), propionate oxidizers (X_{pro}), aceticlastic methanogens (X_{ac}) and hydrogenotrophic methanogens

(X_{h2}). Non microbial particulate species include complex organics that either enter the process in the influent or that result from the death and decay of microbial species and the products of disintegration of the complex organics. This latter group consists of carbohydrates, proteins and LCFAs.

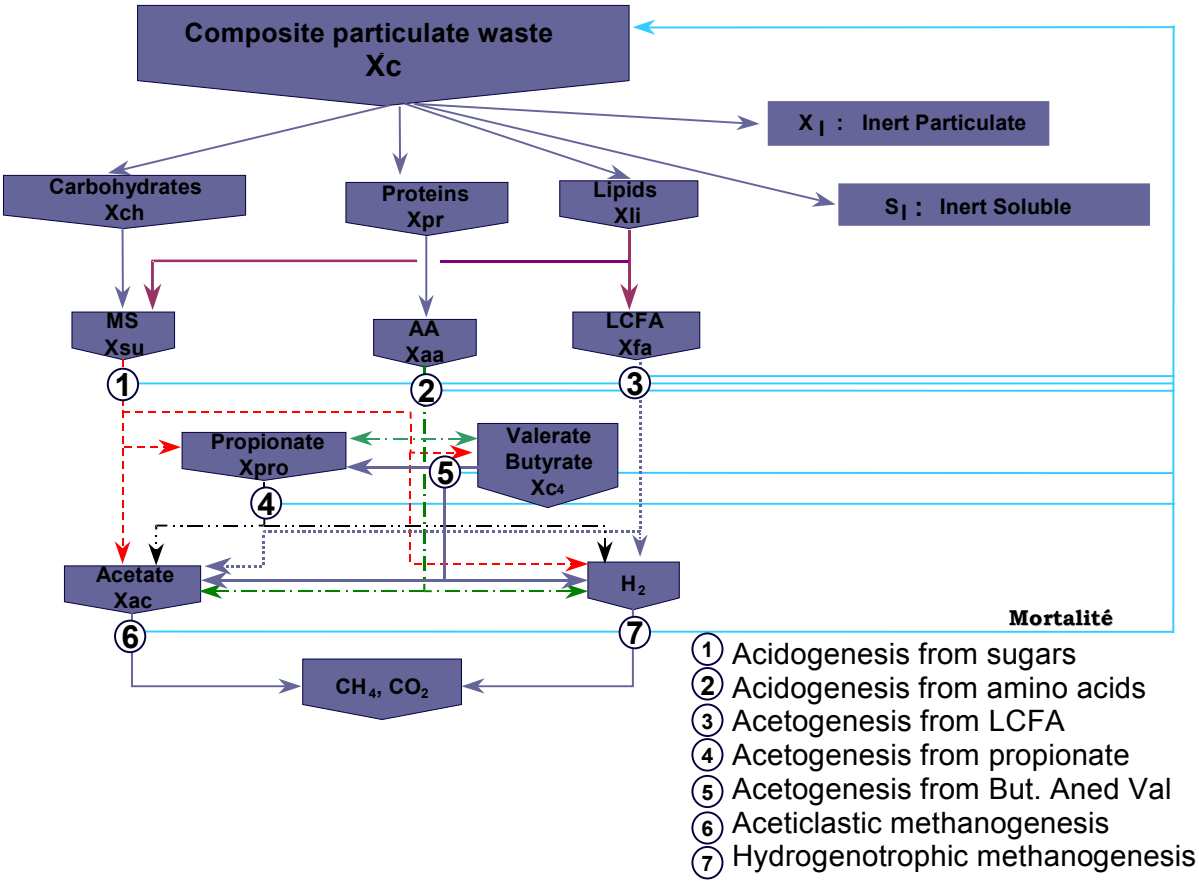

Figure I.5 General reaction pathway of anaerobic digestion included in ADM1.

Substrate conversion processes are described by a number of kinetic expressions that describe the conversion rates in terms of substrate concentrations and rate constants. The disintegration of Xc and hydrolysis of Xch, Xpr and Xli are described by first order rate expressions. Substrat-based uptake Monod-type are used as the basis for all intracellular biochemical reactions. Death of biomass is represented by first order kinetics and dead biomass is maintained in the system as composite particulate material.

It is recognized that a number of conversion processes can be inhibited by the accumulation of intermediate products such as H_2, ammonia or by extremes values of pH. In the model, inhibition functions include pH (for all groups), hydrogen (for the acetogenic groups) and free

ammonia (for the aceticlastic methanogens). Inhibition that is caused by H_2 and free ammonia is implemented in the model using rate multipliers that reflect non-competitive inhibition. An empirical correlation is also employed as a process rate multiplier to reflect the effects of extreme pH.

Liquid–gas mass transfer of gaseous components (CH_4, CO_2 and H_2) is described by mass transfer relationships. Hence, the application of the model equations requires separate mass balances for the liquid and gas phases of the components.

Tha ADM1 doen not describe all the mechanisms ocurring in anaerobic digestion (such as solids precipitation, sulfate reduction for example). However, the main objective was to develop a tool that would predict the dynamics of the key phenomena with sufficient acuracy to be useful in process developement, operation and optimization. Due to the varying demands in these fields, a different degree of model calibration and validation will be required en each case.

I.2.3.2 ADM1 Limitations

Initial work (2002 to 2005) was reviewed in a workshop in Copenhagen (Batstone et al., 2006b), and a number of specific limitations were identified for ADM1, including:

- Glucose fermentation models.
- Physicochemical system modelling (particularly precipitates, and specifically phosphorous modelling).
- Input characterisation.
- Parameter variation and validation in a broader context (validation on primary sludge was well established).

Many of the 30 papers presented at this workshop addressed some of these limitations, and subsequent work has led to significant advances in at least the last two areas.

Glucose fermentation modelling received a partial boost, with publication of a new theoretical model by Rodriguez *et al.,* (2006). This has been partially validated and further developed within the same group (Temudo *et al.,* 2007), but it is evident that there is still no

clear picture of how to represent glucose fermentation in a generalised way. From the hydrogen production perspective, fermentation modelling has decreased in importance, due to the possibility of thermal and electrochemically assisted hydrogen production direct from glucose and acetate (e.g., Liu *et al.,* 2005).

The **physicochemical system** used in anaerobic digestion modelling is fairly sophisticated, but has proven to be inadequate for complex and non-dilute systems. In particular, key limitations mean that divalent ions are particularly poorly represented, which causes problems for modelling of key states, including phosphate. This has not been really addressed well and is becoming a key issue, especially since physicochemical system modelling is being increasingly applied in activated sludge modelling, sensors, alternative systems (e.g., anaerobic ammonium removal, microbial fuel cells), and pure physicochemical systems (e.g., anion removal by precipitation).

Inputs characterization and model interfacing are recognised key issues in anaerobic digestion modeling. In the last five years, there has been a number of approaches proposed, mainly based on maintaining continuity of the major elemental and charge compounds:

- Generalised continuity based interface models (CBIM) have been proposed, and widely applied by the Ghent research group in Belgium (Vanrolleghem et al., 2005, Volcke et al., 2006; Zaher et al., 2007). These models emphatise continuity of elements (CHNOP) and charge. The key issue is that the user must eliminate degrees of freedom when the destination side has more input states than the source side. This is very much the case for almost any model when linked to ADM1.
- CBIM principles can also be applied to input models, and this has been done for general wastewaters (Kleerebezem and van Loosdrecht, 2006), primary sludge (e.g., Huete et al., 2006) and solid waste (Zaher et al., 2007). As an example, the three measures of particulate oxidation state, mass (or carbon mass) and organic nitrogen content can be used to define the three independent states of proteins, carbohydrates and lipids. Additional states can be defined by additional measurements (e.g., VFA, soluble, charge, titration profile). One of the key issues with these models is that small errors in measurements (e.g., TOC) may lead to large errors in individual input states due to accumulation of errors.
- Continued use of the Xc model, particularly for particular substrates (e.g., Yasui et al., 2006). The approach is here a little bit different than previous ones since it tries

to represent the inputs with a minimal set of lumped states (i.e., 1-2 Xc states). While not having the inherent robustness of CBIM-based models, they are simpler and in application have been very effective. Applying knowledge from these to CBIM-based models is also straightforward.

- Iterative, or stepwise CBIM models. This is a type of tailored CBIM model that removes the problem of excessive degrees of freedom on the destination side by using knowledge of the specific system (e.g., primary sludge or ASM1 states to ADM1). Much of this work has been done by the IWA Benchmarking Taskgroup to interface ASM1 and ADM1 models. Copp et al. (2003) proposed the first type of this model, while Nopens et al., (2007) proposed an updated version, which has also been used as an input model (Batstone et al., 2007).

Initial **parameter validation** post publication was mainly performed on primary sludge. This has now moved onto diverse systems under special conditions (e.g., sulfate reduction). The applicability of ADM1 parameters on primary and activated sludge has become more widely accepted, to such an extend that modelling has become benchmark of reactor performance (i.e., model parameters represent the majority of well-functioning systems), particularly for activated primary sludge. Nowadays, model outputs are more limited by input characterisation, than kinetic parameters.

The ability to better define the behaviour of influent substrate fractions and biomass fractions and their reaction stoichiometric and kinetics together with the power of the modern computer have made it possible to develope sophisticated dynamics models for the design, analysis, and performance prediction of complex biological treatment plants. The work of International Water Association Task group on anaerobic digestion modeling (IWA, 2002) is an excellent and continuos achievement of such modeling efforts that has resulted in the development, commercialization and widespread use of software process models, such as WEST (MOSTforWATER, 2007), SIMBA (Ifak System gbmH, Magdeburg, germany) among others.

This bibliographic review has shown that as engineered systems are often more manageable than large-scale ecosystems, and because parallels between engineered environments and other ecosystems exist, we will show in this PhD manuscript that the former can be used to elucidate some unresolved ecological issues.

Mathematical models in which data on micro-scale molecular diversity has been incorporated to more closely represent wastewater treatment processes, can provide a useful tool to reach this goal. Such models can be used to gain insight in the influence of process conditions on the selection of certain types of bacteria. In a later stage, these models can also be used to develop efficient control strategies adapted to model-based population optimisation. In this work, this approach will be demonstrated in the next chapters.

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CHAPITRE II

MODELISATION DE LA DIVERSITE MICROBIENNE EN DIGESTION ANAEROBIE: ADM1_10 ET PREMIERES APPLICATIONS

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RESUME

Le chapitre est composé de deux parties présentées sous forme d'articles scientifiques. La première partie est consacrée à la prise en compte de la diversité microbienne dans un digesteur anaérobie grâce à une approche par modélisation à l'aide du modèle Anaerobic Digestion Model No1 (ADM1). L'ajout d'une composante stochastique dans un modèle de bilan de masse bien défini et structuré tel que l'ADM1 peut être une amélioration importante pour représenter les données expérimentales en situation de fonctionnement normal ou de fonctionnement anormal. Un fonctionnement normal du procédé est défini de telle sorte que des modifications sur les caractéristiques de l'influent ne doivent pas perturber l'équilibre du système. Un fonctionnement anormal est quant à lui défini par la présence d'un agent toxique dans l'inffluent.

Dans le modèle ADM1 standard, chaque réaction considérée est associée à une population microbienne donnée. Sept groupes fonctionnels de micro-organismes sont inclus, correspondant à la dégradation des sucres simples (X_{su}), des acides aminés (X_{aa}), des acides gras à longues chaînes (X_{fa}), du valérate et du butyrate (X_{c4}), du propionate (X_{pro}), de l'acétate (X_{ac}) et de l'hydrogène (X_{h2}). Nous avons modifié le modèle ADM1 traditionnel en intégrant plusieurs espèces associées à chaque groupe fonctionnel, afin de mieux interpréter la diversité microbienne. Le nombre d'espèces par réaction a été attribué arbitrairement dans cette étude et a été fixé à 10, afin de limiter les temps de simulation. Le modèle obtenu est appelé ADM1_10, où '10' reflète la diversité microbienne du nouveau modèle, soit 10 espèces pour chaque groupe fonctionnel. Les espèces au sein d'un même groupe fonctionnel diffèrent par un coefficient de rendement Y, par une vitesse maximale spécifique de consommation de Monod km et par une constante de demi-saturation K_s. Dans cette étude, le coefficient de rendement est supposé constant car la variabilité de ce paramètre est faible en condition réelle. Dans un groupe fonctionnel, les paramètres cinétiques k_m et K_s sont choisis aléatoirement suivant une distribution normale bimodale, avec des moyennes de $\mu_1 = 0.6 * k$, $\mu_2 = 1.4 * k$ et une déviation standard de $\sigma_{1,2} = 0.125 * k$, où k est la valeur du paramètre correspondant dans le modèle ADM standard. L'objectif du modèle étant de simuler le comportement réel d'un digesteur, il est nécessaire de déterminer certains paramètres clefs de l'ADM1, tels que la vitesse maximale spécifique (k_m) et la constante de demi-saturation (K_s) de consommation pour chaque AGV, à partir d'une calibration par ajustement sur des données expérimentales.

Dans la structure de l'ADM1, chaque processus biologique de conversion du substrat est fonction d'une inhibition par des valeurs extrêmes de pH. De plus, les réactions d'oxydation anaérobies sont inhibées par une accumulation d'hydrogène, alors que la méthanogenèse acétoclaste est inhibée par de fortes concentrations en ammoniac. Les inhibitions engendrées par l'hydrogène et l'ammoniac sont intégrées dans l'ADM1 de base par des coefficients multiplicateurs suivant une fonction d'inhibition non-compétitive et les effets de pH extrêmes sont représentés par une fonction empirique. Toutes les fonctions d'inhibition du modèle modifié ADM1_10 sont identiques à celles du modèle ADM1 d'origine.

Une fonction d'inhibition par un toxique a par ailleurs été introduite dans le but d'étudier l'effet d'un agent toxique dans le système. Volontairement, aucune définition précise de l'action de l'agent toxique n'est fournie et il est supposé affecter l'ensemble des populations microbiennes suivant une fonction d'inhibition non-compétitive, de la forme:

$$I_{tox} = \frac{1}{1 + \frac{S_I}{K_I}}$$

où S_I est la concentration de l'agent toxique et K_I est le paramètre d'inhibition.

S_I est simulée par un signal d'impulsion donné et K_I est choisi arbitrairement équal à 2,5 $kg_{DCO}.m^{-3}$ dans ADM1 standard. Les valeurs des paramètres d'inhibition du toxique pour chaque biomasse d'ADM1_10 sont quant à elles choisies aléatoirement suivant une distribution bimodale uniforme entre deux séries de concentrations données: 1 et 4 $kg_{DCO}.m^{-3}$ (populations microbiennes plus tolérantes que la biomasse globale) et entre 10 et 13 $kg_{DCO}.m^{-3}$ (populations microbiennes plus sensibles à la présence d'un agent toxique).

Afin d'être dans des conditions de simulation comparables, les concentrations initiales de biomasse de l'ADM1 ont par ailleurs été distribuées de façon égale pour chaque espèce d'une population microbienne de l'ADM1_10. Les temps de rétention de la biomasse dans le procédé ont été modélisés suivant la méthode simplifiée proposée dans le rapport ADM1 (Batstone et al., 2002). La méthode permet de considérer la différence entre le temps de séjour hydraulique (TSH) et le temps de rétention du solide (TRS) grâce à l'ajout dans l'équation bilan de la biomasse d'un coefficient représentant le temps de rétention du solide ($t_{res,X}$).

La validation de cette approche s'est effectuée à partir des données expérimentales d'un digesteur anaérobie à lit fixe de 1 m^3, traitant des eaux usées de distillerie, en condition continue (DCOt constante et q_{in} variable). Les résultats de simulations ont montré que les deux modèles permettent de modéliser correctement les variations des principaux composés dans la phase liquide mais également dans la phase gazeuse. Par conséquent, le choix entre le modèle ADM1 et le modèle ADM1_10 est difficile, pour ne pas dire impossible. Bien sûr, il est possible de définir le meilleur ajustement obtenu entre les deux modèles mais l'objectif principal de cette étude est d'évaluer la capacité des deux modèles à prédire correctement le comportement de ce procédé et non d'ajuster parfaitement les données simulées sur les données expérimentales.

Les résultats de simulation obtenus après un "pulse" d'agent toxique démontrent que le modèle ADM1_10 est plus robuste à la présence d'un agent toxique que le modèle ADM1. En effet, l'impact du toxique est moins prononcé dans l'ADM1_10 que dans le modèle de base et une fois le toxique disparu, le retour à un fonctionnement normal est plus rapide (dans le chapitre suivant, les termes appropriés en écologie microbienne pour décrire ce comportement seront utilisés).

La deuxième partie de ce chapitre présente la validation des modèles avec des configurations de réacteurs et des caractéristiques d'entrant différentes. Pour cette étude, le procédé utilisé est un réacteur UASB de 9,8 L, dans lequel la partie inférieure est remplie par une couche de boues, la partie supérieure est occupée par des petits supports flottants de polyéthylène et un flux ascendant de substrat est injecté. Le fonctionnement du réacteur est de 232 jours à 33±1°C. L'alimentation continue du réacteur s'est effectuée avec une charge organique initiale de 2,9 $g_{DCO}.L^{-1}$ et a été augmentée par étape afin d'atteindre une charge de 19,5 $g_{DCO}.L^{-1}$ tout en maintenant un TSH constant de 1,05 j. Le réacteur a été équipé avec un système de recirculation interne continue à un debit de $9L.h^{-1}$ (flux = $0,83m.h^{-1}$) à partir du 159ème jour et ensuite le flux a été diminué de moitié à partir du 160ème jour.

Le modèle a été capable de représenter la tendance des données expérimentales observées. Cependant, après le 100ème jour, les concentrations en AGV sont sur-estimées. Il est apparu que les fonctions d'inhibition assocciées à des valeurs de pH faibles tendent à sur-estimer l'impact du pH sur les vitesses de réactions biologiques des bactéries dégradant les composés acides. De plus, le modèle est capable de prédire correctement les cinétiques de débit de biogaz et la composition du biogaz. Les légères déviations présentes dans la prédiction de la production de biogaz et de sa qualité peuvent être expliquées par le fait que le modèle n'intègre pas des coefficients de transferts liquide/gaz spécifiques à chaque composé du biogaz. En réalité, les coefficients de transferts liquide/gaz peuvent être différents et sont dépendants de la configuration du réacteur utilisé. Le modèle est également capable de simuler correctement les données expérimentales observées sur le pH.

Le modèle présenté dans ce chapitre (ADM1_10) offre donc de grandes perspectives en termes de modélisation mais également en termes de contrôle de procédés. L'intégration de la diversité par espèce microbienne dans les modèles mathématiques est en effet une étape clef afin d'optimiser et de conduire les performances des digesteurs anaérobies.

SUMMARY

This chapter consists of two sections presented in form of articles. The first section presents a modeling approach to account for microbial diversity in complex but structured models such as Anaerobic Digestion Model No1 (ADM1). It will be emphasize that adding a stochastic component on top of the mass balance structure of the well defined and well structured model such as the IWA ADM1, could be a powerful approach to represent experimental data, both in normal and abnormal situations. Normal situations are defined as changing input characteristics that do not imply process imbalance while abnormal situations are illustrated by the presence of toxicant into the reactor.

In the traditional ADM1 model, one microbial population is associated to each reaction. Seven functional groups of microorganisms are distinguished, corresponding to the degradation of sugar (by X_{su}), amino acids (by X_{aa}), LCFA (by X_{fa}), valerate and butyrate (by X_{c4}), propionate (by X_{pro}), acetate (by X_{ac}) and hydrogen (by X_{h2}) and one microbial population is associated to each reaction. In order to account for microbial diversity, the traditional ADM1 model was extended in such a way that multiple species are associated to each functional group. The number of species per reaction is arbitrary and in this study has been set to 10, to limit the reduction of computation speed. The resulting model will further be denoted as ADM1_10, where '10' refers to the extension of the original model for microbial diversity with 10 species for each group. Within each functional group, species may differ in terms of their yield coefficient Y as well as Monod maximum specific uptake rate km and half saturation constant K_s. In our case, the yield coefficient was assumed constant as in reality the variability of this parameter is low. Within a functional group, the kinetic parameters k_m and K_s were randomly chosen from a normal bimodal distribution, with means of $\mu_1 = 0.6 * k$, $\mu_2 = 1.4 * k$ and standard deviations of $\sigma_{1,2} = 0.125 * k$ where k is the value of the corresponding standard ADM1 parameter. With the objective of which the models reflect real-world behavior, some key parameters in ADM1, such as the specific maximum uptake rate constant (k_m) and the half saturation constant (K_s) for the VFAs consumption were calibrated to fit the data.

In ADM1, all microbial mediated substrate conversion processes are subject to inhibition by extreme values of pH. Moreover, the anaerobic oxidation processes are subject to inhibition due to accumulation of hydrogen while aceticlastic methanogenesis is inhibited by high free ammonia concentrations. Inhibition caused by hydrogen and free ammonia was originally implemented in ADM1 by rate multipliers that reflect non-competitive inhibition and an empirical correlation was used to reflect the effects of extreme pH. All inhibitions from ADM1 were kept identical in the modified model ADM1_10.

To investigate the effect of toxicant, an additional specific toxicant inhibition function was added. No precise definition was here chosen for the toxicant: it was assumed to affect all microbial populations and modeled as an non-competitive inhibition factor added to all substrate uptake rates:

$$I_{tox} = \frac{1}{1 + \frac{S_I}{K_I}}$$

where S_I is the toxicant concentration and K_I the inhibition parameter. In the following, S_I was simulated as a pulse signal and K_I value was arbitrarily chosen equal to 2.5 kg_{COD}/m^3 for ADM1. In line with the choice of the kinetic parameters of ADM1_10, the values of the

inhibition factors were randomly chosen for each biomass from a uniform bimodal distribution within two sets: 1 and 4 kg_{COD}/m^3 (to represent the fact that some microbial populations can be more tolerant than the global biomass represented in ADM1) and between 10 and 13 kg_{COD}/m^3 (in this last case, it is assumed that microbial populations are much more sensitive to the presence of toxicant).

In order to maintain comparable conditions for simulations, the initial biomass concentrations in ADM1 have been distributed equally among the corresponding microbial populations in ADM1_10. Biomass retention in the reactor has been modeled in the simplified way suggested in the ADM1 report (Batstone et al., 2002), with a term including the solids residence time of solids ($tres_{,X}$) in the biomass mass balance equation to account for the difference between hydraulic retention time (HRT) and solid retention time (SRT).

Experimental data from a 1 m^3 pilot scale up-flow anaerobic fixed bed digester treating wine distillery wastewater under dynamic input (COD_t is constant but q_{in} is variable) are provided to demonstrate the applicability of this approach. Simulation results show that both models can simulate very nicely the dynamic evolutions of the main variables, in the liquid but also in the gas phases. As a consequence, assessing the most appropriate model among ADM1 and ADM1_10 is a tedious, not to say impossible, task. Of course, it could be said that better fit could have been obtained but the main purpose of this study was not to perfectly fit these data but to evaluate the ability of both models to adequately predict the behavior of this particular digestion process.

With regard to models behavior under pulse toxicant, Simulations results demonstrates that ADM1_10 has higher robustness to the presence of toxicant than ADM1. The effect of the toxicant is indeed less pronounced in ADM1_10 than ADM1 and recovery is faster once the toxicant disappears (In the following chapter, we will use ecological terms to describe this behavior).

With the aim of model validating, in the second section of this chapter, we use the same models but with another reactor configuration and another input regime. In this case we work with a 9.8-L hybrid upflow anaerobic sludge-filter bed reactor in which the lower half portion was occupied by a sludge blanket and the upper half by small floating polyethylene media, using the same previous section substrate. The reactor was operated for a total period of 232 days at 33 ± 1°C. Continuous feeding of the reactor was started with an initial OLR of 2.9 g COD/L.d and then it was increased step wise to 19.5 g COD/L.d by increasing the feed COD concentrations, while maintaining a constant HRT (1.05 d). The reactor was equipped with a continuous internal recirculation system from top to the bottom at the rate of 9 L/h (upflow velocity = 0.83 m/h) up to day 159 and then it was reduced to about half on day 160 onwards.

The model was able to reflect the trends that were observed in the experimental data. However, after day 100, the concentrations of VFAs were over-predicted. It appeared that the inhibition functions associated with low pH values tend to overestimate the impact of pH on biokinetic rates for the acid-consuming bacteria. Furthermore, the model predicts well the dynamics of the biogas production rate and composition as a response of the load imposed. Small deviations in predicting the biogas production and quality have been found. The differences can be explained with the non-optimization of several parameters, for instance the application of identical and non-optimized gas transfer coefficients. In fact, gas transfer coefficients may differ in reality and the dependence on the specific reactor configuration

applied has been neglected. The pH was also be quite accurately simulated and the model was able to reflect the trends that were observed in experimental data.

Finally, the model presented in this chapter (ADM1_10) offers wide perspectives in terms of modeling abilities but also in terms of control objectives since microbial population appears nowadays to be a major component that drives anaerobic digesters performances.

II.1 MODELING MICROBIAL DIVERSITY IN ANAEROBIC DIGESTION[1]

II.1.1 *INTRODUCTION*

As mentioned in annexe II, several advantages are recognized to anaerobic digestion (AD) processes: high capacity to treat slowly degradable substrates at high concentrations, very low sludge production, potentiality for valuable intermediate metabolites production, low energy requirements, reduction of odors and pathogens and possibility for energy recovery through methane combustion or even hydrogen production. However, AD processes also have drawbacks: (i) the low sludge production is closely linked to the slow growth of micro-organisms, (ii) AD micro-organisms are highly sensitive to overloads of the process and disturbances of several causes and (iii) AD is a complex process involving many different micro-organisms which is still not completely understood.

These drawbacks explain probably that AD processes are not more widely used at the industrial scale. In the past, the lack of knowledge concerning AD processes led to breakdowns, ranging from minor to catastrophic, mainly due to organic overloads of various origins. In general, anaerobic reactors are indeed affected by changes in external factors, but the severity of the effect is dependent upon the type, magnitude, duration and frequency of the imposed changes (Leitão *et al.,* 2006). The typical responses include a decrease in performance, accumulation of volatile fatty acids (VFAs), drop in pH and alkalinity, change in biogas production rate and composition, sludge washout and shifts in microbial community structure. The accumulation of VFAs during anaerobic digester overload is particularly well documented (see for example Ahring *et al.,* 1995). Without adequate levels of populations that can remove hydrogen and other intermediates, VFAs continue to accumulate, inhibiting methanogenesis and causing further imbalance. Since the microorganisms responsible for VFA consumption (*i.e.,* proton-reducing acetogens) are very sensitive to the accumulation of their own metabolites (hydrogen, formate and acetate – see for example Stams, 1994), the inhibition of methanogenesis by these products causes further VFAs build-up. Therefore, the rapid acidification of an overload digester can bring VFA oxidation and methanogenesis to a complete halt, preventing digester recovery (Zinder 1993). A successful recovery from overload would require adequate levels of VFA-degrading microbes to metabolize the surplus

[1] Paper published by I. Ramirez and J-Ph. Steyer in *Water Science & Technology*, **57**(2):265-270, (2008).

intermediates, sufficient quantities of methanogens to consume the hydrogen and acetate produced during VFAs oxidation, and environmental conditions to encourage their close association. However, until now, very few studies have linked microbial community structures with reactor performances. Such an analysis is indeed difficult due to the lack of adequate tools to study microbes directly in their natural habitat. Cultured-base methods have been especially difficult to use in anaerobic systems because syntrophic interactions, low growth rates, unknown growth requirements and obligate anaerobic conditions make microorganisms difficult to isolate and to identify from digesters. However, these last two decades, molecular tools based on sequence comparison of small sub-units (SSU) ribosomal RNA (rRNA) molecules have made it possible to study complex microbial communities without the need to culture microorganisms, thereby reducing the widely acknowledge biases associated with culturing (Ward *et al.,* 1992). Oligonucleotide probes targeting SSU rDNAs of phylogenetically defined groups of microbes (methanogens, sulfate-reducing bacteria, fiber digesting microbes) have been already used for the quantification of population abundance in bioreactors (Hansen *et al.,*1999). Griffin *et al.,* (1998) used oligonucleotide probe hybridization to evaluate methanogen population dynamics in anaerobic digester and they demonstrated how this technology could be used to link microbial community structure and digester performance. But changes in microbial community structure may also occur without detectable changes in performance (Fernandez *et al.,* 2000). As a consequence, there is nowadays strong evidences that the performance of an anaerobic digestion processes can be closely related to the structure and diversity of its microbial community (McMahon *et al.,* 2001).

Strictly speaking, microbial diversity is the number of different species in a particular area (*i.e.,* microbial richness) weighted by some measure of abundance such as the number of individuals or biomass. However, it is common to speak of species diversity even when it actually refers to species richness which is simply not rich enough. The definition of biological diversity that we have adopted in this work is simply “the variety and abundance of species in a defined unit of study” (Magurran, 2005).

The objective if this paper is to present an approach able to handle microbial diversity in complex models such as the IWA anaerobic digestion model No1 (ADM1) (Batstone *et al.,* 2002). Experimental results will be used to compare classical models such as ADM1 with an

increased complexity model and further simulations will show that microbial diversity can lead to different results and different conclusions about some experimental results.

II.1.2 *MATERIALS AND METHODS*

The effluents used are raw industrial wine distillery wastewaters obtained from local wineries in the area of Narbonne, France. The process is an up-flow anaerobic fixed bed reactor made of a 0.948 m^3 circular column. The following measurements are available on-line: input and recirculation liquid flow rates, pH in the reactor and in the input wastewater, temperature, biogas output flow rate, CO_2, CH_4 and H_2 composition in the gas phase, total organic carbon, soluble chemical oxygen demand, VFAs and bicarbonate concentrations and total and partial alkalinity in the liquid phase. More details on the process can be found in (Steyer *et al.,* 2002).

As already said, the model used for simulation is the ADM1 model. Only few parameters (mainly k_m and K_S related to acetate and propionate degraders) were modified to simulate experimental data available from the process (See Table II.1). The difference between hydraulic and solid retention times (*i.e.,* HRT and SRT) due to biofilm present in the reactor was modeled by adding an extra term (*i.e.,* $t_{res,X}$) in the biomass equation as recommended in the ADM1 report.

Table II.1 Main parameters estimated to fit the experimental data

Parameter	Acetate	Propionate
k_m (kg_{COD}/Kg_{COD}.day)	1.93 (8)*	2.51 (13)
K_S (kg_{COD}/m^3)	1.41 (0.15)	1.41 (0.10)

* Values in parenthesis are the reference values recommended in the ADM1 report

In the ADM1 model, one microbial population is associated to each reaction and seven main grops of bacteria are represented: sugar, amino acids, LCFA, valerate and butyrate, propionate, acetate and hydrogen degraders, each of them having specific kinetic parameters. In order to account for microbial diversity, it was decided to increase the number of species and 10 different species were associated to each reaction. For each specie, the associated kinetic parameters were randomly chosen among 2 sets normally distributed on each side of the kinetic parameters used to simulate ADM1 (*Cf.* Figure II.1). These sets were arbitrarily

centered on 0.6 and 1.4 times the values used in ADM1 (+/- 10%) in order to simulate two distinct populations of each reaction. In the following, this augmented ADM1 model will be called ADM1_10 and in order to obtain similar conditions for simulations, each initial biomass concentrations in ADM1 will be divided by 10 and distributed equally among each microbial population in ADM1_10.

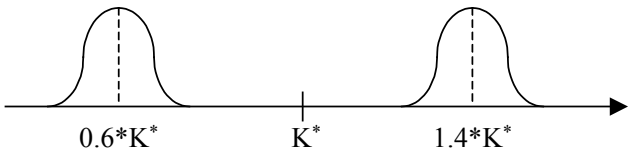

Figure II.1 Each kinetic parameter of ADM1_10 is randomly chosen among 2 sets distributed on each side of the kinetic parameters of ADM1

II.1.3 *RESULTS AND DISCUSSION*

II.1.3.1 ADM1 vs ADM1_10 When Facing Dynamic Inputs

The wine distillery wastewater used as influent during the experiments consisted of carbohydrates, sugars, VFAs, inorganic carbon and nitrogen. The concentrations of these individual components used in ADM1 and ADM1_10 as process inputs are shown in Table II.2. The experimental data over one month are presented in Figure II.2, together with the varying input feed flow and simulations of ADM1 and ADM1_10.

Table II.2 Input concentrations of the wine distillery wastewater used during the experiments

Constituent	Values[1]	Constituent	Values[1]
Sugars	6.64	Inorganic Carbon	0.0025 kmol C/m^3
Total Valerate	0.53	Inorganic Nitrogen	0.0417 kmol N/m^3
Total Butyrate	2.70		
Total Propionate	1.69	CODs	13.90
Total Acetate	2.33	CODx	1.10
Carbohydrates	1.10		

[1] *Unless otherwise stated, kg COD×m^{-3}*

As it can be seen, both models can simulate very nicely the dynamic evolutions of the main variables, in the liquid but also in the gas phases. Even the pH can be quite accurately simulated. As a consequence, assessing the most appropriate model among ADM1 and ADM1_10 is a tedious, not to say impossible, task. Of course, it could be said that better fit

could have been obtained but the main purpose of this study was not to perfectly fit these data but to evaluate the ability of both models to adequately predict the behavior of this particular digestion process.

The main difference from these two models relies in the biomass evolutions. As an illustration, Figure II.3 shows the obtained specific growth rates and the dynamic evolution of acetate degraders during these simulations. Similar results were obtained for all degraders but they are not shown due to space limitation.

II.1.3.2 ADM1 vs ADM1_10 When Facing A Toxicant

Developing and tuning mathematical models in normal situations is nowadays a well defined procedure that can be easily performed, even with complex models such as ADM1. However, developing and tuning a model to adequately represent abnormal situations is still a difficult and challenging task. In particular, when facing inhibition and/or toxicant, AD processes can experimentally present different behaviors that are still not fully understood: one process can indeed show high robustness with respect to toxicant presence while other ones, even though they seem to be very similar, are much more sensitive to disturbances.

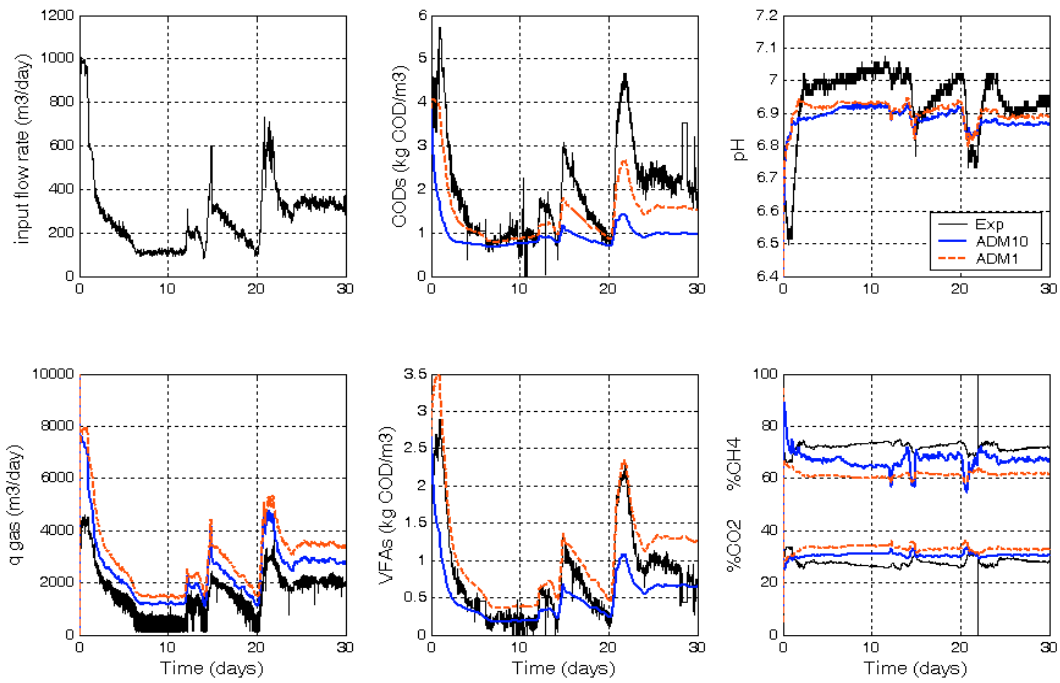

Figure II.2 Experimental data and simulation of ADM1 (dotted lines) and ADM1_10 (continuous lines)

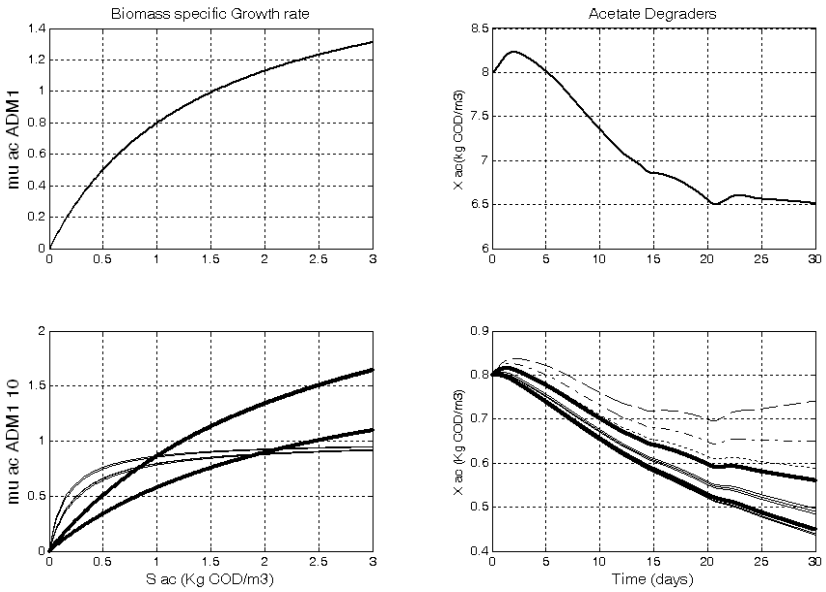

Figure II.3 Specific growth rate and dynamic evolution of acetate degraders during the simulation of ADM1 and ADM1_10.

In ADM1, all microbial mediated substrate conversion processes are subject to inhibition by extreme values of pH. Moreover, the anaerobic oxidation processes are subject to inhibition due to accumulation of hydrogen while acetoclastic methanogenesis is inhibited by high free ammonia concentrations. Inhibition caused by hydrogen and free ammonia was originally implemented in ADM1 by rate multipliers that reflect non-competitive inhibition and an empirical correlation was used to reflect the effects of extreme pH. These different inhibitions were kept in both models but an additional specific toxicant inhibition was added. No precise definition was here chosen for the toxicant: it was assumed to affect all microbial populations and modeled as an non-competitive inhibition factor added to all substrate uptake rates:

$$I_{tox} = \frac{1}{1 + \frac{S_I}{K_I}}$$

where S_I is the toxicant concentration and K_I the inhibition parameter. In the following, S_I was simulated as a pulse signal and K_I value was arbitrarily chosen equal to 2.5 kg_{COD}/m^3 for ADM1. In line with the choice of the kinetic parameters of ADM1_10, the values of the inhibition factors were randomly chosen for each biomass from a uniform distribution within two sets: 1 and 4 kg_{COD}/m^3 (to represent the fact that some microbial populations can be more tolerant than the global biomass represented in ADM1) and between 10 and 13 kg_{COD}/m^3 (in

this last case, it is assumed that microbial populations are much more sensitive to the presence of toxicant). Simulations results are presented in Figure II.4. As it can be seen, ADM1_10 demonstrates a higher robustness to the presence of toxicant (from day 25 to 30) than ADM1. The effect of the toxicant is indeed less pronounced in ADM1_10 than ADM1 and recovery is faster once the toxicant disappears.

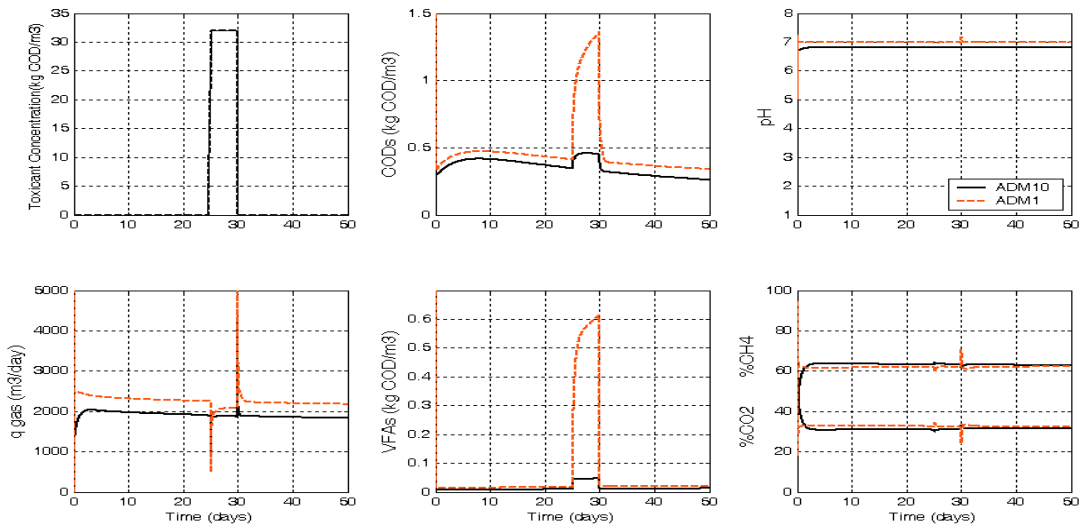

Figure II.4 ADM1 (dotted lines) and ADM1_10 (continuous lines) simulations when facing a pulse of toxicant (top left figure). Except the inhibition parameters related to the toxicant, all other kinetic parameters were identical to those used in the simulations presented in Figures 2 and 3.

It is undeniable that interest and activity in academic and applied anaerobic digestion simulation is rapidly developing. Indeed, there have been more than 750 scientific publications regarding AD modeling over the last 40 years, of which half have been published in the last few years (Batstone *et al.,* 2006). Much of this work has been a driving force for the development of a standardized anaerobic model and ADM1, despite of being introduced very lately, as imposed itself as an excellent simulation platform. This is due to its adequate structure that is able to handle many different situations faced experimentally. However, one of its main drawback is that it is unable to handle microbial diversity and thus, it cannot represent adequately experimental results that apparently seem to be obtained in similar conditions even though being driven by different microbial diversities present during the experiments.

The accounting for microbial diversity in mathematical models has been said by some researchers to be unnecessary and by others to be impossible. The approach presented in this

paper is, to our opinion, an excellent – but not unique – option to handle microbial diversity. It indeed introduces stochastic aspects on top of a well structured model based on first principles (*i.e.,* mass balance). Due to computer limitations, we could not handle more than 10 species for each biological reaction but it is our strong belief that more species (*i.e.,* ADM1_100 to ADM1_1000) should be accounted for to accurately handle microbial diversity. Moreover, by handling a much higher number of species per reaction, one would minimize the efforts for parameter estimation (*i.e.,* only a "global" value of the model parameters such as in ADM1 would be required, microbial diversity being later accounted for by the high number of species handled with random kinetic parameters centered around the average values found to fit ADM1).

Finally, we also believe that engineered operations of AD wastewater treatment systems would be improved if we could predict and manipulate the diversity of such systems. This ability would complement our established ability to predict the optimal process design since, as a single example, it appears nowadays clearly that the nature, composition and distribution of the microbial population ultimately define the main operating characteristics and performances of the reactors.

II.1.4 *CONCLUSIONS*

This paper presented an approach to account for microbial diversity in complex but structured models such as ADM1. It has been emphasized that adding a stochastic component on top of the mass balance structure of the model could be a powerful approach to represent experimental data, even in abnormal situations such as the presence of a toxicant. This offers wide perspectives in terms of modeling abilities but also in terms of control objectives since microbial population appears nowadays to be a major component that drives anaerobic digesters performances.

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II.2 EXPERIMENTAL AND MODELING INVESTIGATIONS OF A HYBRID UPFLOW ANAEROBIC SLUDDGE-FILTER BED (UASFB) REACTOR[2]

II.2.1 *INTRODUCTION*

High-rate anaerobic reactors are becoming increasingly popular for the treatment of various types of wastewater because of their low initial and operational costs, smaller space requirements, high organic removal efficiency and low sludge production, combined with a net energy benefit through the production of biogas. The upflow anaerobic sludge blanket reactor (UASB) and anaerobic filters (AF) are the most frequently used high-rate anaerobic reactors, but both types suffer from technical problems (Jhung and Choi, 1995). Granular sludge formation is the main distinguish characteristics of UASB reactors as compared to the other anaerobic technologies. But with some wastewaters, granulation does not occur readily and problems can be experienced with washout of flocculent biomass (Reynolds and Colleran, 1986). Also, in a UASB reactor, very low flow rate liquid superficial velocity may cause channeling of wastewater through the bed and therefore a poor water-sludge contact, which leads to low treatment efficiencies. In fully packed anaerobic filters, long-term operation may result in excessive biomass entrapment in the interstitial cavities in the matrix bed, with resultant problems of clogging and channeling (Jhung and Choi, 1995). Henceforth, modification of the AF process is required to minimize and overcome the existing deficiencies faced by both UASB and AF. Use of internal packing as an alternative for retaining biomass in the UASB reactor is a suitable solution for the above mentioned problem. The packing medium in the UASB reactor is intended to increase solids retention by dampening short circuiting, improving gas/liquid/solid separation, and providing surface for biomass attachment. A reactor of this kind is referred to as a hybrid upflow anaerobic sludge-filter bed (UASFB) reactor in this study. This kind of reactor hybridizes the advantages of both UASB and upflow anaerobic filter (UAF) processes, while minimizing their limitations (Lo *et al.,* 1994). The use of packing media only in the top portion of the reactor minimizes channeling problem associated with UAF and loss of biomass due to flotation associated with poorly performing UASB reactors.

[2] Paper published by R. Rajinikanth, I. Ramirez, J-Ph. Steyer, I. Mehrotra, P. Kumar, R. Escudie, M. Torrijos in *Water Science & Technology*, **58**(1):109-117, (2008).

The present research work was undertaken to study the biodegradation of wine distillery vinasses in a hybrid upflow anaerobic sludge-filter bed (UASFB) reactor. The following aspects are also discussed in this paper: (i) behaviour of the reactor with respect to the clogging; (ii) the quantity of biomass in the sludge and filter bed zones, and their specific biomass activities; (iii) specific methanogenic activities (SMA) of the granules and attached biomass and (iv) application of IWA's anaerobic digestion model No.1 (ADM1) for simulating and analysing the experimental results.

II.2.2 *MATERIALS AND METHODS*

II.2.2.1 Experimental setup

The schematic diagram of the laboratory scale UASFB reactor used in this study is shown in Figure II.5. The diameter of the reactor was 12 cm and height was 117 cm. The reactor was made-up of plexi-glass with an effective volume of 9.8-L. The reactor column constituted of two compartments viz. bottom part was operated as a UASB reactor; whereas the top part was operated as an anaerobic filter. The top portion of the UASFB reactor was randomly packed with 90 pieces of small buoying polyethylene packing media, which are cylindrical in shape (29 mm high and 30/35 mm diameter) and baffled with 16 partitions. The density and specific area of the media were 0.93 and 320 m^2/m^3 respectively. 50% of the reactor volume (excluding the head space of 30 cm height) was filled with this media. The reactor operated at 33 ± 1^oC, was equipped with a continuous internal recirculation system from top to the bottom at the rate of 9 L/h (upflow velocity = 0.83 m/h) up to day 159. On day 160, the recirculation rate was reduced to 5 L/h (upflow velocity = 0.48 m/h). Recirculation was done mainly to eliminate the possibility of high organic loading close to the feed port and to favour better wastewater/sludge contact. The digester was seeded with granules (15% by total volume) originated from a UASB digester treating cheese wastewaters.

II.2.2.2 Substrate

The experiments were performed with distillery vinasse (wine residue after distillation), which was obtained from a local distillery around Narbonne, France. The reactor was fed with vinasse in the increasing concentrations of total COD from 3.1 g/L to a maximum value of 21.7 g/L by appropriately diluting the raw vinasse with tap water. Around 95% of the total

COD was soluble. The feed was supplemented with nutrients to attain COD:N:P ratio of 400:7:1 in the wastewater. pH of the feed was adjusted to 6-6.5 using a 6N sodium hydroxide.

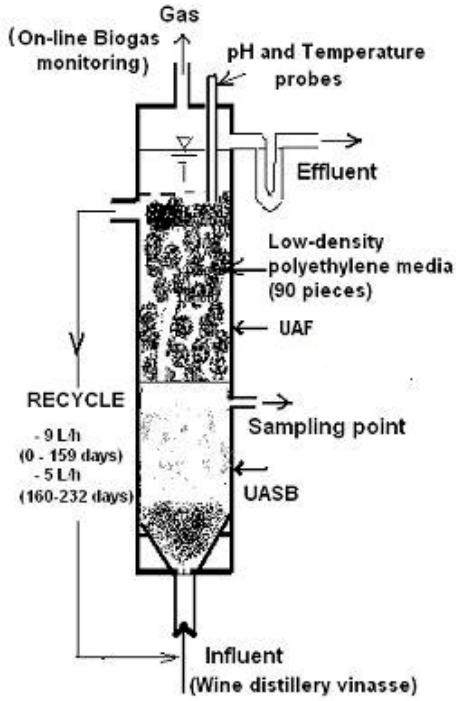

Figure II.5 Schematic diagram of a hybrid UASFB reactor

II.2.2.3 Analytical methods

The performance of the UASFB reactor was evaluated by monitoring total (COD_t) and soluble (COD_s) chemical oxygen demand, suspended solids (SS), volatile suspended solids (VSS), and alkalinity according to the Standard Methods for Examination of Water and Wastewater (1992) at inlet and outlet of the reactor. VFAs were determined using gas chromatograph (GC- 8000 Fisions instrument) equipped with a flame ionisation detector with an automatic sampler AS 800. Biogas production was measured online. Data acquisition and measurement of biogas was performed using the "Modular SPC" software developed by the Laboratory of Environmental Biotechnology (LBE) in Narbonne, France as previously described by Ruiz (2002). The percentage of methane (CH_4) and carbon dioxide (CO_2) in the biogas were determined using a gas chromatograph (Shimadzu GC-8A), with argon as the carrier gas, equipped with a thermal conductivity detector and connected to an integrator (Shimadzu C-R8A).

To analyze the hydrodynamics of the UASFB reactor, the study of the residence time distribution (RTD) was performed by using LiCl solution as previously described by Escudié

et al. (2005). A pulse of tracer lithium chloride (25-mg of Li/L) was introduced at the bottom of the reactor along with the input stream and the Li concentration was measured at the outlet using a Flame photometer (Model 410, Corning). Effluent samples were taken at the outlet using an automatic sampler. The duration of this experiment was more than 5τ, where τ represents the theoretical average retention time.

At the end of the experiment, the reactor was emptied to quantify the amount of biomass (in terms of volatile solids) entrapped in to each support and in granules was quantified gravimetrically by weighing the oven-dried samples at 105°C for 24 h. Oven-dried solid samples were scrapped out from the supports and ignited at 550°C for 2 h to estimate the volatile solid content.

II.2.3 *RESULTS AND DISCUSSION*

II.2.3.1 Operational strategy

A hybrid UASFB reactor was operated for a total period of 232 days at 33±1°C. Continuous feeding of the reactor was started with an initial OLR of 2.9 g COD/L. d and an HRT of about 1.05 d. The OLR was then increased stepwise by increasing the substrate concentration while maintaining a constant HRT. A COD_s removal efficiency of 80% was considered as the threshold level in the present study for the operation of the UASFB reactor. OLR was progressively increased by 20 to 30% once or twice a week until COD_s removal dropped below 80%. Thus, influent COD_t concentration was increased stepwise from 3.1 to 21.7 g/L (max OLR of 19.5 g COD/L. d).

II.2.3.2 Performance of a hybrid UASFB reactor

II.2.3.2.1 Effect of OLR on COD removal efficiencies*:* During the start up period (initial 11 days of operation), solid washout was quite high, which was reflected by an increase in SS (from 0.3 to 1.1 g/L) and COD_t (from 0.5 to 1.3 g/L) concentrations, at a low OLR of 3 Kg COD/m^3.d. Afterwards, those values were decreased gradually to about 0.55 g COD_t/L and 0.4 g SS/L corresponding to a COD_t removal of 85% probably due to the filtration effect of the top packed-bed portion. The OLR was then increased step-wise upto 11.8 g COD/L.d by

increasing the feed COD concentrations. COD_t and COD_s removal efficiencies did not vary during this period with values more than 90 and 93%, respectively (*Cf.* Table II.3).

On day 35, about 300 mL of sludge were discharged out of the reactor due to an accident (connection failure at the bottom of the reactor) and hence the performance of the UASFB was disturbed. As a consequence, the OLR was brought down to about a half in order to balance or minimise the over loading of the reactor due to insufficient microbial biomass in the reactor. Based on the COD removal efficiencies, OLR was then slowly pushed up to 11.8 g COD/L.d. From day 57 onwards, the reactor reached the steady-state conditions and after which the OLR was maintained constant at around 11.8 g COD/L.d with an average COD_t and COD_s removal efficiencies of 86 and 91%, respectively (Table 1).

In order to improve the treatment performance and liquid mixing inside the reactor, fresh feed was mixed with recycled effluent. High recirculation rate of 9 L/h (upflow velocity of 0.83 m/h) was maintained up to day 159, mainly to eliminate high organic over loading and to supply alkalinity by blending the fresh feed with the low COD and high alkalinity recycled stream (Najafpour *et al.,* 2006). On day 160, the recirculation rate was reduced to about half (i.e. 5 L/h, upflow velocity of 0.48 m/h). It was observed that the reduced recirculation rate did not affect the performance of the reactor with a COD_t and COD_s removal efficiencies of 86 ±2.7 and 93 ±0.6 %, respectively (Table 1). On day 180, the OLR was increased from 11.8 to a maximum of 19.5 g COD/L.d with a COD_t and COD_s removal efficiencies of 82 ±4.2 and 88 ±3.4%, respectively.

Table II.3 Performance of UASFB reactor at various OLR*

Days	Influent CODt (g/L)	OLR (g /L. d)	Effluent COD (g/L) [Removal efficiency %]		L CH4/ L of reactor. d	Methane Yield (L CH4/g COD removed)	VFA (g/L)		Alkalinity (g $CaCO_3$/L)	VFA-COD/ Alkalinity
			CODt	CODs	[CH4-COD]		VFA-COD	Non VFA-COD		
0-35	3.1-12.5 (6.4)	2.9-11.8 (6.08)	0.50-2.00 (0.87) [85± 7.5][a]	0.09-1.32 (0.471) [93±1.7][a]	0.89-3.84 (2.23) [2.74-10.83][b]	0.349±0.043	0.01-0.14 (0.07)	0.08-0.69 (0.41)	1.28-1.81 (1.51)	0.04
36-57	6.0-12.3 (7.9)	5.7-11.7 (7.56)	0.64-1.7 (1.03) [87±2.4]	0.41-1.2 (0.720) [90±2.1]	1.60-3.21 (2.22) [5.11-10.16]	0.340±0.025	0.03-0.61 (0.27)	0.35-0.74 (0.53)	1.5-2.1 (1.80)	0.15
58 - 159	11.7-15 (12.9)	10-13.6 (11.66)	1.32-2.2 (1.83) [86±2.3]	0.85-1.34 (1.089) [91±2.1]	3.0-4.47 (3.68) [9.0-12.01]	0.331±0.014	0.07-0.72 (0.28)	0.75-1.07 (0.86)	2.2-3.3 (2.81)	0.10
160–180[+]	12.2-13.7 (13.1)	11-12.3 (11.75)	1.36-2.33 (1.83) [86±2.7]	0.83-1.0 (0.913) [93±0.6]	3.56-4.19 (3.89) [10.0-11.01]	0.334±0.011	0.06-0.08 (0.23)	0.80-1.0 (0.88)	2.5-2.78 (2.60)	0.10
181-195	13.7-17.5 (14.9)	12.3-15.8 (13.42)	1.88-2.8 (2.37) [84±3.3]	0.8-1.3 (1.070) [92±1.0]	4.0-5.9 (4.71) [11.02-14.0]	0.349±0.050	0.06-0.26 (0.16)	0.77-1.09 (0.94)	2.98-3.4 (3.22)	0.07
196-232	18-21.7 (19.2)	16.3-19.5 (17.25)	3.29-4.6 (3.81) [80±2.4]	3.29-4.6 (2.664) [86±3.2]	5.23-6.5 (6.05) [13.0-17]	0.350±0.029	0.83-1.39 (1.18)	1.35-2.41 (1.86)	3.7-3.77 (3.73)	0.28

** HRT was maintained constant at around 1.05 d; [+] Recirculation rate was decreased from 9 to 5 L/h thereafter. Values in parenthesis are ( ) average values; and [ ] [a] Removal efficiencies (%), [b]CH_4-COD (g/L of reactor.d)*

II.2.3.2.2 Biogas production: Methane yield of 0.35 L CH_4/g $COD_{removed}$ was obtained for the maximum OLR showing that the value was found closer to the theoretical yield (Table 1). The biogas was found to have 69-83% CH_4 and the balance being CO_2. A linear relationship was found between the methane production rate and the OLR applied (*Cf.* Figure II.6). A maximum gas production rate of 6.7 L $CH_4/L_{reactor}$.d was achieved with 69% of CH_4 level in the biogas for the highest OLR (Fig. 2). Similar values were reported by Najafpour *et al.* (2006) with a high gas production rate of 6.23 L CH_4/L.d) and 62% of CH_4 level in the biogas for treating palm oil mill effluent.

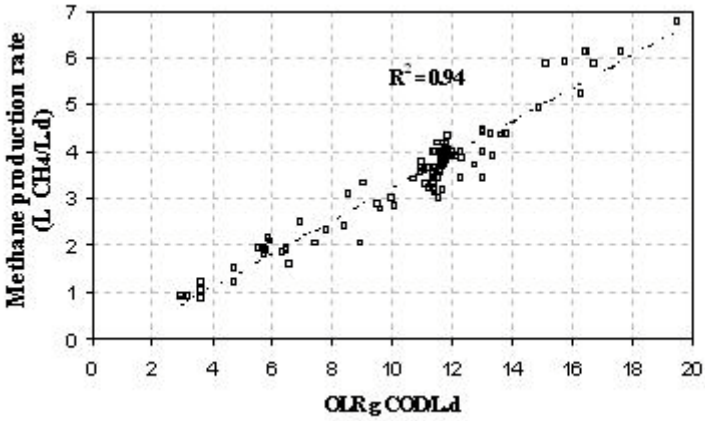

Figure II.6 Methane production rate vs organic load rate

II.2.3.2.3 Effect of OLR on VFA concentrations at outlet*:* The concentrations of VFA-COD and non VFA-COD are presented in Table 1. At steady state conditions, from day 58-159, OLR was maintained constant at around 11.8 g COD/L.d for which the VFA-COD and non VFA-COD did not vary much with a value of 028±0.20 and 0.86 ±0.09 g/L respectively. The 50% reduction in the recirculation rate on day 160, did not affect much on this parameter. With increase in OLR from 11.8 to 19.5 g COD/L.d, the non VFA-COD also increased to a maximum value of 1.9 g/L at these OLR. The increase in the COD concentrations at the outlet was mainly linked to a gradual increase in the VFA-COD and non VFA-COD with the increasing OLR. Acetic acid was the major VFA component in all the reactors but there was a slight build-up in propionic acid concentration with the increasing OLR (i.e. from day 181-232). The acetic acid concentration to the maximum OLR of 19.5 g COD/L.d was 1.39 g/L. Propionic acid concentration remained less than 0.5 g/L at these OLRs. During the entire study period the VFA/alkalinity ratio was always below 0.3 even for the maximum OLRs

studied (Table 1). This indicates that the reactor was operating favourably without the risk of acidification, when this ratio is less than 0.5 (Sanchez *et al*., 2005).

The performance of various anaerobic reactor configurations reported in the literature for the treatment of distillery wastewater was compared and discussed further. García-Bernet *et al.* (1998) reported that the maximum eliminated OLR (i.e. OLR multiplied by COD removal efficiency) of 12.7 g COD/L.d and 75% COD removal efficiency was obtained for the treatment of red wine distillery wastewater in a down-flow anaerobic fluidized bed reactor. Akarsubasi *et al.* (2006) used an UASB reactor for the treatment of alcohol distillery wastewater and they observed the maximum eliminated OLR of 9.9 g COD/L.d and 90% COD removal efficiency; where as Bhavik *et al.* (2007) obtained a maximum eliminated OLR of 14.8 g COD/L.d and 64% COD removal efficiency using an upflow fixed film reactor treating distillery spent wash-water. This shows that a hybrid UASFB reactor used in the present study offers a greater performance advantage in terms of high eliminated OLR (18 g COD/L.d) and COD removal efficiency (80%) in the treatment of high-strength wine distillery vinasses at high OLR and short HRT. Though the reactor shows good treatment possibilities, the organic matter concentrations at the effluent remained above the discharge limits and therefore, it is necessary to include a post-treatment stage for the effluents generated from the UASFB to comply with the limits for discharge into the environment.

II.2.3.2.4 Reactor mixing characteristics : To analyze the hydrodynamics of the UASFB reactor, the study of the residence time distribution (RTD) was performed by using LiCl solution. Tracer studies were realized at days 0, 74, 123, 138, 165 and 223, in order to know the efficiency of the liquid mixing inside the reactor. The normalized concentration of Li in the effluent E(T) was plotted against the normalized time (T) (*Cf.* Figure II.7). The results presented in Fig. II.7 showed that liquid mixing was good through out the experiments and found to be closer to a theoretical continuous stirred tank reactor (CSTR). This result can be explained by the high recirculation ratio as well as the gas production, which is known to have a positive effect on liquid mixing in anaerobic bioreactors. The good liquid mixing showed that there was no clogging of the support or dead zones inside the reactor. Since the liquid mixing was good, the COD_s and VFAs concentrations were found to be homogeneously distributed within the reactor (i.e., both in the UASB and filter-bed sections). RTD study demonstrated that the volume of liquid within the bioreactor did not change during

the experiments. Which means that the liquid volume obtained from the RTD curve (V_E) is almost same to the theoretical liquid volume (V_T) i.e. $V_T \approx 0.99\ V_E$.

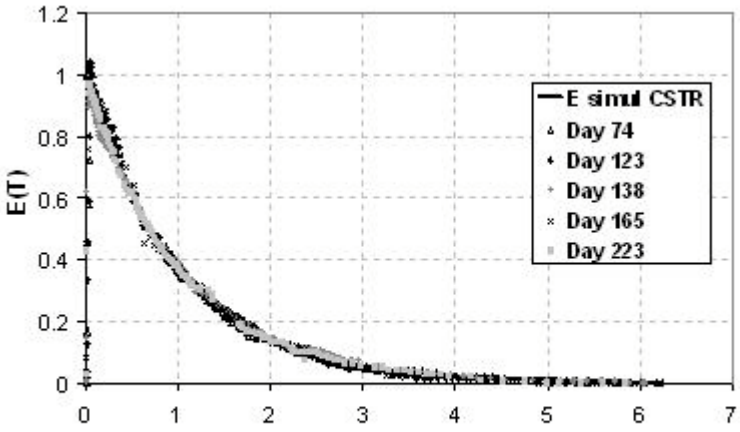

Figure II.7 Residence time distribution (RTD) curves

Whereas, Escudie *et al.* (2005) performed the RTD study in a pilot scale anaerobic fixed-bed reactor (total volume =982 L) packed with Cloisonyle® tubes, which was treating wine distillery vinasses. They observed that the total biofilm volume represented to about 720 L, where as the liquid volume corresponded to about 230 L (which represents only 25% of reactor volume). Similar results were also reported by Rajinikanth *et al.* (2007), in which the upflow anaerobic filter bed reactors packed with small floating polyethylene media were used to treat cheese-dairy and fruit-canning wastewaters. The good results obtained by using UASFB were probably explained by the presence of filter media only in the upper portion, which caused the flocculated biomass to precipitate over the sludge blanket. And thus, helped to enhance the development of granular sludge, while minimize the excess accumulation of biomass onto the media.

II.2.3.2.5 Biomass activity analysis: On day 233, continous mode of feeding with distillery vinasse to the reactor was terminated. And the specific methanogenic activities (SMA) of biomass both in granular and attached forms were determined using 'ethanol' as a substrate in a batch mode. The reactor was fed 4 times (cycle) with 10 mL of ethanol equivalent to 16.8 g of COD. This analysis was based on the online measurement of the rate of biogas production for each cycle as previously described by Ruíz (2002). At the end of the SMA analysis, the reactor was opened and the quantity of volatile solids entrapped on the supports and in granules was measured.

The total quantity of VS in the 9.8 L reactor was found to be 451 g. Granular sludge represented 72% (325 g) of the total biomass, attached biomass represented 26% (116 g) and biomass in suspension was low with only 2% of the total biomass (9 g). Both biomass in suspension and granular sludge had a VS/TS ratio of 0.81 and the mineral content of the attached biomass was higher with a VS/TS of 0.64 only. Each support was able to accommodate quite a high quantity of biomass, with values between 1.4 and 2.2 g dried solids/support.

Specific biomass activity was calculated using the OLR applied at the end of the experiment and the total quantity of VS measured inside the reactor. The average specific activity of total biomass in the reactor was 0.43 g COD/g of VS.d. This activity remained comparable to the specific activity measured by Ruiz (2002) for biomass in suspension treating sugar cane vinasses (0.52 g COD/g of VS.d) or molasses vinasses (0.48 g COD/g of VS.d). Indeed, SMA analysis with ethanol showed that for a load applied (i.e. 10 mL of ethanol equivalent to 16.8 g of COD), the activity of granules was found to be 0.23 g CH_4-COD/g VSS.d and that of attached biomass was 0.20 g CH_4-COD/g VSS.d. This means, about 46% of the activity came from the attached biomass, whereas, it only represented 26% of the total biomass. This shows that the specific activity of the attached biomass was almost 2 times higher than that of the granular sludge. The results further suggest that the amount of attached biomass in the packed bed zone plays a vital role in stabilizing the entire system.

II.2.3.3 Model application

IWA's Anaerobic Digestion Model No.1 (ADM1) represents a universally applicable bio-kinetic model for the mathematical description of anaerobic digestion of different types of organic substrates (Batstone *et al.,* 2002). In order to account for microbial diversity, the traditional ADM1 model was extended by Ramirez *et al.* (2007) in such a way that 10 different species were associated with each degradation reaction (instead of one microbial population in ADM1). This extended ADM1 model called ADM1_10 was used for simulating and analysing the experimental results in the present study.

With the objective of which the models reflect real-world behavior, some key parameters in ADM1, such as the specific maximum uptake rate constant (k_m) and the half saturation

constant (K_s) for the VFAs consumption were calibrated to fit the data (*Cf.* Table II.4). The difference between hydraulic and solid retention times (i.e., HRT and SRT) due to biofilm present in the reactor was modeled by adding an extra term i.e. residence time of solids ($t_{res,X}$) in the biomass equation as recommended in the ADM1 report (Batstone *et al.,* 2002). The wine distillery wastewater used as influent during the experiments described in substrate section consisted of carbohydrates, proteins, lipids, sugars, amino acids, long chain fatty acids, VFAs, inorganic carbon and inorganic nitrogen. The concentrations of these individual components used in the model as process inputs are shown in Table II.5.

Table II.4 Main parameters estimated to fit the experimental data

Parameter	**Acetate**	**Propionate**
k_m (kg_{COD}/Kg_{COD}.day)	2.11 (8)*	2.74(13)
K_S (kg_{COD}/m^3)	1.41(0.15)	1.41 (0.10)

* *Values in parenthesis are the reference values recommended in the ADM1 report*

Table II.5 Input concentrations of the wine distillery wastewater used during the experiments

Constituent	**Values**	**Constituent**	**Values**
Sugars	0.420*CODt_in	Carbohydrates	0.90*CODp_in
Amino acids	0.020*CODt_in	Proteins	0.07*CODp_in
Long Chain Fatty acids	0.010*CODt_in	Lipids	0.03*CODp_in
Total Valerate	0.035*CODt_in	Inorganic Nitrogen	0.05/18*CODt_in
Total Butyrate	0.181*CODt_in	Inorganic Carbon	0.003/18*CODt_in
Total Propionate	0.128 *CODt_in	Total input COD	CODt_in*
Total Acetate	0.152*CODt_in	Input particulate COD	CODp_in*

* *variable input signals*

Both ADM1 and ADM1_10 were applied to simulate the behavior of the reactor. But for the spatial limitations, only the results of ADM1_10 are discussed further. Figure II.8 shows the experimental data for the entire study period, together with the varying input OLR and the simulated results from ADM1_10.

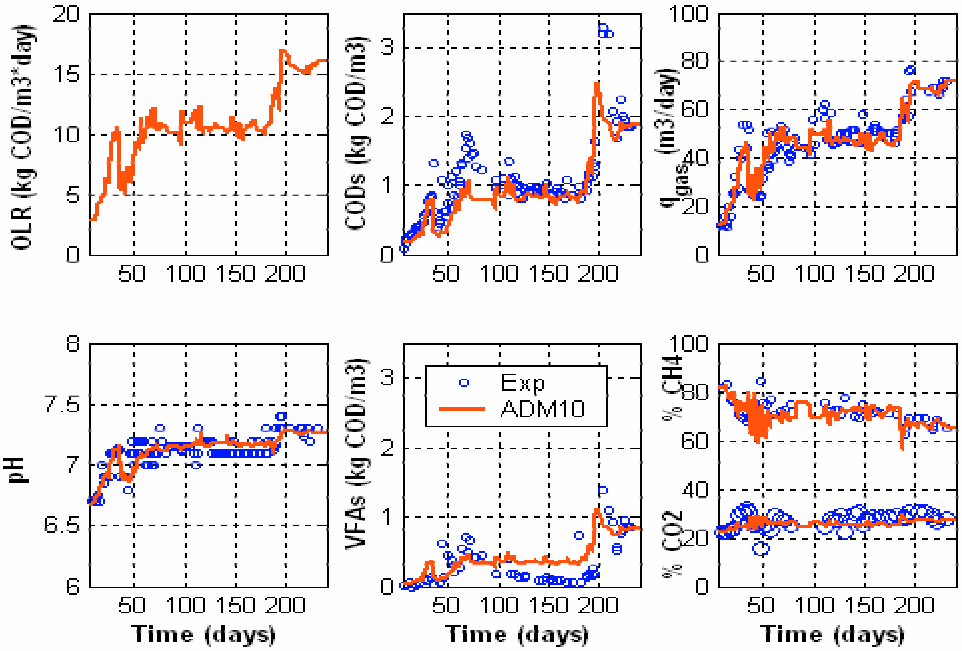

Figure II.8 Experimental data (circles) and Simulated (continuous lines)

As it can be seen from the Fig. 4, the model can simulate nicely the dynamic evolutions of the main variables, in the liquid and also in the gas phases. The disturbance in the performance of the UASFB described in the previous section (i.e. sludge washout on day 35) was not included in the model and may be this explains the differences mainly in COD_s and VFAs between the simulated and experimental data in the period 35-57 days. After day 100, the model over-predicted VFAs concentrations (meanly acetate). It appeared that the simulated rate at which acetate was converted to methane under the load imposed was somewhat under-estimated. This may have resulted from either under-estimation of the substrate consumption coefficients for acetoclastic methanogenesis or an over-estimation of the inhibition of this activity by ammonia. (Parker, 2005). The model predicts well the dynamics of the biogas production rate and composition as a response of the load imposed. Small deviations in predicting the biogas production and quality have been found. The differences can be explained with the non-optimization of several parameters, for instance the application of identical and non-optimized gas transfer coefficients. In fact, gas transfer coefficients may differ in reality and the dependence on the specific reactor configuration applied has been neglected. The pH was also be quite accurately simulated and the model was able to reflect the trends that were observed in experimental data. The pH prediction is closely related to the cation and anion concentrations in the reactor, and actually, the difference between the two

concentrations. Since the ion concentrations were not measured, it was then calculated using the pH value and taking into account the concentration of ammonia, alkalinity and VFAs concentration in the reactor. The value of the input cation from the reactor minus the input anion concentration in the feed was arbitrarily increased in the models, so that the pH values were calibrated.

II.2.4 *CONCLUSIONS*

This study reveals that a hybrid UASFB reactor was efficient in the treatment of high strength wine distillery vinasses at high OLR (max. 19.5 g COD/L.d) and short HRT (~1 d). High COD_t and COD_s removal efficiencies of 82 and 88%, respectively were attained during this study. The efficiency of liquid mixing was good through out the experiments and found to be closer to a theoretical continuous stirred tank reactor (CSTR). The specific activity analysis depicts that the attached biomass was 2 times higher than the specific activity of the granular sludge. The packing medium had a dual role in the retention of the biomass inside the reactor: entrapment of biomass within the support and filtration of the granular biomass, preventing it from going out of the reactor. ADM1_10 model was able to simulate well the dynamic evolutions of the main variables, in the liquid and also in the gas phases.

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CHAPITRE III

MODELISATION DE LA DIVERSITE MICROBIENNE EN DIGESTION ANAEROBIE:

QUELQUES IMPLICATIONS

EN ECOLOGIE MICROBIENNE

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RESUME

Le chapitre III est composé de trois parties présentées sous forme d'articles scientifiques. La première partie présente l'étude de la diversité microbienne dans deux procédés biologiques de traitement de la pollution: la nitrification et la digestion anaérobie. Les discussions ont été réalisées à partir des données expérimentales dans le cas de la nitrification et à partir des résultats de simulation pour la digestion anaérobie. La présence d'un agent toxique, qui affecterait chaque espèce de micro-organismes, a été analysée dans le cas de la digestion anaérobie. Enfin, l'utilisation de la modélisation comme outil d'optimisation des populations microbiennes dans un bioprocédé a été évaluée dans les deux types de traitement.

Le fonctionnement d'un bioprocédé résulte d'une chaîne trophique de plusieurs espèces microbiennes. Jusqu'à présent, la diversité microbienne a été peu étudiée au cours des réactions de conversion des substrats et généralement négligée dans les modèles mathématiques. Néanmoins, les résultats expérimentaux disponibles ont montré que les conditions opératoires d'un bioprocédé peuvent favoriser la sélection de différents types de bactéries, modifiant ainsi la diversité microbienne et donc le comportement de l'écosystème.

L'étude de la diversité microbienne lors de la nitrification s'est effectuée à partir de résultas expérimentaux obtenus dans deux réacteurs à lit turbulé inverse ayant des taux de rétention solide différents. Les deux réacteurs ont montré des performances de nitrification et des observations microbiologiques différentes: une accumulation de nitrite a été observée dans un des réacteur alors que dans l'autre, seule une production de nitrate en tant que composé final de la nitrification a été observée. Les observations de la diversité microbienne dans les deux réacteurs après 4 mois d'opération ont montré des différences concordant avec les performances de nitrification observées: les espèces du genre *Nitrospira* étaient présentes dans les deux réacteurs mais à une concentration très faible dans le premier réacteur par rapport au second réacteur. De plus, deux types de microorganismes convertissant l'ammoniac ont été détectés dans les réacteurs: le genre *Nitrosomonas europaea* dans le premier et le genre *Nitrosomonas sp.* dans le second. Les simulation d'un modèle comprenant deux espèces oxydant l'ammoniaque et une oxydant le nitrite ont été calés avec les observations expérimentales. Ils ont montré l'importance des conditions opératoires car les différences obtenues sur l'activité nitrifiante et la diversité microbienne s'expliquent par la modification d'un seul paramètre, à savoir le taux de rétention de solides.

Dans le cas de la digestion anaérobie, les observations ont été réalisées avec des simulations du modèle ADM1_10 pour deux réacteurs parfaitement agités possédant des inoculums identiques et fonctionnant à un taux de charge organique constant et un taux de charge organique variable. Le premier réacteur a été alimenté avec des vinasses d'une DCO totale de 15 $kg_{DCO}.m^{-3}$ et a fonctionné à un temps de séjour hydraulique (TSH) de 2,5 j. Le second réacteur a fonctionné à un TSH entre 0 et 2,5 j et avec un taux de charge organique variable constitué de cinq phases de perturbation. Chaque perturbation avait une durée de 10 jours: 5 jours d'alimentation à un taux de charge organique deux fois supérieur à celui du premier réacteur, puis de 5 j à un taux de charge organique nul avant l'alimentation suivante. Ainsi, le taux de charge organique moyen a été égal pour chaque réacteur. Un système avec un contrôleur proportionnel a été intégré dans le modèle afin de maintenir le pH au-delà d'une valeur limite de 6,9 pour éviter une inhibition des méthanogènes acétoclastes. Après 50 jours de fonctionnement, les performances du second réacteur étaient supérieures à celles du premier, avec un meilleur abattement des composés solubles, une production de biogaz plus importante et une concentration en AGV totaux en sortie plus faible. De plus, la communauté

microbienne du second réacteur est apparue plus variée avec des variations temporelles importantes. Par contre, la diversité microbienne du premier réacteur était plus homogène et moins variée pour les deux types de micro-organismes, *Bacteria* et *Archaea*.

Le second réacteur fonctionnant avec un taux de charge organique variable, induisant une population microbienne plus diverse et moins stable, a donc montré de meilleures performances que celles du premier réacteur. Ainsi, une diversité moins stable est corrélée à des fonctions plus stables (i.e., de meilleures performances). Dans cette étude, la diversité microbienne est induite par des variations temporelles plus importantes des populations *Bacteria* et *Archaea* en réponse aux variations brutales du substrat.

Pour résumer, les deux réacteurs parfaitement agités ont montré des différences d'un point de vue macroscopique et microbiologique, dues à la stratégie d'alimentation qui est le seul paramètre opératoire modifié lors des simulations.

Un autre résultat important de cette partie a été que la biodiversité permettait de préserver les performances du réacteur parfaitement agité par rapport à des variations temporelles comme la présence d'un agent toxique affectant l'ensemble des espèces. Ce phénomène a été mis en évidence par les résultats de simulations obtenus sur deux réacteurs fonctionnant avec les mêmes conditions opératoires aux précédents et l'ajout d'un agent toxique à une concentration de 50 $k_{DCO}.m^{-3}$ à partir du jour 12,5 pendant 2,5 j. Après 50 jours de fonctionnement, les résultats de modélisation ont montré que les vitesses de production de biogaz et l'abattement de la DCO soluble diminuaient dans les deux réacteurs dès l'ajout de l'agent toxique. Après élimination du toxique, le second réacteur a retrouvé un fonctionnement normal alors que les performances du premier réacteur ont diminué. La diversité microbienne du second réacteur est apparue plus variée avec des variations temporelles importantes. Par contre, celle du premier réacteur apparaît plus homogène avec une faible diversité principalement pour les bactéries de type *Bacteria*. Ainsi, la diversité microbienne élevée du premier réacteur, engendrée par une charge organique variable, a permis de retrouver les performances initiales de fonctionnement après le passage d'un toxique au sein du réacteur. La simulation a donc montré qu'un réacteur ayant une population microbienne de grande diversité mais moins stable obtienait un fonctionnement plus stable vis-à-vis d'un agent toxique.

Il existe différentes méthodes de sélectionner les populations microbiennes désirées afin d'augmenter l'hétérogénéité du système. Le développement localisé de micro-organismes, permettant une efficacité importante du procédé grâce à une organisation spatiale spécifique des différentes populations microbiennes et de leur fonction, est largement utilisé dans les procédés de granulation, les réacteurs à biofilm ou des réacteurs à membrane. Les travaux de cette partie ont présenté une autre méthode, qui consiste à introduire une hétérogénéité dans le système à intervalle de temps régulier, c'est-à-dire d'effectuer des injections (ou « pulses ») de substrat pour favoriser la croissance des micro-organismes désirés. Bengtsson *et al.* (2002) ont également démontré dans un écosystème différent, les avantages d'un développement des communautés microbiennes à partir de cycles de perturbations, assurant ainsi un système plus robuste et plus stable qui est le résultat de la variation temporelle et spatiale des conditions opératoires.

La deuxième partie s'est intéressée à la présence d'un agent toxique spécifique (l'ammoniaque), inhibant un niveau trophique particulier de la digestion anaérobie (les méthanogènes). L'étude consistait donc à modéliser les cinétiques des bactéries

méthanogènes lors d'une augmentation graduelle de la concentration en ammoniac dans un réacteur parfaitement agité en condition mésophile, alimenté en eaux usées d'abattoirs. La concentration en ammoniac dans le réacteur a été augmentée en faisant varier la concentration en azote ammoniacal totale de l'alimentation et la valeur de pH a été régulée à 7,7 grâce à un contrôleur proportionnel. Les fonctions d'inhibition du modèle ADM1 d'origine ont été conservées dans le modèle modifié, exceptée la constante d'inhibition de l'ammoniac ($k_{I,NH3}$) qui a été choisie de façon aléatoire à partir d'un distribution bimodale normale dont les deux modes sont $\mu_1 = 0.6 * k_{I,NH3}$ (ce cas suppose que les espèces *Archaea* sont plus sensibles à la présence de l'ammoniac), $\mu_2 = 1.4 * k_{I,NH3}$ (les espèces *Archaea* peuvent être alors plus tolérantes) et une déviation standard $\sigma_{1,2} = 0,125 * k_{I,NH3}$.

Le modèle ainsi modifié a été évalué à partir des résultats de simulation sur les variations de biomasses et les performances du réacteur, suivant différents scénarios : (i) avant variation de la concentration en azote ammoniacal total, (ii) pendant une période de transition de la concentration et (iii) après adaptation aux nouvelles conditions. Les périodes de transition et d'adaptation ont été évaluées à partir des paramètres de performances tels que la vitesse de production de méthane, l'abattement de DCO soluble et la concentration en AGV totaux dans l'effluent. Les courbes d'abondance de la biomasse et l'indice de diversité de Simpson ont été utilisés pour décrire la biodiversité du système. Deux tests de simulation ont été réalisés afin d'observer les variations de concentration en azote ammoniacal total. Pour le premier test, deux réacteurs ont été utilisés pour évaluer l'impact d'une variation brutale de la concentration : le réacteur R1 avec une variation de concentration de 13 à 58 mM, et le réacteur R2 avec une variation de la concentration en deux étapes, de 13 à 40 mM puis après stabilisation de 40 à 58 mM. La dernière variation a été réalisée dans le but de comparer l'effet de la différence de variation entre 13 à 58 mM et de 40 à 58 mM. Le second test a permis d'étudier l'influence du nombre d'espèces résistantes à une concentration élevée en azote ammoniacal totale sur l'adaptation d'un réacteur parfaitement agité après une variation brutale de la concentration en ammoniac de 13 à 40 mM (R3).

Les résultats de l'étude ont montré une adaptation de la biomasse vis-à-vis d'une augmentation de la concentration en ammoniac. En effet, les performances du réacteur R2, lors de la variation de la concentration en ammoniac de 40 à 58 mM, ont subi une perturbation plus faible que dans le cas de la variation de la concentration de 13 à 58 mM du réacteur R1. L'abattement de DCO soluble ainsi que la vitesse de production de méthane ont diminué pendant la phase de variation de la concentration en ammoniac et après adaptation, les valeurs de ces paramètres étaient plus faibles que les valeurs initiales. De plus, le temps nécessaire pour obtenir une adaptation complète (c'est-à-dire un retour à un fonctionnement normal des paramètres tels que la concentration en AGV totaux en sortie, l'abattement de DCO soluble et la vitesse de production de méthane) est plus long dans R1 par rapport à R2. Le phénomène d'adaptation à une concentration élevée en ammoniaque résulte donc d'un changement de la composition des communautés bactériennes, c'est-à-dire d'une sélection des méthanogènes acétoclates résistantes présentes initialement dans l'inoculum (les espèces 7, 8 et 9 sont sélectionnées dans le réacteur R1 et seulement l'espèce 10 dans le réacteur R2).

La sélection d'espèces méthanogènes acétoclastes dominante a été analysée en détail dans le réacteur R3. La méthode d'étude a consisté dans un premier temps à simuler les performances du réacteur en présence des 10 bactéries dégradant l'acétate. Ensuite, les simulations ont été refaites en supprimant à chaque modélisation la bactérie dominante dégradant l'acétate. Les résultats ont montré que le temps d'adaptation est plus faible lorsque le nombre d'espèces résistantes à une concentration élevée en ammoniac augmente. Ainsi, le temps d'adaptation

est influencé significativement par la diversité microbienne. De plus, lorsque toutes les espèces résistantes (espèces 6 à 10) ont été supprimées, les performances du réacteur n'ont pas été rétablies. Il est important de noter que l'élimination de l'espèce dominante à chaque simulation (l'ordre d'élimination étant 7-9-10-6-8) n'était pas en accord avec les valeurs du coefficient *J* calculées pour chaque espèce à partir de l'expression des travaux de Hsu *et al.* (1977). Le coefficient *J* permet de déterminer l'ordre des espèces dominantes. Cependant, les travaux de Hsu *et al.* (1977) ont été validés sur une seule réaction et sans inhibition, alors que dans notre étude, le système est composé de plusieurs réactions de dégradation avec la présence d'une inhibition par l'ammoniaque. Ainsi, pour notre étude, un terme d'inhibition a dû introduit dans l'expression de Hu pour calculer le coefficient *J*.

La principale différence entre les deux modèles ADM1 et ADM1_10 est liée à l'évolution des biomasses. Dans ADM1_10, deux types de biomasses sont présentes : les *K_strategists* (pour les espèces 1 à 5) et les *μ_strategists* (pour les espèces 6 à 10), combinant ainsi des espèces ayant un K_S élevé avec un μ_{max} élevé et des espèces ayant un K_S faible et un μ_{max} faible. Les résultats de simulation ont montré, qu'après une diminution de toutes les sous-espèces reliée à une diminution de la biomasse totale (au alentour du 150ème jour), les espèces 6 à 10 ne sont plus en compétition avec les espèces 1 à 5, ce qui peut être dû à leurs vitesses de croissance plus élevées. Dans le même temps, la concentration en acétate a atteint une valeur élevée, ce qui a permis des conditions favorables au type de biomasse de *μ_strategists*.

Un résultat important est que la biodiversité simulée est proportionnelle à la stabilité de l'écosystème. Ainsi, la biodiversité permet de préserver les performances d'un réacteur parfaitement agité lors de variations des conditions opératoires tel que l'augmentation de la concentration en ammoniaque. Cette conclusion est similaire à celle de la première partie mais il est important de noter qu'ici, l'agent toxique affecte un niveau trophique et non l'ensemble des espèces.

Ces résultats peuvent permettre de conclure que l'ADM1_10 est un outil intéressant pour suivre les transformations au sein du écosystème anaérobie et pour évaluer l'effet de la présence d'un agent toxique et le rôle de la biodiversité sur les performances du réacteur. Le modèle permet donc d'approfondir les connaissances sur les relations entre la biodiversité et les performances du réacteur. Ainsi, l'adaptation des micro-organismes à des composés inhibiteurs, présentée dans ce chapitre, peut permettre une amélioration importante de l'efficacité de traitement d'un déchet. Il a été également suggéré que l'adaptation à une concentration élevée en ammoniac peut résulter de la sélection des méthanogènes acétoclastes résistantes initialement présentes dans l'inoculum. L'influence des paramètres microbiens des espèces méthanogènes acétoclastes résistantes à une concentration élevée en ammoniaque affectant la compétition entre espèces a été clairement évaluée.

Dans la troisième partie de ce chapitre, l'ADM1_10 a été utilisé pour simuler le comportement de cinq réacteurs batchs anaérobie thermophile alimentés avec une boue activée brute et prétraitée thermiquement, afin d'évaluer les relations entre les performances du réacteur et la composition de la communauté microbienne. Les cinétiques des AGV totaux et individuels, le pH et les productions cumulées de méthane et de dioxyde de carbone, mesurées sur chaque réacteur, ont été utilisées pour calibrer et valider le modèle. Le modèle, utilisant les valeurs de paramètres déterminées dans le chapitre III, a modélisé correctement les données expérimentales issues de la dégradation de boues ayant subies différentes conditions de prétraitement thermique. La production de méthane cumulée est optimale lors de la dégradation de la boue prétraitée à 165°C par injection de vapeur. L'ensemble des

réacteurs, excepté le réacteur présenté précédemment, ont montré une concentration en AGV significative et similaire en sortie avec une prédominance de l'acétate et du propionate. L'accumulation d'AGV indique une activité limitée des micro-organismes dégradant les AGV lors de la méthanogenèse.

L'analyse des résultats a permis de montrer que la modification de la température du prétraitement thermique entraîne un changement dans la composition de la communauté microbienne du réacteur thermophile. Il a ainsi été démontré que la diversité microbienne, basée sur l'indice de diversité de Simpson, est un paramètre important dans le développement d'une communauté microbienne anaérobie fonctionnelle. La diversité des populations bacteriennes a été plus importante au début de la digestion (correspondant à des vitesses de production de méthane élevées) que lorsque la production de méthane atteint un plateau où le maintient des conditions batchs semble provoquer une faible présence de micro-organismes dominants. Une diminution de l'indice de diversité de Simpson durant le début de la digestion peut indiquer que l'inoculum initial détermine la composition de la communauté bactérienne des étapes ultérieures de la digestion.

Dans l'ensemble des réacteurs, la composition des espèces *Bacteria* et *Archaea* a varié durant la digestion en fonction des changements des conditions opératoires telles que la disponibilité du substrat et/ou le pH. Pour une température optimale de prétraitement du substrat à 165°C, la communauté microbienne dégradant ce substrat assure une conversion importante du carbone organique en méthane, augmentant ainsi les performances du réacteur.

SUMMARY

This chapter consists of three sections presented in form of articles. The first section will assess microbial diversity in biological wastewater treatment systems by means of two case studies: nitrification and anaerobic digestion. Experimental data will be provided for the former and results from simulation for the latter. Situation where a toxicant affects all species is carefully analyzed for anaerobic digestion and for both case studies, the potential of process control to optimize microbial populations will be discussed.

Key microbiological conversion processes do not result from the work of a single bacterial species but are performed by a wide variety of bacteria. Up till now, this microbial diversity is usually not tracked during reactor operation and mostly neglected in mathematical models. Nevertheless, experimental evidence is available that different process conditions may favor the selection of different types of bacteria, modifying the microbial diversity and consequently the behavior of the ecosystem.

In the nitrification case, we consider experimental data from two Inverse Turbulent Bed Reactors (ITBRs), only differing in their solid hold-up ratio, i.e., the ratio of static to expanded bed height: 0.1 (R10) and 0.3 (R30). Synthetic wastewater containing 250 mgN L^{-1} as ammonium sulfate was supplied at a constant flow rate of 0.3 L h^{-1}. Temperature was maintained at 30°C, pH was controlled at 7.5 and the airflow rate was kept constant at 30 L h^{-1}. The two ITBRs showed a different nitrifying performance, both from a macroscopic and microbiological point of view. The reactor R30 (highest support concentration) accumulated nitrite whereas R10 produced only nitrate as a final nitrification product. The comparison of microbial communities in both reactors after 4 months of operation was in agreement with this result: the same population of nitrite-oxidizing *Nitrospira* was present in both reactors but in very low proportion in R30 compared with R10. The major ammonium-oxidizer was different in both reactors, *Nitrosomonas europaea* in R30 and *Nitrosomonas* sp. in R10. The reactors' solid hold-up, was the only operating parameter different between both reactors, and it act upon nitrifying activity and on the major ammonium oxidizer present. The simulation results agree with the experimental observations: nitrite accumulates in R30 while complete oxidation to nitrate is achieved in R10.

In the anaerobic digestion case, the response of two CSTRs with identical inoculums was simulated using ADM1_10, for constant and pulsed organic loading rate (OLR) operation, respectively. In this section, these reactors will be called R1 and R3 respectively. R1 was operated at HRT = 2.5 days and fed with vinasses with CODt = 15 kg COD/m^3. R3 was operated with a HRT between 0 and 2.5 days and the multiple-pulse OLR consisted of five sequential pulses with a duration of 5 days with 5 days between pulses, and amplitude twice the constant OLR, in such a way that the average organic loading rates for the perturbation cycle for the reactors were equal. A P-controller was implemented in the model to maintain the reactor pH above a lower limit of 6.9, in order to avoid pH inhibition of aceticlastic methanogenesis. After 50 days of operation : R3 performed better than R1 because it had a higher soluble removal efficiency and a higher gas production for a lower mean accumulated VFAs concentration. The R3 microbial community appeared to be more diverse, with higher temporal variations. In contrast, the R1 microbial community appears more homogeneous with less diversity in both domains : *Bacteria* and *Archaea*.

Pulsed OLR reactor (R3) with high diversity and less stable microbial community thus displayed a better performance than the reactor with constant OLR (R1), i.e., the less stable

community was correlated to more stable function (better performance). In this case, the less stable community was the one that displayed greater temporal variations of *Bacterial* and *Archaeal* populations in response to substrate shock. To sum up : the two CSTRs showed a different performance, both from a macroscopic and microbiological point of view. The input OLR profile, was the only operating parameter different between both reactors, and it acted upon microbial community.

Another important outcome of this section is that biodiversity acts as insurance (buffer) for CSTR functions against temporal changes in environmental factors like pulsed toxicant that affect all species. This is illustrated with the response of two CSTRs, with the same constant and pulsed loading rate operation as before, but now with a pulse toxicant concentration applied at day 12.5 for 2.5 days with amplitude 50 kg COD/m^3. In this section, these reactors will be called R2 and R4, respectively. After 50 days of operation, the simulated results showed that biogas production rate and soluble COD removal decrease in both reactors upon toxicant adition, then recover to their pre-perturbed values once the toxicant has been removed in R4, while the new steady state values in R2 were lower than the pre-toxicant ones, i.e., the pulsed OLR reactor (R4) has better performance than the constant OLR (R2) towards toxicant addition. The microbial community in the reactor R4 appeared to be more diverse, with higher temporal variations. In contrast, the microbial community in the reactor R2 appeared more homogeneous with significantly less diversity mainly in *Bacteria*'s domain. The main difference between these communities is that R4 microbial community was able to return to the pre-toxicant conditions, while this was not the case for the one in R2. To sum up : the simulation results indicated that the reactor with a less stable community but with higher diversity was more functionally stable towards pulsed toxicant disturbances.

Enhancing system heterogeneity by fostering the right populations can be achieved in several ways. One way is to take advantage of spatial effects, as in granulation or biofilm development or membrane reactors, which can be effective due to the different location of different populations and functions in these systems. Another possibility to be explored is to introduce heterogeneity at temporal scale, e.g. by means of providing substrate pulses to encourage the growth of desired microorganisms, as suggested in this section. The development of communities that are more resilient in the long term due to the pulse disturbances has been demonstrated in other ecosystems, and the stability developed therein is the result of heterogeneity operating in both temporal and spatial scales (Bengtsson et al., 2002).

A situation where a non-reactive toxicant such as ammonia inhibits only one specific trophic level (in our case methanogenes) will be analyzed for anaerobic digestion in the second section. For this, methanogenic population dynamics under gradually increasing free ammonia levels, in mesophilic CSTRs seeded with slaughterhouse wastes, were simulated. Free Ammonia Nitrogen (FAN) in the reactors was gradually increased by increasing Total Ammonia Nitrogen (TAN) in the feed and keeping reactor pH at 7.7 by means of a proportional controller. All inhibitions from ADM1 were kept identical in the modified model except the inhibition constant for ammonia ($K_{I,NH3}$) that was randomly chosen from a normal bimodal distribution with two means: $\mu_1 = 0.6 * K_{I,NH3}$ (in this case, it is assumed that some *archaea* species are more sensitive to the presence of ammonia) and $\mu_2 = 1.4 * K_{I,NH3}$ (to represent the fact that some *archaea* species can be more tolerant) and standard deviations $\sigma_{1,2} = 0{,}125 * k_{I,NH3}$.

The basic rational design was based on simulations of biomass evolution and reactor performance (i) prior to a change in TAN concentration, (ii) during a transition period and (iii) after adaptation to the change has occurred. The transition period and adaptation was judged by reactor performance indicators such as the methane production rate (MPR), soluble COD removal and effluent total volatile fatty acids (VFAs) concentrations. Abundance Biomass Curves (ABC) comparison and Simpson's diversity index were used to describe the microbial community structure. Two simulation trials were performed to assess the effect of changes in TAN concentration. In the first trial, two reactors were used to assess the effects caused by a sudden change from 13 to 58 mM (called R1) and by a two step increase, first from 13 to 40 mM and, once the reactor is stabilized, from 40 to 58 mM (called R2). This latter change was performed in order to compare the effects of switching ranges of 13 to 58 mM to 40 to 58 mM. The second trial was performed to study the influence of the number of resistant species (richness) at high TAN concentrations on the adaptation process in a CSTR where ammonia concentration was suddenly changed from 13 to 40 mM (called R3).

Simulation results will show the adaptation to increased TAN concentrations, indicated by the fact that when the TAN was changed from 40 to 58 mM in R2, the reactor performance is not as disturbed as in R1 when the TAN was changed from 13 to 58 mM. As a result, the time required for complete adaptation (*i.e.,* return to steady state as noted by effluent VFAs concentration, soluble COD removal and MPR) was longer in R1 than in R2. It will be also clear from the simulation results that MPR and soluble COD removal efficiency decreased in the transition period (*i.e.,* the time required for adaptation) and that these indices returned to lower levels than those obtained prior to the change in TAN concentration. Furthermore, a shift in the microbial communities (R1 indeed selects species 7, 8 and 9 while R2 selects only the 10th one) suggests that adaptation to elevated ammonia concentrations results from the selection of resistant aceticlastic methanogens already present in the seeded sludge and that the methanogenic activity was affected the most whereas the acetogenic and fermentative activities were not affected.

The selection of dominant aceticlastic methanogen species has been analysed in more detail in R3. We begin with the reactor performance when all 10 acetate degraders are present. Subsequently, the simulations have been redone for a gradually restricted group of acetate degraders, successively elimitating the winning species from the previous simulation. The dynamic behaviour in terms of the lenght of the acclimatization was significantly influenced by the microbial properties. The simulations also reveal that the adaptation period gets lower when the number of resistant species at high TAN concentration levels (richness) increases. Moreover, when all resistant species (6th to 10th sps) where supressed, the performance of the rector did not recover. It is important to notice that simulation results (selection of species in the order 7-9-10-6-8) do not agree with the ranking of the calculated *J*-values, which already indicates the findings of Hsu et al., (1977) are valid for one-step reactions without inhibition, while in our case the acetate degradation reaction is one step in a network and we have ammonia inhibition. So, it cannot be garanteed that the same results will hold. We have calculated *J*-values without inhibition. We would suggest to calculate alternative *J*-values introducing the inhibition term in the Hu-expresion.

The main difference between the ADM1 and ADM1_10 models lies in the biomass evolutions. In ADM1_10, we have two biomass groups : *K_strategists* (1st to 5th sps) and *μ_strategists* (6th to 10 th sps), which is related to the fact that we have combined high K_S values with high μ_{max} values and low K_S values with low μ_{max} values. Simulation results showed that after an initial decrease of all subspecies, related to the decrease of total biomass,

around day 150, species 6 to 10 outcompete species 1 to 5, may due to the former species having higher growth rates. At the same time, acetate concentration switches from low values to high ones, leading to a competitive advantage of the biomass group of *μ_strategists*.

An important finding of this section is that biodiversity is positively related to ecosystem stability. The biodiversity acts as insurance for CSTR functions against temporal changes in environmental factors like pulsed TAN. Although the same conclusion was already obtained in the first part of this chapter, it is important to notice that in this case, the toxicant only affects one tropical level and not all as previously.

These results allow us to conclude that ADM1_10 is a helpful tool to get insight in the system and to investigate the impact of increasing toxicant concentrations and the role of biodiversity on the performance of the anaerobic reactor. The model showed its capacity to elucidate relationships between biodiversity and reactor performance. Adaptation of microorganisms to inhibitory substances, as suggested in this chapter, can significantly improve the waste treatment efficiency. We suggested too that adaptation to elevated ammonia concentrations resulted from the selection of resistant acetoclastic methanogens already present in seed sludge. The influence of microbial parameters of resistant aceticlastic metahanogens specie at high ammonia levels affecting interspecie competition has been assesed explicitly.

In the third section, ADM1_10 was modified to simulate thermophilic anaerobic digestion of WAS with and without thermal pretreated (as in chapter 3 with ADM1) and used to simulate the behavior of the five batch reactors previously described in order to find relationships between thermophilic anaerobic batch reactor performance and Microbial Community Structure (MCS). The dynamics of total and individual VFAs, pH and cumulative volumetric methane and carbon dioxide productions obtained from batch thermophilic anaerobic digestion of untreated and thermal pretreated sludges during a period of 20 days were used to calibrate and validate the model. Predictions by the model using the parameters established in chapter 3 agreed well with the data measured under different preteatment conditions. The cumulative volumetric methane production was found to be the highest in 165°C vapour mode WAS reactor. With regard to VFA levels, except for the previous reactor, all remaining reactors exhibited significant and very similar concentrations of VFA in the effluents, with acetate and propionate being the most abundant acids. Finding of accumulated acids indicates a limited capability of the VFA-degrading microorganisms to take an active part in the methanogenesis.

The results of our analysis indicated that a variation in pretreatment temperature of WAS caused changes on the microbial community structure of the thermophilic reactor. Microbial diversity based on the Simpson's reciprocal diversity index was shown to be important in developing a functionally successful anaerobic microbial community. The diversity of the *Bacterial* community was higher at the start-up of digestion than in routine operation where the maintenance of batch conditions seems to have caused the emergence of a few dominant microorganisms. This selection of a few *Bacterial* populations, compared to *Archaeal* populations which maintain higher microbial diversity, may result from the batch conditions. A decrease in Simpson's reciprocal diversity index during the reactor start-up suggests that the initial inoculum determine the structure of the microbial composition at later steps of operation.

In all reactors, during the digestion, the microbial community structures in the reactors in both domains *Bacteria* and *Archaea* changed over the time as consequence of the changes in the

environment conditions, such as substrate availability and/or pH. Until a temperature of 165°C, changes in the structure and activity of the microbial community of the thermophilic batch treating thermally pre-treated WAS occurred, and under the applied operational conditions, the microbial community succeeded in maintaining the increased flow of carbon into methane as was reflected by the improved reactor performance.

III.1 MODELING AND MONITORING OF MICROBIAL DIVERSITY IN ECOSYSTEMS– APPLICATION TO BIOLOGICAL WASTEWATER TREATMENT PROCESSES[3]

III.1.1 *INTRODUCTION*

Biological wastewater treatment nowadays is considered as a proven technology. Different processes can be applied for the removal of organic substrate (quantified in terms of COD, i.e. chemical oxygen demand) and nutrients such as nitrogen (N) and phosphorus (P). These processes rely on distinct biological conversion reactions, carried out by different types of bacteria. Besides, microbial diversity can also be distinguished within the microbial communities responsible for a certain function.

For instance, the nitrification reaction, which plays a central role in biological nitrogen removal, consists of two subsequent steps, carried out by two types of bacteria: ammonium oxidizing bacteria (AOB) convert ammonium nitrite, while nitrite oxidizing bacteria (NOB) perform subsequent oxidation to nitrate. Nevertheless: within these functional groups, different species can be distinguished, like *Nitrosomonas* and *Nitrosospira* for AOB, while *Nitrobacter* and *Nitrospira* are typical NOB.

Another wastewater treatment process which will be dealt with in this contribution is anaerobic digestion. Its main advantages lie in the possibility to convert even slowly degradable COD and at the same time produce methane gas which can be further used for energy recovery or even electricity production. For complete conversion of complex organic material to methane, five groups of bacteria are required: acidogenic bacteria, propionate and butyrate-utilizing acetogens, as well as H_2- and acetate-utilizing methanogens. These bacteria must work syntrophically, as they are linked physiologically, kinetically, and thermodynamically (Sekiguchi *et al*, 2004). In comparison to nitrification, anaerobic digestion is a complex process, involving many different bacteria which interact through a network of reactions, which is still not completely understood.

In general, anaerobic reactors are affected by external changes, although the severity of the effect is dependent on the type, magnitude, duration and frequency of the imposed changes

[3] Paper published by I. Ramirez, E.I.P. Volcke and J-Ph. Steyer at the *17th IFAC World Congress,* 6-11 July 2006, Seoul, Korea.

(Leitão et al., 2006). Typical responses indicating reactor failure include a decrease in performance, accumulation of reaction intermediates such as volatile fatty acids (VFAs), drop in pH and alkalinity, change in biogas production rates and compositions, sludge washout and shifts in microbial community structure.

The availability of new molecular biological tools for studying microbial communities in bioreactors and other engineered systems without cultivation, has resulted in remarkable insights linking microbial diversity and dynamics to process stability. Fernandez et al. (1999) monitored the community dynamics of Bacteria and Archaea in a functionally stable, continuously mixed methanogenic reactor, fed with glucose, over a 605 day period. Even though the reactor maintained constant pH and COD removal during this period, they found differences in the levels of diversity and dynamics between the Bacterial and Archaeal domains, indicating that functional stability does not imply community stability, i.e. levels of individual populations fluctuate in a functionally stable community. Similar results were observed in another methanogenic reactor system, a fluidized bed reactor fed with vinasse (wine distillation waste) in which the biomass was immobilized on powder from porous volcanic stone (Zumstein et al., 2000).

Another aspect concerns the effect of operational disturbances on the underlying microbial community. Fernandez et al. (2000) experimentally investigated the effect of substrate loading shocks on population dynamics. For continuously mixed methanogenic reactors that maintained two different communities, they found that the less stable community structure resulted in more stable functioning. These results were attributed to the substrate processing structure that was developed in each reactor type prior to perturbation: substrate processing through parallel pathways was associated with a functionally more stable (resilient) system, in contrast to serial processing of substrate.

An important outcome of these and other experiments is the realization that population diversity alone does not drive ecosystem stability. The positive relationship between the presence of multiple pathways towards a product (parallel processing of substrate) and functional stability parallels theoretical concepts in higher ecological organization (Peterson et al., 1998). Ecosystem stability is not the outcome of population diversity as such, but of functional redundancy, which is ensured by the presence of a reservoir of species able to perform the same ecological function. Recognizing the diversity and the links within each key

functional group of a system can lead to better ways to model diversity and functioning, and can help to improve process stability (Watanable et al., 2002).

It is our belief that the engineering of wastewater treatment systems would be improved if one could predict and manipulate the associated microbial diversity. Mathematical models in which data on micro-scale molecular diversity has been incorporated to more closely represent wastewater treatment processes, can provide a useful tool to reach this goal. Such models can be used to gain insight in the influence of process conditions on the selection of certain types of bacteria. In a later stage, these models can also be used to develop efficient control strategies adapted to model-based population optimisation. In this contribution, this approach is demonstrated for two different wastewater treatment applications.

III.1.2. *MICROBIAL COMPETITION IN NITRIFYING BIOFILM REACTORS*

III.1.2.1 Materials and methods

A first case study considers experimental data from two inverse turbulent bed reactors (ITBRs). In this type of reactors, biomass is grown on low density particles, fluidised by an upward current of gas. The reactors were filled with Extendosphere™ particles as solid carrier material. Biological ammonium oxidation was carried out in two ITBRs, only differing in their solid hold-up ratio, i.e. the ratio of static to expanded bed height: 0.1 (reactor R10) and 0.3 (reactor R30) (Bernet et al., 2004). Synthetic wastewater and containing 250 $mgN.L^{-1}$ as ammonium sulfate was supplied at a constant flow rate of 0.3 $L.h^{-1}$. Temperature was maintained at 30°C, pH was controlled at 7.5. The airflow rate was kept constant at 30 $L.h^{-1}$. Nitrate, nitrite and ammonium were analyzed by an ion chromatography system (DIONEX 100) using conductivity detection. Bacterial communities were monitored by total DNA extraction and 16S rDNA-targeted PCR-SSCP (single strand conformation polymorphism) (Dabert et al., 2001).

III.1.2.2 Experimental observations

The two ITBRs showed a different nitrifying performance, both from a macroscopic and microbiological point of view (Bernet *et al.*, 2004). The reactor R30 (highest support concentration) accumulated nitrite whereas R10 produced only nitrate as a final nitrification

product. The comparison of microbial communities in both reactors after 4 months of operation (Figure III.1) was in agreement with this result: the same population of nitrite-oxidizing *Nitrospira* (NOB) was present in both reactors but in very low proportion in R30 compared with R10. The major ammonium-oxidizer was different in both reactors, *Nitrosomonas europaea* (AOB1) in R30 and *Nitrosomonas* sp. (AOB2) in R10.

The question arises how the reactors' solid hold-up, being the only operating parameter different between both reactors, can act upon nitrifying activity and on the major ammonium oxidizer present? Note that the different solid hold-up of the reactors R10 and R30 results in different liquid volumes (1.27 L and 1.1 L respectively), leading to different ammonium loading rates (1420 and 1640 $gN.m^{-3}.d^{-1}$ respectively). The 15% higher loading rate in R30 compared to R10, for the same aeration flow rate, results in a lower oxygen: ammonium influent ratio in R30. The latter has likely caused oxygen depletion in R30, on its turn causing nitrite accumulation. It is postulated that the difference in the major ammonium oxidizer is also due to a selection pressure driven by the different oxygen concentration.

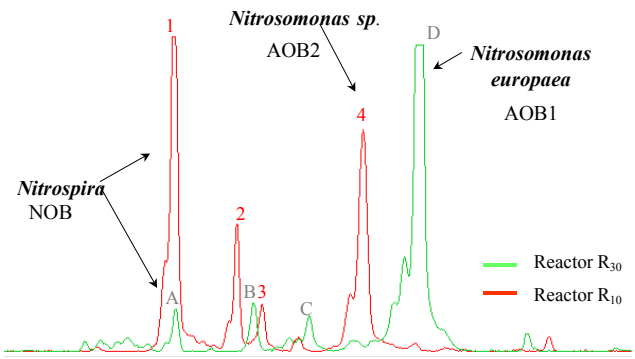

Figure III.1 Comparison of bacterial SSCP profiles from reactors R10 and R30 after start-up period (4 months of operation) with identification of nitrifying populations (Bernet *et al.*, 2004). The reactor R30 profile has been artificially increased to be able to detect the presence of peak A.

III.1.2.3 Reactor model

In order to describe the observed experimental behavior, a 0-dimensional biofilm model has been set up to describe the behavior of soluble components (ammonium, nitrite and nitrate) as well as biomass. The oxygen concentration is assumed constant in each of the reactors. The terminology '0-dimensional' indicates that a homogenous distribution of all components throughout the biofilm reactor has been assumed. Biomass retention in the reactor has been

modelled in a simplified way, equivalent to the one suggested in the ADM1 report (Batstone *et al.*, 2002). The nitrifying population considered consists of two ammonium oxidizing species and one nitrite oxidizing species, with respective concentrations X_{AOB1}, X_{AOB2} and X_{NOB}. In this way, the model contains the same number of nitrifying species as observed experimentally. The first ammonium oxidizing species (AOB1) was assumed to have a higher maximum growth rate than the second one (AOB2), which in turn had a higher oxygen affinity. Biomass detachment and biomass decay are assumed proportional to biomass concentrations. More details on the resulting model can be found in Volcke *et al.* (2008).

III.1.2.4 Dynamic simulation results

Figure III.2 displays the simulated behaviour of the R30 and R10 reactors. The oxygen level in the reactor has been set to 0.2 $gO_2.m^{-3}$ for R30 and to 3 $gO_2.m^{-3}$ for R10. Precise oxygen measurements have not been recorded during experiments, but it was verified that the oxygen level in reactor R30 was indeed limiting and that this was not the case in reactor R10. The initial conditions are the same for both reactors. The simulation results agree with the experimental observations: nitrite accumulates in R30 while complete oxidation to nitrate is achieved in R10; the dominating microbial populations correspond to the ones in Figure III.1.

The survival of only the ammonium oxidizer AOB1 at low oxygen concentrations can describe the occurrence of *Nitrosomonas europea* in the R30 reactor. For high oxygen concentrations, as prevailing in reactor R10 due to the lower load, both AOB2 (*Nitrosomonas* sp.) and NOB (*Nitrospira*) colonize the reactor. Note the different timescales at which different phenomena take place: soluble component concentrations display fast changes, while total biomass concentrations take longer to reach their steady state.

An important outcome of these simulations is that dynamics resulting from interspecies competition are even slower: individual AOB1 and AOB2 have not completely reached steady state values even after 4 months and this was confirmed by experimental findings (Volcke *et al.*; 2008).

As a possible control strategy to maintain the two different types of AOBs in the reactor, one could opt to switch the oxygen concentration in the reactor between two levels, e.g. by

controlling the oxygen level between large boundaries rather than on a strict set point (see for example Bougard et al. 2006).

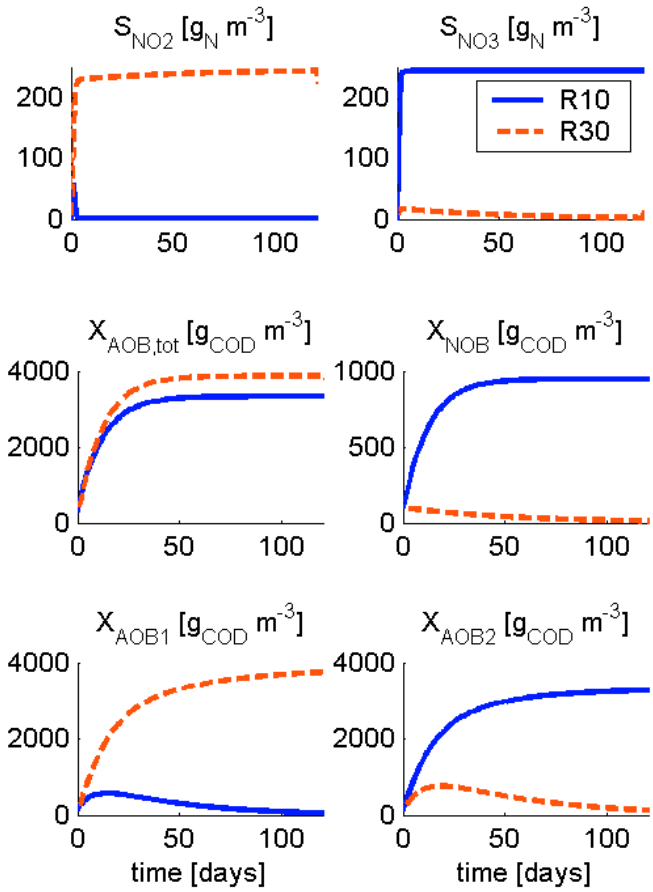

Figure III.2 Simulated behaviour of the R10 and R30 reactors : concentrations of ammonium (S_{NH}), nitrite (S_{NO2}), nitrate (S_{NO3}), total ammonium oxidizers ($X_{AOB,tot}$), individual nitrite oxidizers (X_{AOB1} and X_{AOB2}) as well as nitrite oxidizers (X_{NOB}).

III.1.3 *MICROBIAL DIVERSITY IN ANAEROBIC DIGESTION*

III.1.3.1 Modelling diversity in anaerobic digestion

In parallel, the IWA Anaerobic Digestion Model No. 1 (ADM1, Batstone *et al.*, 2002) was modified to handle microbial diversity (Ramirez and Steyer, 2008). The simulation software package MATLAB™/Simulink was used to study the relationship between reactor performance and microbial community structure.

In the traditional ADM1 model, one microbial population is associated to each reaction. Seven main groups of microorganisms are represented, corresponding to the degradation of

sugar, amino acids, LCFA, valerate and butyrate, propionate, acetate and hydrogen, each group of microorganisms having specific kinetic parameters. The microorganisms corresponding to the first five conversions are classified as bacteria, the ones corresponding to the latter two as archaea.

In order to account for microbial diversity, the traditional ADM1 model was extended in such a way that 10 different species were associated to each degradation reaction. For each species, the associated kinetic parameters were randomly chosen among 2 sets, normally distributed on each side of the kinetic parameters used to simulate ADM1 (*Cf.* Figure III.3). These sets were centered on 0.6 and 1.4 times the values used in ADM1 (± 10%) in order to simulate two distinct populations of each reaction. In the following, this extended ADM1 model will be called ADM1_10. In order to maintain comparable conditions for simulations, the initial biomass concentrations in ADM1 will be distributed equally among the corresponding microbial populations in ADM1_10.

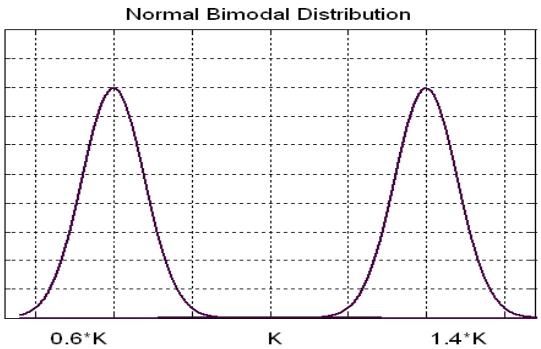

Figure III.3 Kinetic parameters in ADM1_10 (Ramirez and Steyer, 2008).

All inhibitions from ADM1 were kept in the model but an additional specific toxicant inhibition was added. No precise definition was here chosen for the toxicant since it was assumed to affect all microbial populations and modelled as a non-competitive inhibition factor added to all substrate uptake rates:

$$I_{tox} = \frac{1}{1 + \frac{S_I}{K_I}} \qquad (1)$$

where S_I is the toxicant concentration and K_I the inhibition constant. In the following, S_I was simulated as a pulse signal and the K_I mean value was arbitrarily chosen equal to 8 kgCOD/m^3. In line with the choice of the kinetic parameters of ADM1_10, the values of the

inhibition factors were randomly chosen for each biomass from a uniform distribution within two sets of mean values: 5 kgCOD.m^{-3} and 11 kgCOD.m^{-3}, to represent the fact that some microbial populations (in this case the latter) can be more tolerant than the global biomass represented in ADM1.

The resulting model was applied to simulate the behavior of four identical continuous stirred tank reactor (CSTR) configurations with a fixed headspace volume of 20 L, and nominal reactor size of 948 L, at mesophillic temperature (35°C), with identical inoculum. The composition of the simulated influent was based on the characterisation of vinasses from local wineries in the area of Narbonne, France (see Ramirez and Steyer, 2008, for details).

Traditional performance parameters such as biogas production, VFAs concentration and removal soluble COD were used to evaluate CSTR's performance. Abundance Biomass Curves comparison and Simpson's diversity index (Magurran 2005) were used to describe the microbial community structure. In order to quantify microbial diversity, the Simpson diversity index (D) was calculated as follows:

$$D = \frac{1}{\sum_{j=1}^{N} p_i^{\,2}} \qquad (2)$$

The ratios p_i have been calculated by dividing the biomass concentration of each species in a given family (*Bacteria* and *Archaea*) by the total biomass concentration at a given time instant.

III.1.3.2 Continuous versus pulsed loading rate operation

The response of two CSTRs with identical inocula was simulated for constant and pulsed organic loading rate (OLR) operation, respectively. In the following, these reactors will be called R1 and R3 respectively. R1 was operated at a hydraulic retention time (HRT) of 2.5 days and fed with vinasses with total COD of 15 kg COD/m^3. R3 was operated with a HRT between 0 and 2.5 days and the multiple-pulse OLR consisted of five sequential pulses with a duration of 5 days with 5 days between pulses, and amplitude twice the constant OLR, in such a way that the average organic loading rates for the perturbation cycle for all reactors were

equal (Fig. III.4). A P-controller was implemented in the model to maintain the reactor pH above a lower limit of 6.9, in order to avoid pH inhibition of aceticlastic methanogenesis. Figure III.4 presents the simulation results over a period of 50 days.

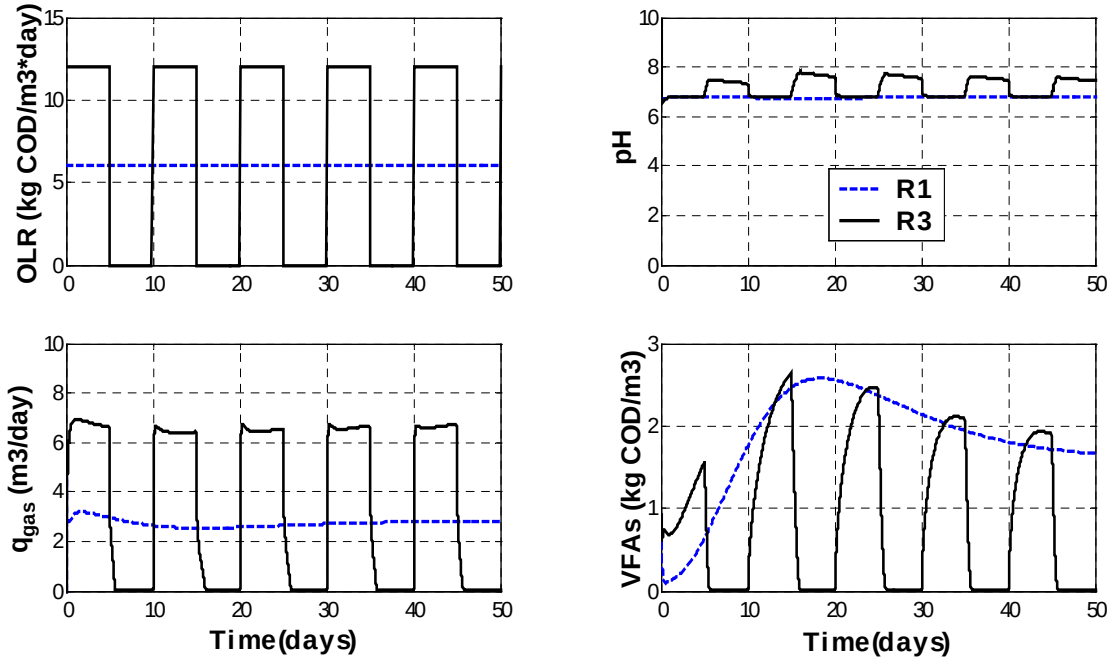

Figure III.4 ADM1_10 predicted VFAs concentrations, Biogas production rate, pH and OLR of R1 and R3 feed with vinasses, for constant and pulsed OLR respectively.

Every incremental increase in OLR during pulsed operation caused inhibition in the reactor performance during a short period after the loading. This may be attributed to the increase in the substrate concentration to be converted, which requires sufficient acclimatization period for native microflora to sustain to the changed environmental condition of the system. During each substrate shock load, the model showed increases in effluent VFA and soluble COD (not shown, but mainly consisting of VFAs) while the gas production increased but the methane content decreased. Nevertheless, the reactor always recovered to its normal performance within the next cycle, meaning that the shock were not too severe and assimilated by the reactor "buffer" for load capacity.

Considering the averaged behaviour over the cycles, R3 performs better than R1: it has a higher soluble removal efficiency, a higher gas production (33.8 m^3 in R3 versus 27.4 m^3 in R1), for a lower mean concentration of accumulated VFAs. Another difference from these two reactors lies in the biomass evolutions. Figure III.5 displays the dynamic evolution of

acetate degraders together with Abundance Biomass Curves and Simpson's diversity index for the *Bacteria* and *Archaea* domains, corresponding to the operation of R1 and R3. Similar results were obtained for all degraders but they are not shown due to space limitation. The R3 microbial community appears to be more diverse, with higher temporal variations. In contrast, the R1 microbial community appears more homogeneous with less diversity in the *Bacteria* and *Archaea* domains.

Summarizing, the pulsed OLR reactor (R3) displays a better performance than the one with constant OLR (R1), despite having a more diverse and less stable microbial community.

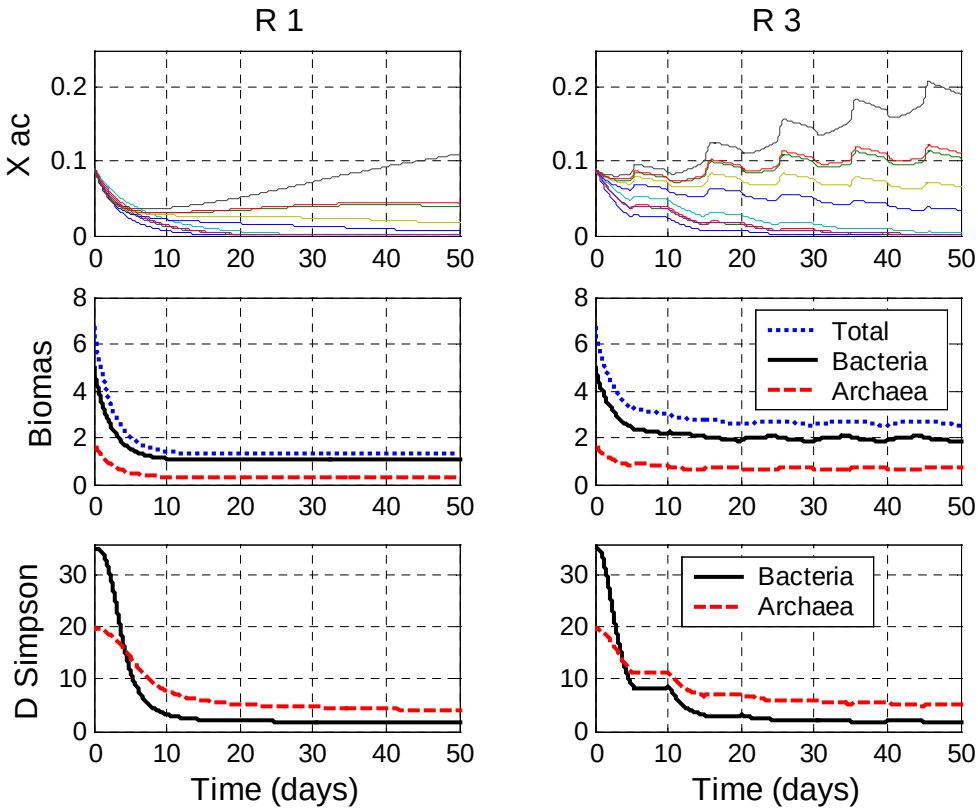

Figure III.5 ADM1_10 simulated biomass behaviour of R1 and R3 fed with vinasses, under constant and pulsed OLRs.

III.1.3.3 Effect of a toxicant pulse

The response of two CSTRs, with the same constant and pulsed loading rate operation, has now been simulated to a pulse toxicant concentration applied at day 12.5 for 2.5 days with amplitude 50 kg COD/m^3. In the following these reactors will be called R2 and R4, respectively. Figure III.6 shows the simulated behavior of both reactors over 50 days.

Upon toxicant addition, the concentration of VFAs (and consequently COD) increases rapidly in both reactors. This is associated with a pH decrease (not shown). In R4, VFA and COD concentrations decrease to their pre-perturbed values once the toxicant has been removed, while the new steady state values in R2 are higher than the pre-toxicant ones. The gas production displays a similar behaviour: it decreases in both reactors upon toxicant addition, then recovers to its pre-toxicant value in R4, while staying at a lower value than before the shock in R2. From the third cycle on, the gas produced per cycle was 34.5 m^3 for R4 and 20.7 m^3 for R2.

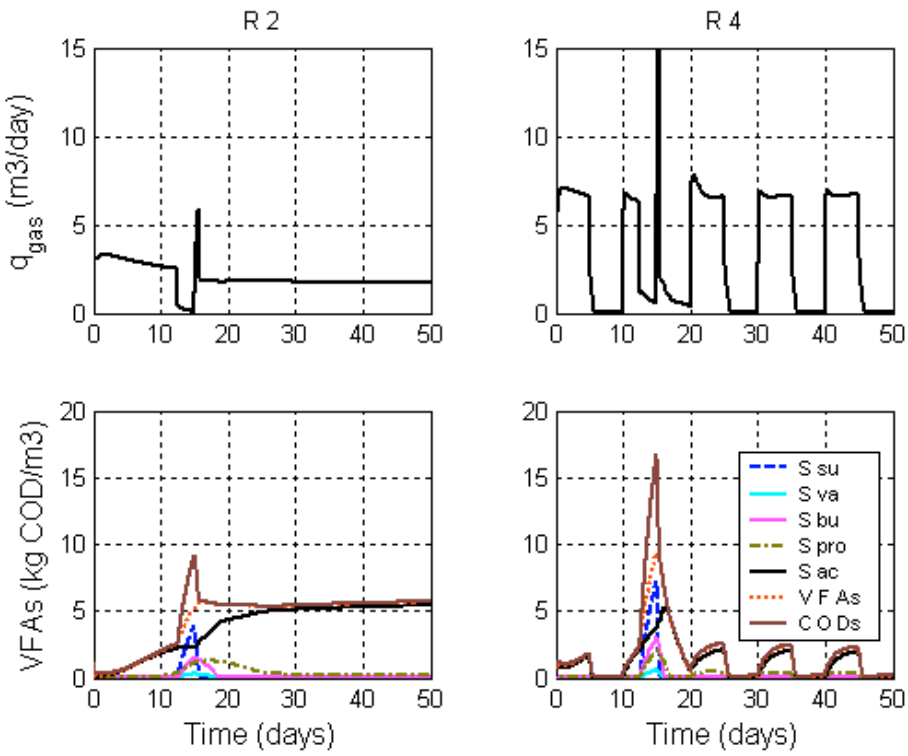

Figure III.6 ADM1_10 predicted VFAs, CODs and intermediate products concentrations and Biogas production rate of R2 and R4 reactors, under step input and pulsed OLR with a 2.5 day toxicant pulse applied at day 12.5

As in the previous section, the main difference between both reactors lies in the biomass evolution. Figure III.7 shows the dynamic evolution of acetate degraders together with Abundance Biomass Curves and Simpson's diversity index evolution for the *Bacteria* and *Archaea* domain. The rapid accumulation of VFAs in both reactors (Fig. III.6) results from a clear decrease in the activity of acetate-utilizing methanogens (Fig. III.7) and acetogens. Fermentative bacteria were also affected by the toxicant substrate perturbation. The H_2-utilizing methanogens appeared to be less affected by the substrate perturbation, furthermore

no significant accumulation of H_2 was observed during the entire experiment (results not shown).

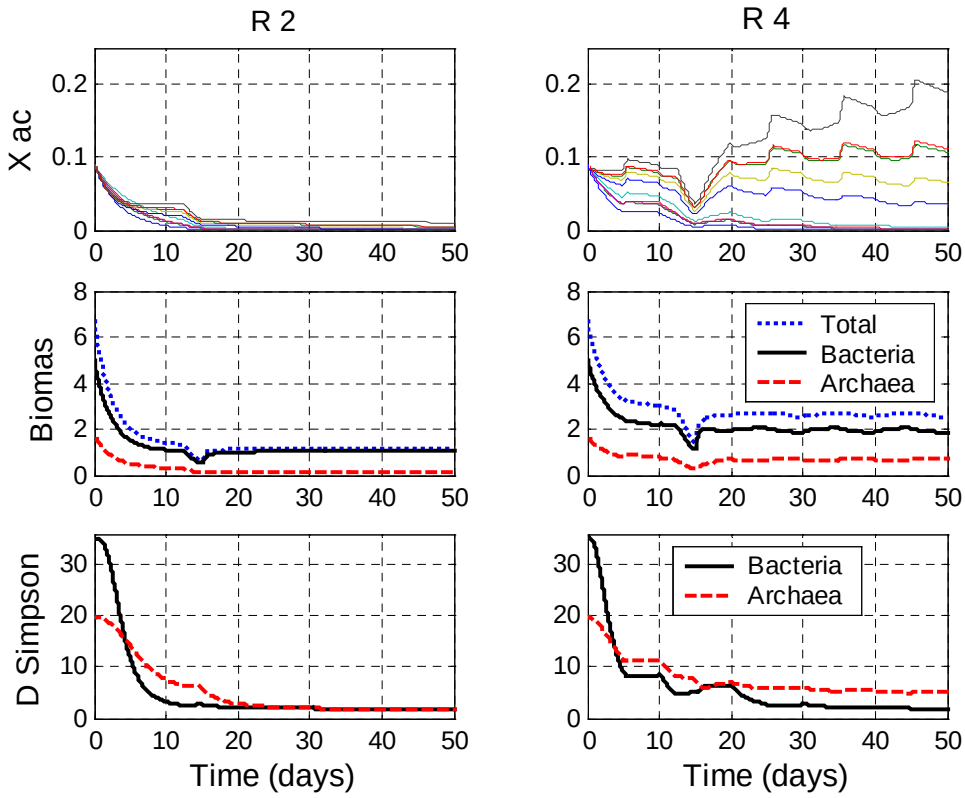

Figure III.7 ADM1_10 predicted biomass behaviour R2 and R4 feed with vinasses, under constant and pulsed OLR with a toxicant pulse applied at day 12.5

The observed changes in individual VFAs concentrations (Fig. III.6) indicate that all the major groups were impacted by the toxicant perturbation. Sugar accumulation points out that the fermentative bacteria were also affected by the toxic substrate perturbation. The most evident sign of this was the dramatic change in the products of sugar fermentation and this may contribute to explain the ability of R4 to adapt to the toxic substrate perturbation. The microbial community in the reactor R4 appeared to be more diverse, with high temporal variations. In contrast, the microbial community in the reactor R2 appeared more homogeneous with significantly less diversity mainly in Bacteria's domain.

Summarising, the pulsed OLR reactor (R4) has better performance than the constant OLR (R2) towards toxicant addition, despite that its microbial community was more diverse and less stable. The main difference between these communities is that R4 microbial community was able to return to the pre-toxicant conditions, while this was not the case for the one in R2. The simulation results thus indicate that the reactor with a less stable community but with

higher diversity was more functionally stable towards pulsed toxicant disturbances. The differences found between the fermentation pathways of accumulated sugar during the toxicant disturbance period suggest that the R4 community structure was more flexible than the R2 one.

In order to further emphasize the influence of microbial diversity in response to toxicant pulse, Figure III.8 shows simulations results with ADM1 and ADM1_10 under pulse feeding regime. As it can be seen, the diversity of ADM1_10 is higher than ADM1 for both domains: *Bacteria* and *Archaea* and although the biomass was able to recover in both models, higher biogas production and lower VFAs accumulation is obtained with ADM1_10, demonstrating a better tolerance to the toxicant pulse.

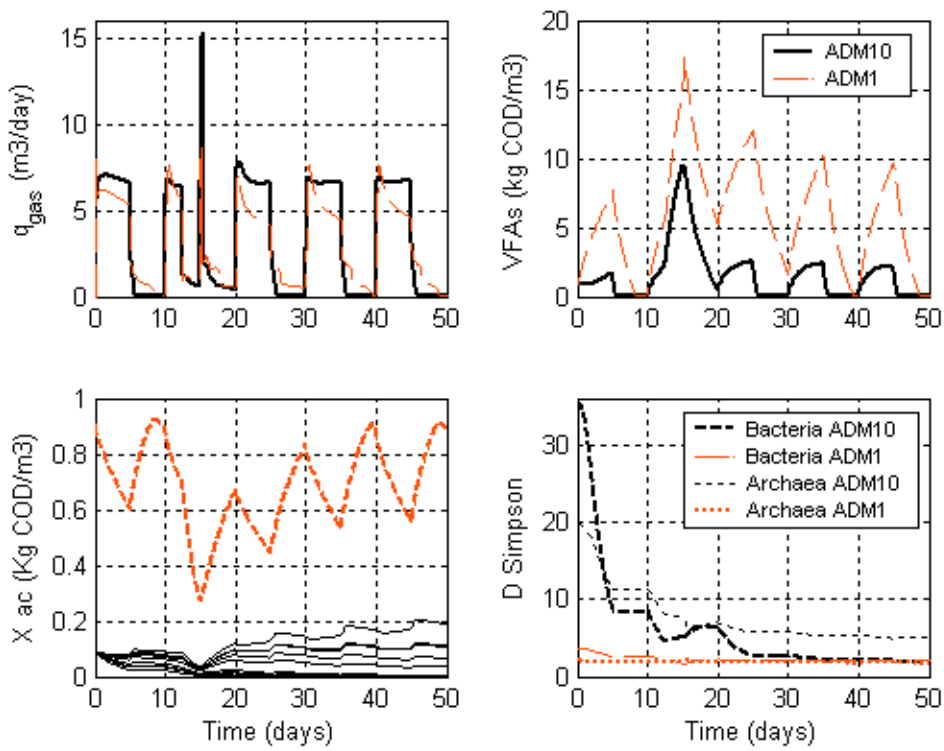

Figure III.8 ADM1 (thin dotted lines) and ADM1_10 (thick continuous lines) simulations: Comparison of VFAs and acetate degraders concentrations, biogas production rate and Simpson diversity indices under pulsed OLR with a 2.5 day toxicant pulse applied at day 12.5

III.1.4 *CONCLUSIONS*

Enhancing system heterogeneity by fostering the right populations can be achieved in several ways. One way is to take advantage of spatial effects, as in granulation or biofilm development or membrane reactors, which can be effective due to the different location of

different populations and functions in these systems. Another possibility to be explored is to introduce heterogeneity at temporal scale, e.g. by means of providing substrate pulses to encourage the growth of desired microorganisms, as suggested in this paper. The development of communities that are more resilient in the long term due to the pulse disturbances has been demonstrated in other ecosystems, and the stability developed therein is the result of heterogeneity operating in both temporal and spatial scales (Bengtsson *et al.*, 2002).

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III.2 MODELING MICROBIAL DIVERSITY IN ANAEROBIC DIGESTION THROUGH AN EXTENDED ADM1 MODEL[4]

III. 2.1 *INTRODUCTION*

The anaerobic digestion process for wastewater treatment can nowadays be considered as a state-of-the art technology. Because of it is yet widely applied and its sustainable characteristics, i.e. high capacity to treat slowly biodegradable substrates at high concentrations, low energy requirements, reduction of odors and the possibility for energy recovery and reduced CO_2 emissions compared to other techniques, its application is expected to further increase in future.

Anaerobic digestion is a multi-step process in which organic carbon is converted into biogas, being a mixture of mainly carbon dioxide (CO_2) and methane (CH_4). Besides physicochemical reactions, the process comprises two types of biochemical reactions: extracellular (disintegration and hydrolysis) and intracellular ones. The latter type involves a variety of microorganisms, namely fermentative bacteria (i.e. acidogens, responsible for the uptake of sugar and amino acids), hydrogen-producing and acetate-forming bacterias (i.e. acetogens, degrading long chain fatty acids, valerate, butyrate and propionate), and archaea which convert acetate or hydrogen into methane (i.e. methanogens). Other types of anaerobes play important roles in establishing a stable environment at various stages of methane fermentation. An example of the latter are homoacetogens, which can oxidize or synthetize acetate depending on the external hydrogen concentration (Kotsyurbenko, 2005).

Despite their distinct advantages, the application of anaerobic digestion systems is often limited by the fact that they are sensitive to disturbances and may suffer from instability. Such instability is usually witnessed as a drop in the methane production rate, a drop in the pH and/or a rise in the volatile fatty acid (VFA) concentration, leading to digester failure. Such failure can be caused by various inhibitory substances, one of them being ammonia (Chen et al., 2008). High ammonia concentrations, originating from the degradation of organic proteineous material, are often encountered during anaerobic digestion of animal wastes such as slaughterhouse waste, swine manure, cattle and poultry wastes and industrial wastes

[4] Paper published by I. Ramirez, E.I.P. Volcke,R. Rajinikanth and J-Ph. Steyer in *Water Research* (2009) http://dx.doi.org/10.1016/j.watres.2009.03.034.

originating from food processing. Although ammonia is an important buffer in the process, and it is an essential nutrient for anaerobic microbes, high ammonia concentrations can decrease microbial activities, particularly for methanogens (Angelidaki et al., 1993); resulting in a disturbed balance between fermentation and methanogenesis which may lead to a fatal upset of the anaerobic treatment process. Within two distinct methanogenic groups, acetate-consuming methanogens are usually found to be more sensitive to high ammonia concentrations than hydrogen-utilizing ones (Hansen et al., 1998; Sprott et al., 1986), altough some studies also indicate hydrogen-utilizing methanogens as the most sensitive group (Wiegant et al., 1986).

Given that Free Ammonia Nitrogen (FAN) rather than Total Ammonia Nitrogen (TAN) is suggested as the actual toxic agent, an increase in pH will result in increased toxicity (Borja et al., 1996). Process instability due to ammonia often results in volatile fatty acids (VFAs) accumulation, which again leads to a decrease in pH and thereby declining concentration of FAN. The interaction between FAN, VFAs and pH may lead to an ‘‘inhibited steady state’’, a condition where the process is running stably but with a lower methane yield (Angelidaki et al., 1993).

Most of studies on inhibition of anaerobic digestion by ammonia reported in literature determine inhibition thresholds rather than the dynamic behavior of microorganisms upon toxicant addition and their adaptation to elevated ammonia concentrations. Nevertheless, an example of the species selection during process start-up is described by Calli et al. (2005). They detected a shift in the archaea population, from *Methanosaeta*-related species to *Methanosarcina*-like acetoclastic methanogens during start-up of five upflow anaerobic reactors seeded with different sludges, to gradually increasing free ammonia levels, through cloning, denaturing gradient gel electrophoresis (DGGE) and fluorescent in-situ hybridization (FISH) techniques.

Mathematical models have proven their effectiveness in biological process design and operation. With respect to anaerobic digestion, the Anaerobic Digestion Model No.1 (ADM1, Batstone et al., 2002), developed by the corresponding International Water Association (IWA) Task Group, has become widespread and generally accepted. However, ADM1 does not distinguish between microorganisms performing the same reaction – which implies all of them are assumed to have the same properties-, and can therefore not adequately represent or

predict experimental results concerning this type of interspecies diversity. The need for incorporation of detailed micro-scale data into current wastawater tretment models was also indicated previously by Yuan and Blackall (2002), regarding the influence of plant design and operation on microbial and microbial properties in activated sludge systems.

This contribution presents an approach for modelling microbial diversity in the anaerobic digestion process, applied to the standard ADM1 which has been extended with multiple species for each reaction. The extended model has subsequently been applied to handle microbial diversity in both normal conditions (i.e., not leading to process imbalance) and abnormal situations (e.g., characterized by the presence of inhibiting ammonia levels in the reactor).

III.2.2 *MATERIALS AND METHODS*

III.2.2.1 Experimental setup

A laboratory scale Upflow Anaerobic Sludge Filter Bed (UASFB) reactor (diameter 12 cm; height 117 cm; effective volume 9.8 L) was used in this study. The reactor column was made in plexi-glass and constituted of two compartments: the bottom part was operated as a UASB reactor whereas the top part was operated as an anaerobic filter. The top portion of the UASFB reactor was randomly packed with 90 pieces of small cylindrical, buoyant polyethylene packing media (height: 29 mm; diameter: 30-35 mm; density: 0.93 kg/m3), and baffled with 16 partitions. 50% of the reactor volume (excluding the head space of 30 cm height) was filled with the packing media. The reactor operated at $33\pm1^{o}C$, was equipped with a continuous internal recirculation system from top to the bottom (recirculation rate: 9 L/h). Recirculation was done mainly to eliminate the possibility of high organic loading close to the feed port and to favour better wastewater/sludge contact. The digester was seeded with granules (15% by total volume) originating from a UASB digester treating cheese wastewaters.

This hybrid UASFB reactor was operated for a total period of 232 days. Continuous feeding of the reactor started with an initial OLR of 3.1 gCOD/L.d. OLR was then increased stepwise by increasing the substrate concentration from 3.1 to 21.7 g/L (around 95% of the total COD was soluble), while maintaining a constant HRTof 1.15 days. Soluble COD removal of 80%

was here considered as the threshold level between low and high efficiency of the UASFB reactor. The OLR was progressively increased by 20 to 30% once or twice a week until the COD_s removal dropped below 80%. The feed was supplemented with nutrients to attain a COD:N:P ratio of 400:7:1 in the wastewater. The feed pH was adjusted to 6-6.5 using a 6N sodium hydroxide. The performance of the UASFB reactor was monitored as described by Rajinikanth et al. (2008).

In this type of wastewater soluble COD is mainly present as monosaccharides (*i.e.,* S_{su} in ADM1) and little as amino acids (S_{aa}) and long chain fatty acids (S_{fa}). The particulate COD is mainly present in the form of carbohydrates (X_{ch}), besides some composites (X_c), proteins (X_{pr}) and lipids (X_{li}). The input VFAs values were calculated from measured concentrations of acetate (S_{ac}), propionate (S_{pro}), butyrate (S_{bu}) and valerate (S_{va}). The initial pH was calculated from the ionized forms of VFAs, bicarbonate, ammonia and cation/anion concentrations. Ammonia (S_{IN}) and bicarbonate (S_{IC}) were measured by Kejdahl's method and using a TOCmeter, respectively. Anion concentration (S_{an}) was taken equal to S_{IN} and cation concentration (S_{cat}) was adjusted in each case according with initial experimental pH. The concentrations of these individual components used in the model as process inputs can be found in Rajinikanth et al. (2008).

III.2.2.2 Model structure

The IWA ADM1 was extended to handle microbial diversity within functional groups. In the traditional ADM1 model, one microbial population is associated to each reaction. Seven functional groups of microorganisms are distinguished, corresponding to the degradation of sugar (by X_{su}), amino acids (by X_{aa}), LCFA (by X_{fa}), valerate and butyrate (by X_{c4}), propionate (by X_{pro}), acetate (by X_{ac}) and hydrogen (by X_{h2}) and one microbial population is associated to each reaction. In order to account for microbial diversity, the traditional ADM1 model was extended in such a way that multiple species are associated to each functional group. The number of species per reaction is arbitrary and, in this study, has been set to 10 to limit the reduction of computation speed. This approach is detailed in Appendices A & B, for the sugar degraders (X_{su}, state variable 17) involved in sugar degradation (reaction 5), and subject to decay (reaction 13). Its application to the remaining populations (state variables 18 to 23) with respect to the corresponding degradations (reactions 6 to 12) and decay reactions (reactions 14 to 19) is straightforward. Whereas the original ADM1 possesses 24 state

variables, of which 7 biomass types, the extended model includes 70 different microorganisms and 87 state variables in total. The number of associated reactions is extended from 19 to 154. The resulting model will further be denoted as ADM1_10, where '10' refers to the extension of the original model for microbial diversity with 10 species for each group. Within each functional group, species may differ in terms of their yield coefficient Y as well as Monod maximum specific uptake rate k_m and half saturation constant K_s. In this sense, species may be different not only in the sense handled by microbial taxonomists (e.g. using 97% sequence similarity in 16S rRNA genes), but also when belonging to the same genus i.e. individuals that share a common set of kinetic and stoichiometric characteristics.

In our case, the yield coefficient was assumed constant as in reality the variability of this parameter is low. Within a functional group, the kinetic parameters k_m and K_s were randomly chosen from a normal bimodal distribution, with means of $\mu_1 = 0.6 * k$, $\mu_2 = 1.4 * k$, and standard deviations of $\sigma_{1,2} = 0.125 * k$ where k is the value of the corresponding standard ADM1 parameter. The distribution type and their parameters values were stablished following a curve-fitting process using experimental data from a UASFB reactor. Compared to the deterministic ADM1, this approach adds a stochastic component to ADM1_10. It is clear that many other approaches to define the microbial properties within functional groups can be thought of. They are all likely to be stochastic since microbial properties cannot be defined with certainty. In order to maintain comparable conditions for simulations, the initial biomass concentrations in ADM1 will be distributed equally among the corresponding microbial populations in ADM1_10.

Biomass retention in the UASFB reactor has been modeled in the simplified way suggested in the ADM1 report (Batstone *et al.,* 2002) with a term including the solids residence time of solids ($t_{res,X}$) in the biomass mass balance equation to account for the difference between hydraulic retention time (HRT) and solid retention time (SRT). The resulting model has been implemented in MATLAB^{TM}/Simulink. Its applicability has first been tested by Ramirez and Steyer (2008) to model anaerobic digestion in a fixed bed reactor. In this contribution, a thorough model validation has been performed on experimental data for UAFSB reactor. It is important to note that the presented modelling approach is generic and can also be applied to other processes as ASM. Volcke et al. (2008) demonstrated the applicability of a model

including different species performing the same reaction, describing experimental nitrification data through a model with two types of ammonium oxidizers.

Developing and tuning mathematical models in normal situations are nowadays a well defined procedure that can be easily performed, even with complex models such as ADM1. However, developing and tuning a model to adequately represent abnormal situations is still a difficult and challenging task. In particular, when facing inhibition and/or toxicant, anaerobic digestion processes can experimentally present different behaviors that are still not fully understood: one process can indeed show high robustness with respect to toxicant presence while an other one, even though they seem to be very similar, are much more sensitive to toxicant. It is indeed likely that different species will exhibit different behaviours towards these substances. The effect of nonreactive toxicant affecting all species has been examined by Ramirez and Steyer (2008). Other non-reactive toxicant such as ammonia inhibits a specific populations, in this case methanogens.

In ADM1, all microbial mediated substrate conversion processes are subject to inhibition by extreme pH values. Moreover, the anaerobic oxidation processes are subject to inhibition due to accumulation of hydrogen while acetoclastic methanogenesis is inhibited by high free ammonia concentrations. Inhibition caused by hydrogen and free ammonia was originally implemented in ADM1 by rate multipliers that reflect non-competitive inhibition and an empirical correlation was used to reflect the effects of extreme pH. All inhibitions from ADM1 were kept identical in the modified model ADM1_10.

III.2.3 RESULTS AND DISCUSSION

The behaviour of the modified anaerobic digestion model, ADM1_10, has been compared to the one of the standard ADM1 and to experimental results in simulating the behaviour of a pilot-scale UASFB reactor operated under varying input OLR over 260 days. Further comparison of the ADM1 and ADM1_10 has been performed for abnormal conditions, by simulating the effect of ammonia pulses. Finally, simulation results of ADM1_10 for a reactor exposed to increasing levels of ammonia were analysed with respect to the relationship between reactor performance and microbial community structure. The results are described in what follows.

III.2.3.1 Simulation of UASFB with varying OLR: ADM1 vs ADM1_10

Previous experience in simulating the behavior of a reactor fed with the same wine destillary wastewater (Ramirez and Steyer, 2008) led to the identification of the main ADM1 parameters which need to be modified in order to reasonably reflect the experimental data. In this case, only the maximum specific substrate uptake rate (k_m) and the half saturation constant (K_s) for acetate and propionate were calibrated to fit the data. The resulting values were used in all simulations, with ADM1 as well as ADM1_10 (in the latter case as center values).

Figure III.9 compares the experimental data with the simulation results obtained with both models for the UASFB reactor operated at a varying input loading rate by varying the influent concentration while maintaining a constant HRT. As it is seen both models can simulate very nicely the dynamic evolutions of the main variables, in the liquid and also in the gas phase. As a consequence, assessing the most appropriate model among ADM1 and ADM1_10 is a tedious, not to say impossible, task. Of course, it could be said that better fit could have been obtained but the main purpose of this study was not to perfectly fit these data but to evaluate the ability of both models to adequately predict the behavior of this particular digestion process. Soluble COD, VFAs and biogas production values are higher in ADM1 than in ADM1_10 due to the amount of biomass from ADM1 is lower than the biomass from ADM1_10. This is in agreement with the diversity-productivity hypothesis of Tilman et al (2002) and the phenomenon is known as “overyielding”.

Between day 100, and 200 both models over-predicted VFA concentrations. It appeared that the simulated rate at which acetate was converted into methane under the load imposed was somewhat under-estimated. This may have resulted from either under-estimation of the substrate consumption coefficients for acetoclastic methanogenesis or an over-estimation of the inhibition of this activity by ammonia.

The models predict well the dynamics of the biogas production rate and composition as a response of the load imposed. Small deviations in predicting the biogas production and quality have been found, which may be attributed to the fact that the standard ADM1 uses the same gas/liquid transfer coefficients for all gases (CO_2, CH_4, H_2), while this is not the case in reality. Besides, the dependence of these coefficients on the specific reactor configuration applied has been also neglected. Similarly, the pH was quite accurately simulated and both models were able to reflect the trends that were observed in experimental data. The pH

prediction is closely related to the cation and anion concentrations in the reactor and actually, to the difference between the two concentrations. Since the ion concentrations were not measured, it was then calculated using the pH value and taking into account the concentration of ammonia, alkalinity and VFAs concentration in the reactor. The value of the input cation from the reactor minus the input anion concentration in the feed was arbitrarily increased in the models, so that the pH values were calibrated. On day 35, about 300 mL of sludge were accidentally discharged out of the reactor (connection failure at the bottom of the reactor) and hence the performance of the UASFB was disturbed. This disturbance was not included in the simulations and may be this explains the differences mainly in COD_s and VFAs between the simulated and experimental data in the period 35-57 days.

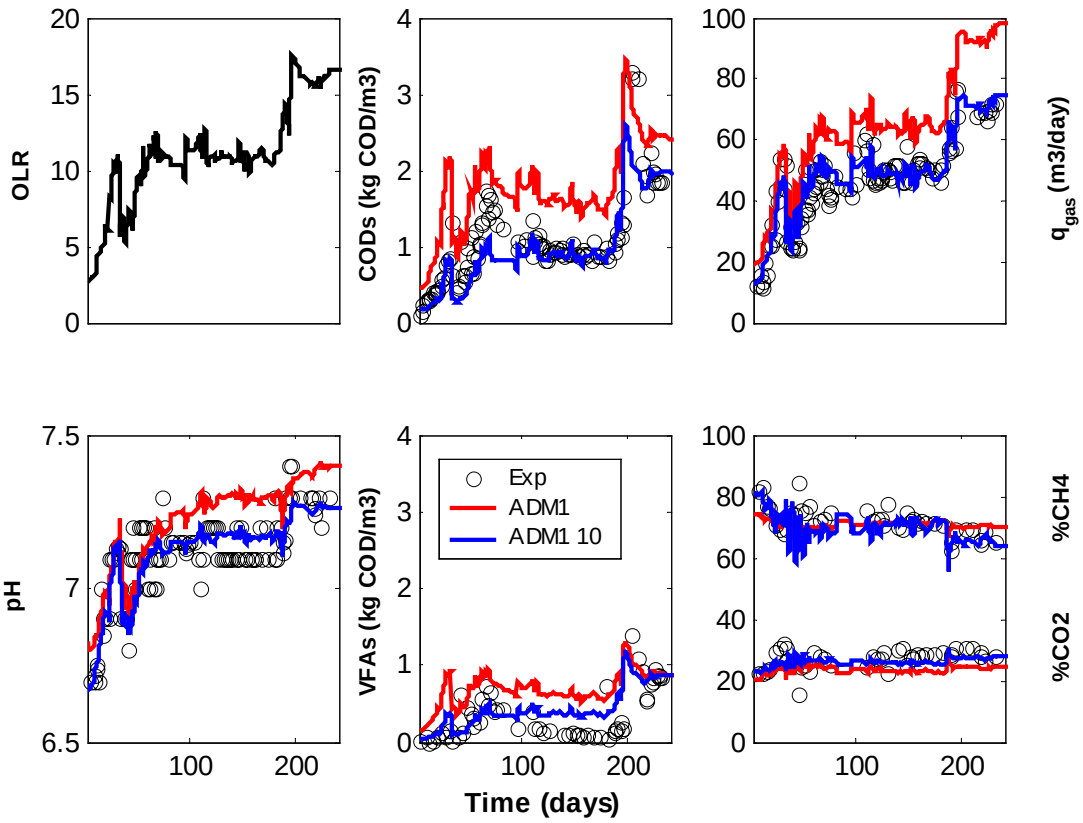

Figure III.9 Behavior of a UAFSB reactor: experimental data versus simulation results with ADM1 and ADM1_10.

The main difference between the ADM1 and ADM1_10 models lies in the biomass evolutions. Figure III.10 shows the obtained specific growth rates and the dynamic evolution of acetate degraders during these simulations. Similar results were obtained for other degraders (not shown). The specific growth rate in terms of substrate concentrations (Monod curves) are depicted too. As it is seen in Figure III.10.c we have two biomass groups :

$K_strategists$ (1st to 5th species) versus $\mu_strategists$ (also known as R-strategists, 6th to 10th species) which is related to the fact that we have combined high K_S values with high k_m values and low K_S values with low k_m values. After an initial decrease of all species, related to a decrease of total biomass, from day 150, species 6 to 10 outcompetes species 1 to 5, (Figure III.10.d) may due to the former species have high growth rate as we can see in Figure III.10.c. At the same time, acetate concentration switches from low values to high ones (data not shown), leading to a competitive advantage of the biomass group of $\mu_strategists$.

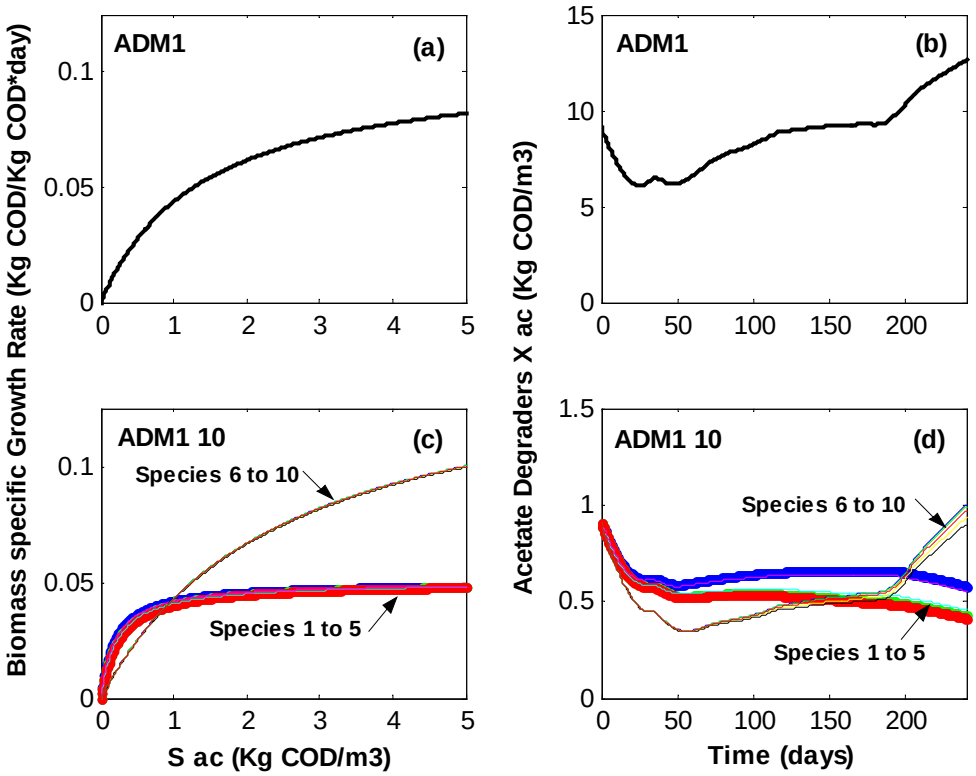

Figure III.10 Acetate degrading biomass evolution and corresponding specific growth rates: ADM1 vs ADM1_10.

This competitive advantage is also maintained for a longer simulation period: even after 3000 days, species 6 to 10 all survive (data not shown).

III.2.3.2 <u>ADM1 vs ADM1_10 when facing a toxicant</u>

In this section, both models were applied to evaluate the performance of a digester facing a toxicant in the feeding line. To avoid effect of biomass retention on the microbial population dynamics, it was decided to simulate the behavior of a CSTR. Nominal reactor volume was

arbitrarly chosen equal to 4.4 L and headspace volume equal to 1.6 L. Working temperature was in the mesophilic range (*i.e.,* 35°C). The composition of the simulated influent was based on the characterization of slaughterhouse wastewater with a COD concentration of 15 kgCOD/m^3 and operated for 750 days under constant loading rate of 1.75 kgCOD/m^3.day. This influent consisted mainly of carbohydrates, proteins, lipids, VFAs, inorganic carbon and inorganic nitrogen. The concentrations of these individual components used during simulations as process inputs are shown in Table III.1.

Table III.1 Input concentrations of the slaughterhouse wastewater used during the simulations of the toxicant present in the feeding line

Component	Values (kgCOD/m^3)	Component	Values(kgCOD/m^3)
Total VFAs	1.08	Inorganic Carbon	2.51 mM C
Carbohydrates	2.35	Inorganic Nitrogen	8.91 mM N
Proteins	6.71	Total dissolved COD	1.54
Lipids	2.51	Total particulate COD	13.47

The behaviour of ADM1 and ADM1_10 has been compared in terms of biomass evolution and reactor performance before, during and after a temporarily increase in the influent TAN concentration (from 13 to 110 mM applied between day 150 and 200). The transition period and acclimatization was judged by traditional reactor performance indicators such as methane production rate (MPR), soluble COD removal and effluent volatile fatty acid (VFAs) concentrations.

The diversity of microbial community structure has been quantified by Simpson's reciprocal diversity index (D), calculated as follows:

$$D = \frac{1}{\sum_{j=1}^{N} p_i^{\,2}}$$

where the ratios p_i are calculated by dividing the biomass concentration of each species in a given family (*Bacteria* and *Archaea*) by the total biomass concentration at a given time instant. It is clear that a higher Simpson diversity index corresponds with a more diverse population. The usefulness of this index to encode accurate information from microbial

fingerprinting profiles has recently been demonstrated by Haegeman et al. (submitted). Microbial population concentrations lower than 10^{-3} kgCOD/m3 were not considered in diversity indices calculations, to avoid accounting of species that are too diluted to be measured in practice.

To investigate the effect of nonreactive toxicant such as ammonia that acts on specific populations, in this case methanogens, the inhibition constant for ammonia $K_{I,NH3}$ was modified. In line with the choice of the kinetic parameters of ADM1_10, the values of the inhibition constant for ammonia was randomly chosen from a normal bimodal distribution with means: $\mu_1 = 0.6 * K_{I,NH3}$ (in this case it is assumed that some archaea species are much more sensitive to the presence of ammonia), $\mu_2 = 1.4 * K_{I,NH3}$ (to represent the fact that some archaea species can be more tolerant) and standard deviations $\sigma_{1,2} = 0.125 * K_{I,NH3}$.

To measure functional stability, we adopted parameters described in ecology (Grimm, 1992; Neubert, 1997) in terms of the amplification envelope of key intermediate products in response to a perturbation (*Cf.* Figure III.11). The two main parameters obtained from this envelope are resistance and resilience. Resistance of a community with respect to an intermediate product is defined as the maximum accumulation of the product. It is a measure of the buffering capacity of the community with respect to the corresponding intermediate products (in our case, the different VFAs). Resilience is defined as the time taken by the accumulated intermediate product to return to its referential state (Neubert, 1997). In this way, a higher numerical value denotes lower resistance or resilience.

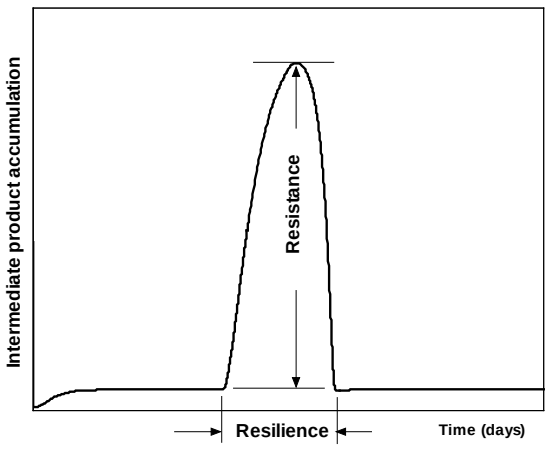

Figure III.11 Ecological parameters of functional stability.

Simulation results are presented in Figure III.12. As it is seen, ADM1_10 demonstrates a higher robustness to the presence of toxicant (from day 150 to 200) than ADM1.

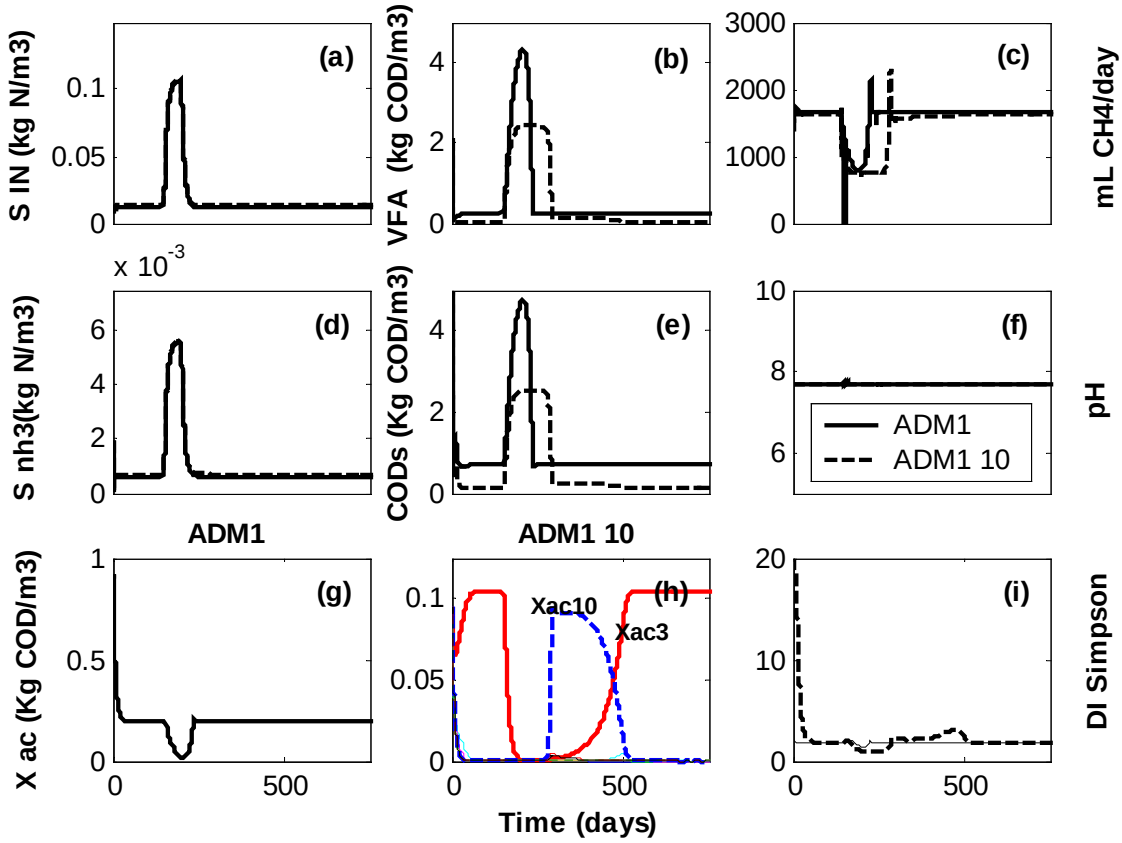

Figure III.12 ADM1(continuous line) and ADM1_10 (dotted lines) simulations when facing a pulse of total ammonia nitrogen (top left figure). Except the inhibition constant for ammonia, all other kinetic parameters were identical to those used in the simulations presented in Figures III.9 and III.10.

The biodiversity is also positively related to ecosystem stability, i.e resistance and resilience (Reinthaler et al., 2005; Saikaly et al., 2005). Therefore, the high bacterial diversity in the CSTR could allow better and more stable performance as can be seen in Figure III.12. In our case, the microbial community diversity from ADM1_10 was higher than the ADM1 (Figure III.12.i), but the former displayed more resistance (less accumulation of VFA and CODs) and less resilience in response to TAN shocks (reflected by a larger recovery period). In other words, the biodiversity acts as an "insurance" for CSTR functions against temporal changes in environmental factors like pulsed TAN,. because removal soluble COD from ADM1 is lower than the ADM1_10 one during the perturbation period.

These simulation results agree with previous findings (Fernandez et al., 2000) where the responses of two continuously mixed methanogenic reactors, designated as high-spirochete (HS) and low-spirochete (LS) sets, were analysed with respect to substrate (glucose) shock loads. The microbial community diversity of the latter (LS) was higher than the former one (HS) but displayed more resistance and less resilience in response to these glucose shocks.

Figure III.12.g and III.12.h display the evolution of acetate degraders for ADM1 and ADM1_10, respectively. For both models, the pulse increase of ammonia in the reactor results in a temporarily decrease of the total amount of acetate degraders. The time to return to the total amount of acetate degraders present before the pulse is longer for ADM1 10 than for ADM1, again indicating the lower resilience of the former. Figure 4.h also reveals a population shift induced by the ammonia pulse: whereas species 3 is initially dominating, it is replaced with species 10, which is less inhibited by ammonium (higher $K_{I,NH3}$ value, see Table 3); once the ammonium concentration has decreased again, species 3 again wins the competition.

III.2.3.3 Relationships between reactor performance and microbial community structure (facing increasing levels of ammonia with ADM1_10)

In this section, ADM1_10 is applied to simulate the behaviour of a CSTR for three different TAN concentration feeding strategies summarized in Figure III.13.

III.2.3.3.1 One-step increase vs two-step increase in TAN

The destabilizing effect of a one or two step increase in TAN concentration on the reactor performance is shown in Figure III.14 in terms of total ammonia (*Cf.* Figure III.14.a), VFAs concentration (*Cf.* Figure III.14.b), MPR (*Cf.* Figure III.14.c) and soluble COD removal (*Cf.* Figure III.14.f). Biomass adptation to increased TAN concentration is indicated by the fact that when the TAN was changed from 40 to 58 mM in R2 the reactor performance are not as disturbed as in R1 when the TAN was changed from 13 to 58 mM. As a result, the time required for complete adaptation (i.e. return to steady state as noted by effluent VFAs concentrations, removal soluble COD and MPR) was longer in R1 than in R2.

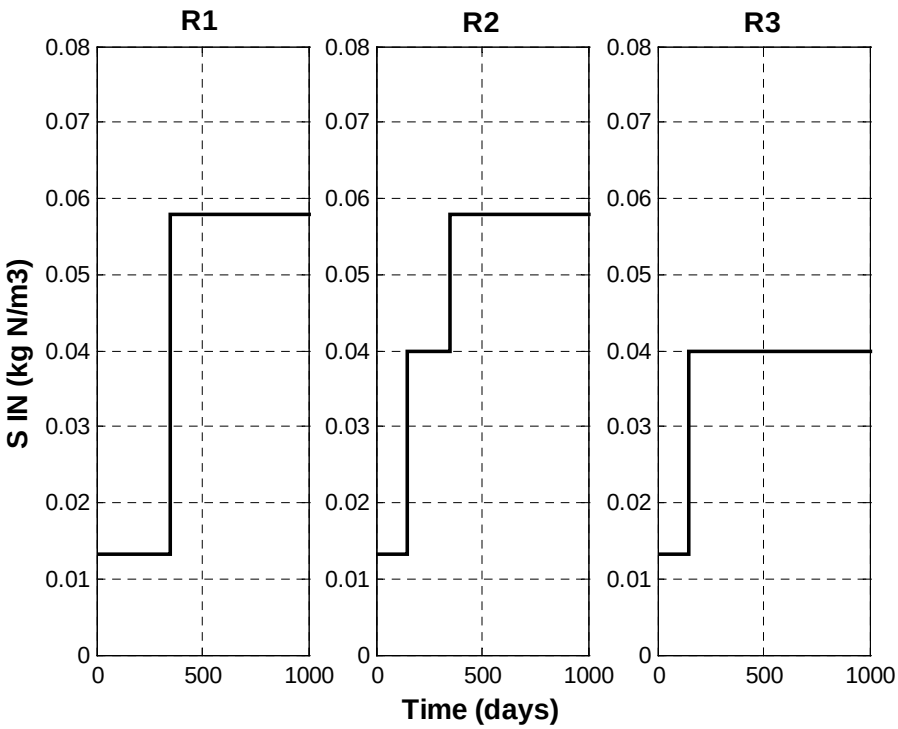

Figure III.13 TAN concentration patterns for three feeding strategies of increasing ammonia in the feeding line.

These observations on slaughterhouse reactors are similar to those of van Velsen (1979) who studied municipal sludge and piggery wastes degradation with respect to both adaptation time and disturbation grade. It is also clear from the simulation results that MPR and soluble COD removal efficiency decreased in the transition period (*i.e.,* the time required for adaptation) and that these indices returned to lower levels than those obtained prior to the change in TAN concentration.

Regarding the composition of the acetate degrading community (*Cf.* Figure III.14.g and III.14.h), species 3 (i.e. Xac3) is dominant at the initial low TAN reactor concentrations, followed by a population shift for increasing TAN concentrations. The nature of this population shift depends on the different feeding strategies applied : R1 selects species 7 (i.e. Xac7), 8 (i.e. Xac8) and 9 (i.e. Xac9) (*Cf.* Figure III.14.g), while R2 selects only the 10th onespecies 10 (i.e. Xac10) This suggests that adaptation to elevated ammonia concentrations resulted from the selection of resistant aceticlastic methanogens (i.e. the species with high $K_{I,NH3}$) already present in seed sludge. The diversity indices plot indicates that the methanogenic activity was most affected (*Cf.* Figure III.14.e), whereas the acetogenic and fermentative activities were not affected (*Cf.* Figure III.14.d). These findings are in agreement

with those of Calli et al. (2005), who, as already mentioned found a shift in archaea population during adaptation period under gradually increasing FAN levels.

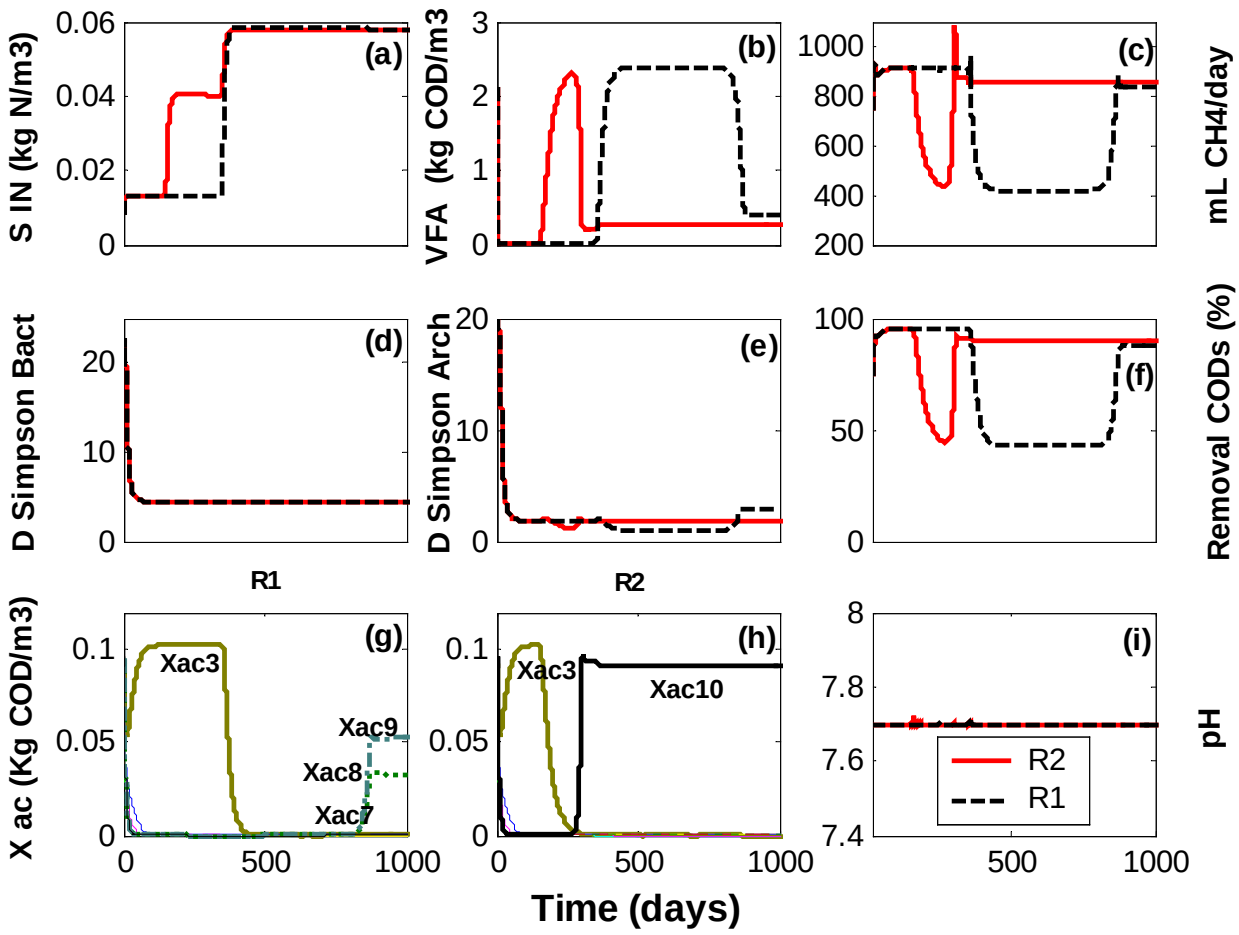

Figure III.14 Reactor 1 & 2 performance. One-step 13 to 58 mM (R1: dotted lines) vs two-step 13 to 40 mM and 40 to 58 mM (R2: continuous line).

III.2.3.3.2 Effect of the suppression of species (R3)

The selection of dominant aceticlastic methanogen species has subsequently been analysed in more detail for a CSTR with a step increase in the TAN concentration from 13 to 40 mM at day 150 (Figure III.13, R3). Figure III.15.a shows the evolution of the 10 groups of acetate degraders initially present. Subsequently, the simulations have been rerun for a gradually restricted group of acetate degraders, successively elimitating the winning species from the previous simulation. The results presented in Figures III.15.b to III.15.f reveal the following ranking in order of decreasing competitive power: 10-9-6-8-7. Table III.2 shows the values of the affinity constants and the maximum growth rates, which differ between the species. A common feature of all surviving species is their relatively high tolerance towards ammonium (high $K_{I,NH3}$); the reactor did not recover when only the more sensitive species (1 to 5) are

present (Figure III.15.f). Regarding the order of species selection, one may expect that species with a high substrate affinity (low K_S) or a high maximum growth rate have a competitive advantage. However, the ranking cannot be explained only in terms of either decreasing K_S or increasing μ_{max}, which would yield an order 10-6-9 rather than 10-9-6. As we explained below, the inhibition constant for ammonia (KI,NH3) also plays a role.

Table III.2 Biochemical parameters for the different acetate degraders

Number of species	$\mu_{max,ac}$	$K_{S,ac}$	$K_{I,NH3}$	$Inhib_{ac}$	J_{ac}^*
1	0.2892	0.0197	0.0014	0.3937	0.2208
2	0.3281	0.0284	0.0012	0.3672	0.7854
3	0.3114	0.0477	0.0010	0.3246	0.2492
4	0.2856	0.0195	0.0011	0.3366	0.0844
5	0.2563	0.0467	0.0009	0.2925	0.1167
6	0.5635	0.2704	0.0023	0.5186	0.2021
7	0.4850	0.2775	0.0026	0.5510	0.2439
8	0.5391	0.2941	0.0027	0.5619	0.2066
9	0.5590	0.2884	0.0028	0.5695	0.1865
10	0.5714	0.2472	0.0025	0.5400	0.1683

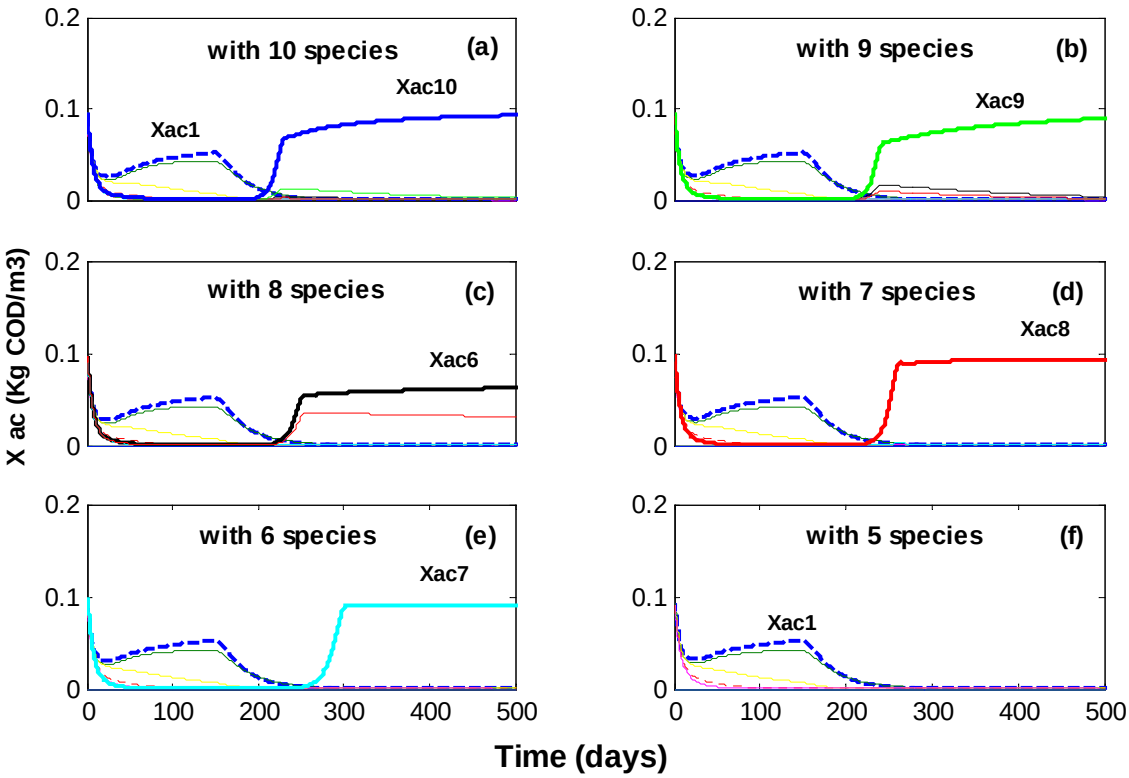

Figure III.15 Acetoclastic methanogens Biomass behavior in R3 (*i.e.,* one-step increase from 13 to 40 mM). In each case, the dominant species was successively suppressed.

A theoretical basis to understand species selection was given by Hsu *et al* (1977), who have defined criteria for the outcome of microbial competition for a single limiting substrate in a CSTR operated under constant with a constant dilution rate *D* and for a constant influent substrate concentration . They have defined,

$$J_{ac}(i) = K_{S,ac}(i)\frac{D}{\mu_{\max,ac}(i) - D}$$

in which *D* represents the dilution rate.

If the number of competing species is such that their J_ac's are ordered, with:

$$J_{ac}(1) < J_{ac}(2) < \ldots < J_{ac}(10)$$

all species die out if $S_{ac}(0) < J_{ac}(1)$. On the other hand, if $S_{ac}(0) > J_{ac}(i)\ \forall i$, then only specie 1 (*i.e.,* the one associated to $J_{ac}(1)$) survives and outcompetes all rival species. This principle has been verified experimentally by Hansen and Hubbel (1980) .

An analogous *J*-expression has been defined for our case where inhibition is present as follows:

$$J_{ac}(i)^* = K_{S,ac}(i)\frac{D}{\mu_{\max,ac}(i) * Inhib_{ac}(i) - D}$$

The maximum growth rate has been corrected for inhibition effects through the same inhibition factor that we used for uptake of acetate in both models, i.e. $Inhib=I_{pH}.I_{IN}.I_{nh3}$ (see appendix III.B). Note that the mathematical rigorousness of the criterion of Hsu et al (1977), valide for a single substrate, expires in our case since NH_3 acts as an additional substrate during acetate degradation (even though not being limiting). Moreover, the acetate degradation reaction is only one step in the anaerobic digestion reaction network, while Hsu's criterion holds for single reaction systems. Despite these uncertainties, the obtained species ranking 10-9-6-8-7 in terms of increasing $J_{ac}*$ values, agrees with the simulation results. This may explain why washout of the archaea species is more sensitive to the presence of ammonia (i.e. have a lower inhibition constant). The results indicate the advantage of the above criteria to predict the outcome of interspecies competition and may stimulate further research in this

direction for models involving multiple reactions in series and/or parallel and including inhibition.

Figure III.16 displays the macroscopic reactor performance corresponding to Figure III.15 when the dominant species were successively suppressed. Note that the total initial biomass concentrations are the same in all simulations. The steady state behaviour before and after the step was slightly influenced by the properties of the underlying microbial species. Nevertheless, the dynamic behaviour in terms of the lenght of the acclimatization was significantly influenced by the microbial properties. The plot also reveal that the adaptation period gets lower when the number of resistant species at high TAN concentration levels (i.e. richness) increase. When all resistant species (6th sps to 10th sps) where supressed, the performance of the rector did not recover. Nevertheless, the process was running in stably conditions but with VFAs accumulation, lower MPR and higher effluent soluble COD, a condition termed « inhibited steady state » (Angelidaki et al., 1993).

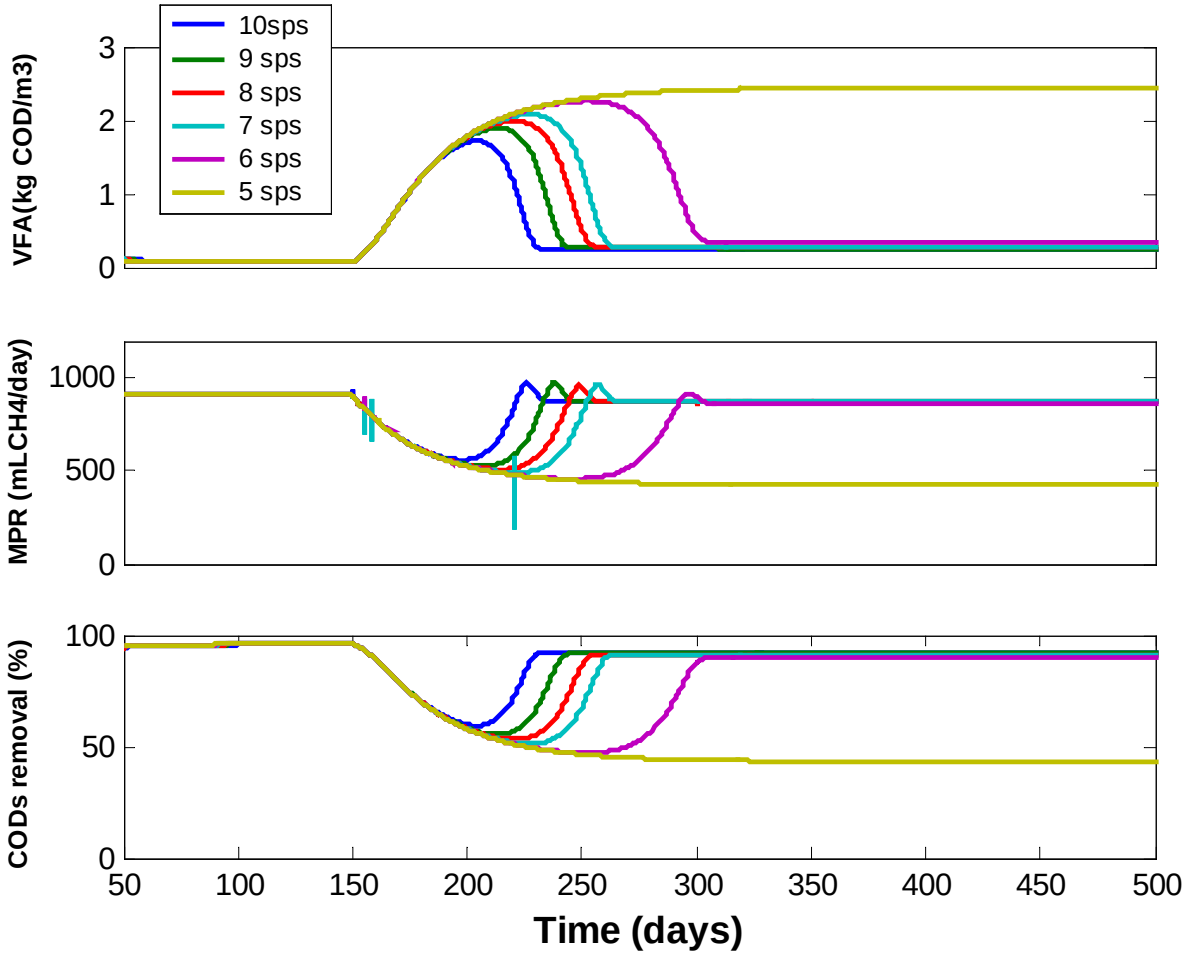

Figure III.16 Performance of Reactor 2. At day 150, TAN was changed from 13 to 40 mM.

This example clearly illustrates that, although a different microbial composition may somethimes not seem to influence the macroscopic reactor behaviour (the steady state conditions before and after the influent ammonia step increase are indeed the same), they may induce significantly different effects (i.e. different response to increased toxic loads) in other operating conditions. This strengthens our belief that the engineering of wastewater treatment systems would be improved if one could predict and manipulate the associated microbial diversity. This ability would complement our established capacity to predict the optimal process design. Mathematical models in which data on micro-scale molecular diversity, as gained with modern molecular tools (such as denaturant gradient gel electrophoresis – DGGE -, fluoresent in situ hybridization with DNA probes – FISH -. If this late is combined with a confocal laser-scanning microscope will allow the visualization of three-dimentional microbes structures, Sanz and Kochling, 2007), has been more closely incorporated to represent wastewater treatment processes can provide a useful tool to reach this goal.

A credible model to predict the nature, composition and distribution of the microbial community can indeed allow us to explain how microbial diversity could vary with environmental conditions. Since the type of microorganisms present in a reactor ultimately defines its operational performance, this information can be of the utmost importance. . Even though we do not yet know exactly the diversity of the different functional groups nor how this diversity is sustained, the appproach applied in this paper can be used to gain insight in the influence of process conditions on the selection of certain types of species and in our general belief, handle microbial diversity. In a later stage, this model can also be used to develop efficient control strategies adapted to model-based population optimisation, but further work is clearly needed before engineers could use it to design a system.

III.2.4 *CONCLUSION*

A methodology to account for microbial diversity in complex but structured models such as the anaerobic digestion model ADM1 has been presented. This approach consists in extending the number of mass balances for an arbitrary number of species having the same function (performing the same reaction), while using aa stochastic mechanism to select the corresponding microbial parameters. The resulting model remains powerful in representing macroscopic experimental data, but is moreover able to get insight in underlying microscopy. This has been demonstrated by investigating the impact of increasing toxicant concentrations and assessing the relationship between biodiversity and reactor performance.

Adaptation of microorganisms to inhibitory substances, as suggested in this paper, can significantly improve waste water treatment efficiency. For instance adaptation to elevated ammonia concentrations may result from the selection of resistant acetoclastic methanogens already present in seed sludge. The influence of microbial parameters of resistant aceticlastic metahanogens species at high ammonia levels affecting interspecies competition has been assesed explicitly.

To deal with microbial diversity, the number of species considered for each biological reaction is arbitrary and in this study was set to 10, which is sufficient to demonstrate the potential of modelling microbial diversity. Besides, the number of species considered may differ between different functional groups (reactions). Moreover, handling a very high number of species per reaction (e.g. 100-1000) can be seen as a way toreduce efforts required for parameter estimation. Indeed,, only a "global" value of the model parameters such as in ADM1 would be required, microbial diversity being later accounted for by the high number of species handled with random kinetic parameters centered around the average values found to fit ADM1.

Application of the presented methodology to include biodiversity in other structured models, such as activated sludge models (ASM) is straightforward. This offers wide perspectives in terms of modeling abilities but also in terms of control objectives since microbial population appears nowadays to be a major component that drives processes performance.

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Appendix III.A Process kinetics and stoichiometry for sugar uptake and decay of sugar degraders in ADM1_10 (i=1-16,j=1-154)

	ESTATES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Component i	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Ssu	Saa	Sfa	Sva	Sbu	Spro	Sac	Sh2	Sch4	S_IC	S_IN	S_I	X_xc	X_ch	X_pr	X_li
j	process																
1	Disintegration										*	**	f_SI_Xc	-1	f_ch_Xc	f_pr_Xc	f_li_Xc
2	Hydrolysis Carbohydrates	1									*				-1		
3	Hydrolysis Proteins		1								*					-1	
4	Hydrolysis Lipids	1-f_fa_li		f_fa_li							*						-1
5(1)	Uptake of Sugars byXsu(1)	-1				(1-Y_su(1))f_bu_su	(1-Y_su(1))f_pro_su	(1-Y_su(1))f_ac_su	(1-Y_su(1))f_h2_su		*	(-Y_su(1))*N_bac					
5(2)	Uptake of Sugars byXsu(2)	-1				(1-Y_su(2))f_bu_su	(1-Y_su(2))f_pro_su	(1-Y_su(2))f_ac_su	(1-Y_su(2))f_h2_su		*	(-Y_su(2))*N_bac					
5(3)	Uptake of Sugars byXsu(3)	-1				(1-Y_su(3))f_bu_su	(1-Y_su(3))f_pro_su	(1-Y_su(3))f_ac_su	(1-Y_su(3))f_h2_su		*	(-Y_su(3))*N_bac					
5(4)	Uptake of Sugars byXsu(4)	-1				(1-Y_su(4))f_bu_su	(1-Y_su(4))f_pro_su	(1-Y_su(4))f_ac_su	(1-Y_su(4))f_h2_su		*	(-Y_su(4))*N_bac					
5(5)	Uptake of Sugars byXsu(5)	-1				(1-Y_su(5))f_bu_su	(1-Y_su(5))f_pro_su	(1-Y_su(5))f_ac_su	(1-Y_su(5))f_h2_su		*	(-Y_su(5))*N_bac					
5(6)	Uptake of Sugars byXsu(6)	-1				(1-Y_su(6))f_bu_su	(1-Y_su(6))f_pro_su	(1-Y_su(6))f_ac_su	(1-Y_su(6))f_h2_su		*	(-Y_su(6))*N_bac					
5(7)	Uptake of Sugars byXsu(7)	-1				(1-Y_su(7))f_bu_su	(1-Y_su(7))f_pro_su	(1-Y_su(7))f_ac_su	(1-Y_su(7))f_h2_su		*	(-Y_su(7))*N_bac					
5(8)	Uptake of Sugars byXsu(8)	-1				(1-Y_su(8))f_bu_su	(1-Y_su(8))f_pro_su	(1-Y_su(8))f_ac_su	(1-Y_su(8))f_h2_su		*	(-Y_su(8))*N_bac					
5(9)	Uptake of Sugars byXsu(9)	-1				(1-Y_su(9))f_bu_su	(1-Y_su(9))f_pro_su	(1-Y_su(9))f_ac_su	(1-Y_su(9))f_h2_su		*	(-Y_su(9))*N_bac					
5(10)	Uptake of Sugars byXsu(10)	-1				(1-Y_su(10))f_bu_su	(1-Y_su(10))f_pro_su	(1-Y_su(10))f_ac_su	(1-Y_su(10))f_h2_su		*	(-Y_su(10))*N_bac					
6	Uptake of Amino Acids		-1		(1-Y_aa)f_va_aa	(1-Y_aa)f_bu_aa	(1-Y_aa)f_pro_aa	(1-Y_aa)f_ac_aa	(1-Y_aa)f_h2_aa		*	Naa-Y_aa*N_bac					
7	Uptake of LCFA			-1				(1-Y_fa)*0.7	(1-Y_fa)*0.3		*	(-Y_fa)*N_bac					
8	Uptake of Valerate				-1		(1-Y_c4)*0.54	(1-Y_c4)*0.31	(1-Y_c4)*0.15		*	(-Y_c4)*N_bac					
9	Uptake of Butyrate					-1		(1-Y_c4)*0.8	(1-Y_c4)*0.2		*	(-Y_c4)*N_bac					
10	Uptake of Propionate						-1	(1-Y_pro)*0.57	(1-Y_pro)*0.43		*	(-Y_pro)*N_bac					
11	Uptake of Acetate							-1		1-Y_ac	*	(-Y_ac)*N_bac					
12	Uptake of Hydrogen								-1	1-Y_h2	*	(-Y_h2)*N_bac					
13(1)	Decay of Xsu(1)										*	N_bac-N_xc		1			
13(2)	Decay of Xsu(2)										*	N_bac-N_xc		1			
13(3)	Decay of Xsu(3)										*	N_bac-N_xc		1			
13(4)	Decay of Xsu(4)										*	N_bac-N_xc		1			
13(5)	Decay of Xsu(5)										*	N_bac-N_xc		1			
13(6)	Decay of Xsu(6)										*	N_bac-N_xc		1			
13(7)	Decay of Xsu(7)										*	N_bac-N_xc		1			
13(8)	Decay of Xsu(8)										*	N_bac-N_xc		1			
13(9)	Decay of Xsu(9)										*	N_bac-N_xc		1			
13(10)	Decay of Xsu(10)										*	N_bac-N_xc		1			
14	Decay of Xaa										*	N_bac-N_xc		1			
15	Decay of Xfa										*	N_bac-N_xc		1			
16	Decay of Xc4										*	N_bac-N_xc		1			
17	Decay of Xpro										*	N_bac-N_xc		1			
18	Decay of Xac										*	N_bac-N_xc		1			
19	Decay of Xh2										*	N_bac-N_xc		1			
											$* \sum_{i=1-9}^{11-87} C_i \nu_{i,j}$						

Appendix III.B Process kinetics and stoichiometry for sugar uptake and decay of sugar degraders in ADM1_10 (i=17-87,j=1-154)

17(1)	17(2)	17(3)	17(4)	17(5)	17(6)	17(7)	17(8)	17(9)	17(10)	18	19	20	21	22	23	24		
										18	19	20	21	22	23	24		
										Xaa	Xfa	Xc4	Xpro	Xac	Xh2	X_I	RATE	
																f_XI_Xc	kr_dis*Xc	
																	k_hyd_ch*Xch	
																	k_hyd_pr*Xpr	
																	k_hyd_li*Xli	
Y_su(1)																	k_m_su(1) * Ssu/(K_S_su(1)+Ssu) *Xsu(1)*inhib[1]	
	Y_su(2)																k_m_su(2) * Ssu/(K_S_su(2)+Ssu) *Xsu(2)*inhib[1]	
		Y_su(3)															k_m_su(3) * Ssu/(K_S_su(3)+Ssu) *Xsu(3)*inhib[1]	
			Y_su(4)														k_m_su(4) * Ssu/(K_S_su(4)+Ssu) *Xsu(4)*inhib[1]	
				Y_su(5)													k_m_su(5) * Ssu/(K_S_su(6)+Ssu) *Xsu(5)*inhib[1]	
					Y_su(6)												k_m_su(6) * Ssu/(K_S_su(6)+Ssu) *Xsu(6)*inhib[1]	
						Y_su(7)											k_m_su(7) * Ssu/(K_S_su(7)+Ssu) *Xsu(7)*inhib[1]	
							Y_su(8)										k_m_su(8) * Ssu/(K_S_su(8)+Ssu) *Xsu(8)*inhib[1]	
								Y_su(9)									k_m_su(9) * Ssu/(K_S_su(9)+Ssu) *Xsu(9)*inhib[1]	
									Y_su(10)								k_m_su(10)* Ssu/(K_S_su(10)+Ssu) *Xsu(10)*inhib[1]	inhib[1] = I_pH_aa*I_IN_lim
																		inhib[2] = inhib[1]*I_h2_fa
										Y_aa							k_m_aa * Saa/(K_S_aa+Saa) *Xaa*inhib[1]	inhib[3] = inhib[1]*I_h2_c4
											Y_fa						k_m_fa * S fa/(K_S_fa+Sfa) *Xfa *inhib[2]	inhib[4] = inhib[1]*I_h2_pro
												Y_c4					k_m_c4 * Sva/(K_S_c4+Sva) *Xc4*Sva/(Sva+Sbu+eps)*inhib[3	inhib[5] = I_pH_ac*I_IN_lim*I_nh3
												Y_c4					k_m_c4 * Sbu/(K_S_c4+Sbu)*Xc4*Sbu/(Sbu+Sva+eps)*inhib[3	inhib[6] = I_pH_h2*I_IN_lim
													Y_pro				k_m_pro* Spro/(K_S_pro+Spro)*Xpro*inhib[4]	
														Yac			k_m_ac * S ac/(K_S_ac+Sac)*Xac*inhib[5]	I_IN_lim = 1/(1+K_S_IN/SIN)
															Y_h2		k_m_h2* Sh2/(K_S_h2 +Sh2)*Xh2*inhib[6]	I_h2_c4 = 1/(1+Sh2/K_lh2_c4)
																		I_h2_c4 = 1/(1+Sh2/K_lh2_c4)
-1																	k_dec_Xsu(1) *Xsu(1)	I_h2_pro = 1/(1+Sh2/K_lh2_pro)
	-1																k_dec_Xsu(2) *Xsu(2)	
		-1															k_dec_Xsu(3) *Xsu(3)	
			-1														k_dec_Xsu(4) *Xsu(4)	
				-1													k_dec_Xsu(5) *Xsu(5)	
					-1												k_dec_Xsu(6) *Xsu(6)	
						-1											k_dec_Xsu(7) *Xsu(7)	
							-1										k_dec_Xsu(8) *Xsu(8)	
								-1									k_dec_Xsu(9) *Xsu(9)	
									-1								k_dec_Xsu(10) *Xsu(10)	
										-1							k_dec_Xaa *Xaa	I_nh3 = 1/(1+xtemp[33]/K_I_nh3)
											-1						k_dec_Xfa *Xfa	
												-1					k_dec_Xc4 *Xc4	**(Nr_xc-f_xI_xc*N_I-f_sI_xc*N_I-f_pr_xc*N_aa)
													-1				k_dec_Xpro *Xpro	***(Ns_xc-f_xI_xc*N_I-f_sI_xc*N_I-f_pr_xc*N_aa)
														-1			k_dec_Xac *Xac	
															-1		k_dec_Xh2 * Xh2	

III.3 RELATIONSHIP BETWEEN MICROBIAL COMMUNITY STRUCTURE AND BATCH THERMOPHILIC ANAEROBIC DIGESTION PERFORMANCE OF THERMALLY PRETREATED WAS: MODELING APPROACH[5]

III.3.1 INTRODUCTION

The anaerobic digestion (AD) process generally consists of five stages: disintegration, hydrolysis, acidogenesis, acetogenesis and methanogenesis (Batstone et al., 2002). In anaerobic digestion of waste activated sludge (WAS), the biological hydrolysis is often identified as the rate-limiting step (Tiehm et al., 2001; Li et Noike, 1992). To reduce the impact of this rate-limiting step, pretreatment of WAS is required such as thermal, alkaline, ultrasonic or mechanical disintegration (Tanaka et al., 1997; Kepp et al., 2000; Nah et al., 2000; Schieder at al., 2000; Kim and Lee, 2005). With the pretreatment, the organic part of the waste is splited up in a first step into short-chain fragments that are biologically well suited for microorganisms. The following fermentation runs much faster and more complete than in conventional digestion processes and the biogas yield is increased. In other words, these treatments can accelerate the solubilization (hydrolysis) of WAS and reduce the particle size, which subsequently improves the anaerobic digestion (Tanaka et al., 1997; Tiehm et al., 2001).

Many studies have investigated the pretreatment of WAS for anaerobic digestion and mostly dealt with a single pretreatment method in comparison with non pretreatment (Li et al., 1992 ; Lin et al., 1997).

More recently, the microbial ecology of anaerobic reactor systems has also been investigated in detail (Delbes et al., 2001; Ahring et al., 2001; Gerardi, 2003; Collins et al., 2003; McHugh et al., 2004). Other limited number of studies have covered the microbial ecology of acidification reactors (Cha and Noike, 1997; Ince and Ince, 2000; Solera et al., 2002; Carbone et al., 2002). In addition, few other studies have specifically focused on the start-up of AD processes (Anderson et al., 1994; Liu et al., 2002, Cresson et al. 2008).

[5] Paper in preparation by I. Ramirez, A. Mottet, H. Carrère, S. Déléris, F. Vedrenne and J-Ph. Steyer.

Furthermore, changes in operational and environmental conditions of anaerobic reactors and within the microbial populations present in the reactor definitely affect each other mutually (Burak at al., 2006). As a consequence, further evaluation of these variations seems extremely useful from a microbiological point of view. Demirel and Yenigün (2006) investigated the behaviour of the microbial populations within an anaerobic reactor, operated at a hydraulic retention time (HRT) range between 24 and 12 h, in terms of changes in numbers of total bacterial community, autofluorescent methanogens, non-methanogens and morphology of the autofluorescent methanogens, using epifluorescence microscopy and microbiological enumeration techniques. They showed that the numbers of the total bacterial community and autofluorescent methanogens both decreased during start-up. Also, the proportion of the number of autofluorescent methanogens in the total bacterial community varied from 5% to 16% during operation.

Sewage sludge consists of primary sludge and waste activated sludge. WAS are known to be more difficult to degraded than primary sludge. As mentioned above, although improvement of biodegradability of WAS has been widely investigated, the microbiology of thermophilic digestion of WAS has been poorly studied. Anaerobic digestion of WAS is a special biological phenomenon in which anaerobic *Bacteria* degrade the mixed microorganisms produced in activated sludge systems. Kobayashi et al., (2008) studied the structure of anaerobic microbial community during the thermophilic anaerobic digestion (TAD) of WAS. They suggested that bacterial communities of WAS and TAD sludge were different, maybe due to the fact that most of the *Bacteria* present in the WAS could not survived the condition of the thermophilic anaerobic digestion. They clearly showed that the bacterial community of thermophilic digester had less diversity than a mesophilic digester. However, few studies have been performed the influence of pretreatment on the microbial diversity. Kim et al. (2005) showed that the methanogenic activity of anaerobic granules was increased by the application of ultrasound. Mladenovska et al., (2006) studied the impact of thermal pretreatment at 140°C on the microbial community of thermophilic anaerobic digestion of manure. They showed that the bacterial and archaeal populations identified in both CSTR (non treated manure and treated at 140°C) were found to be identical, but a change in the abundance of the species was detected. The thermal pretreatment had thus a positive impact for the development of an active hydrogenotrophic methanogens in the case of manure treatment.

It is obvious that the pretreatment has an important impact on the substrate composition. Moreover, the substrate composition clearly affects the microbial community structure of anaerobic digester (Riviere at al., 2007). Thus, in order to know the pretreatment impact, it is necessary to determine the links between microbial community structure, operating conditions, substrate composition and anaerobic digestion process performance. Mathematical models in which data on micro-scale molecular diversity have been incorporated to more closely represent anaerobic digestion processes can provide a useful tool to reach this goal. Such models can be used to gain insight on the influence of process conditions on the selection of certain types of microorganisms. In a later stage, these models can also be used to develop efficient control strategies adapted to model-based population optimisation. In this contribution, this approach is demonstrated for thermophilic anaerobic digestion of thermally pretreated WAS.

In a previous work, the IWA Anaerobic Digestion Model (ADM1 – see Batstone et al. 2002) model has been slightly modified with changes in the hydrolysis kinetics and addition of a Hill function to better account for disintegration/hydrolysis and ammonia inhibition in thermophilic anaerobic treatment of thermally pretreated WAS at 110°C, 165°C (electric and steam modes) and 220°C (Ramirez et al. 2008, Annexe III). In another study, ADM1 was adapted to account for microbial diversity and was then called ADM1_10 (Ramirez and Steyer, 2008). In the present work, ADM1_10 is used to analyze the relationship between batch reactor performance and microbial community structure (MCS). More specifically, relationships between (i) inoculum's MCS and reactor performance and (ii) MCS and reactor performance during both start-up and routine operations will be assessed.

III.3.2 *MATERIALS AND METHODS*

III.3.2.1 Experimental approach

WAS samples from a wastewater treatment plant (France), working with high load process, were used during the experiments. Thermal pretreatments were performed in a 10 L agitated autoclave (Autoclave, class IV), allowing a temperature increase by electric mode or by steam mode. Sample volume was around 6 L. Temperatures of treatment were 110°C, 165°C (in both electric and steam modes) and 220°C. Once temperature was reached, treatments lasted for 30 min.

Biochemical Methane Potential (BMP) tests were used to measure the final methane potential, e.g. anaerobic biodegradability, of untreated and pretreated samples. The method was based from Buffiere et al. (2006). Anaerobic batch reactors were kept at 55°C (thermophilic conditions) by water circulation in a water jacket. Five reactors, with a volume of 3.5 L each, were used in parallel. The inoculum was taken from a full scale sludge anaerobic digester. One reactor was used with no feed to quantify the endogenous activity of the inoculum. Others reactors were fed with untreated sludge and with sludge treated at 110°C, 165°C (electric and steam modes) and 220°C. Organic loading was 0.5 g_{COD} of WAS per g_{VS} of inoculum. For each condition, four successive 22 days batch experiments were carried out to minimise the effect of the inoculum. At the beginning of each BMP test, the reactors were purged with N_2/CO_2 (75/25) gas mixture. Biogas production and pH were measured continuously. An electronic volumetric gas counter was used to monitor biogas production. During anaerobic digestion, total and soluble Chemical Oxygen Demand (COD), total and individual Volatile Fatty Acids (VFA) and volumetric biogas production were daily monitored in order to follow the formation of by-products, involved in the biological reactions. The fourth batch experiments were used to modeling anaerobic digestion kinetics because inoculum effect had been minimised.

The soluble and particulate fractions were separated by centrifugation at 50 000 g, 15 min and 5°C, then by filtration through a cellulose acetate membrane with 0.45 µm pore size. Substrate characterisation was realised on the sludge samples to determine initial variables. Some measurements were performed on total and soluble fractions: COD; proteins were measured according to the Lowry method (Lowry et al., 1951); total sugars were measured with the anthrone reduction method (Dreywood, 1946). Ammonia nitrogen; inorganic carbon and VFA were measured on soluble fraction. Lipids were measured with Soxhlet method and petroleum ether as solvent, on total and particulate fractions.

VFA concentrations were measured by using gas chromatograph (GC-8000 Fisons instrument), equipped with a flame ionisation detector with an automatic sampler AS 800. The internal standard method allowed to measure acetate, propionate, butyrate and iso-butyrate, valerate and iso-valerate concentrations. The composition of biogas was determined with a gas chromatograph (Shimadzu GC-8A), with a CTRI Alltech column, with argon as the

carrier gas, equipped with a thermal conductivity detector and connected to an integrator (Shimadzu C-R8A).

III.3.2.2 Modeling approach

As already mentioned, the IWA ADM1 model was previously modified in order to handle the microbial diversity present in AD reactors. Ten populations were associated to each reaction instead of one as in the standard ADM1 and the model was thus called ADM1_10. Validation was done using experimental data obtained during the treatment of wine distillery wastewater (Rajinikanth et al, 2008).

In the present work, this ADM1_10 was modified with new disintegration/hydrolysis kinetics and Hill function for ammonia inhibition as depicted in Ramirez et al. (2008) for ADM1. This new ADM1_10 is used in the following to analyze the relationship between batch reactor performance and MCS in thermophilic anaerobic digestion of thermally pretreated WAS. This modified ADM1_10 was applied to simulate the behaviour of five batch reactor configurations, at thermophilic temperature (55°C), and fed with untreated sludge and with sludge treated at 110°C, 165°C (electric and steam modes) and 220°C.

Reactor performance was judged by indicators such as BMP and effluent VFAs concentrations. Abundance Biomass Comparison (ABC) curves and Simpson's reciprocal diversity index (Magurran, 2005) for both domains *Bacteria* and *Archaea* were used in order to display changes between communities of different pretreted WAS samples.

In order to quantify microbial diversity, the Simpson's reciprocal index diversity (*D*) was calculated as follows:

$$D = \frac{1}{\sum_{j=1}^{N} p_i^{\,2}}$$

The ratios p_i have been calculated by dividing the biomass concentration of each species in a given family (*Bacteria* and *Archaea*) by the total biomass concentration at a given time instant.

The model formulation involves three disintegration biochemical parameters ($k_{m,Xc}$, $K_{S,Xc}$ and $k_{dec,Xc}$), nine hydrolysis biochemical process parameters ($k_{m,ch}$, $K_{S,ch}$, $k_{dec,ch}$, $k_{m,pr}$, $K_{S,pr}$, $k_{dec,pr}$, $k_{m,li}$, $K_{S,li}$ and $k_{dec,li}$) and four stoichiometric parameter values (Y_{Xc}, Y_{ch}, Y_{pr} and Y_{li}). Corresponding parameters values can be found in Ramirez et al. (2008) where the sixteen parameters were established following a curve-fitting process using experimental data from a batch reactor fed with untreated WAS (model calibration) and experimental data from the other batch reactors run with pretreated WAS were then used to validate the model using the parameters estimated from the first reactor.

III.3.3 *RESULTS AND DISCUSSION*

III.3.3.1 Model validation

Figures III.17 to III.19 show the experimental data over 20 days, together with predicted varying Simpson's reciprocal diversity indices for both domain *Bacteria* and *Archaea*, predicted ABC curves and simulated results, for untreated WAS and pretreated WAS at 165°C (electric mode) and 220°C.

As can be seen, ADM1_10 predictions follow very closely the temporal trends in the measured variables from thermophilic batch reactors. The model predicts even better than the modified ADM1 depicted in (Ramirez et al., 2008) the dynamics of the biogas produced as a response of the pretreatment imposed. Very small deviations in predicting the cumulative biogas production are indeed observed. Of course, it could be said that a better fit could have been obtained but the main purpose of this study was not to perfectly fit these data but to evaluate the ability of the modified ADM1_10 to adequately predict the behavior of this particular digestion process. This agreement validates the modeling approach as well as the sixteen model parameters previously established in (Ramirez et al., 2008).

Acetic, propionic, butyric and valeric acids were commonly produced VFAs during thermophilic acidogenesis of untretated and thermal pretreated WAS. Our findings are in agreement with the results of Liu et al. (2008). They clearly showed that these VFAs were produced jointly from a rich-protein substrat. Besides, the changes in VFA production could also be explained by a population selection according to the type of substrate (Dinopoulou et al., 1988; Bengtsson et al., 2008). For complex types of wastes, high propionic acid

production can indeed be encountered. Propionate persistance is often observed in thermophilic anaerobic digestion (Speece et al., 2006).

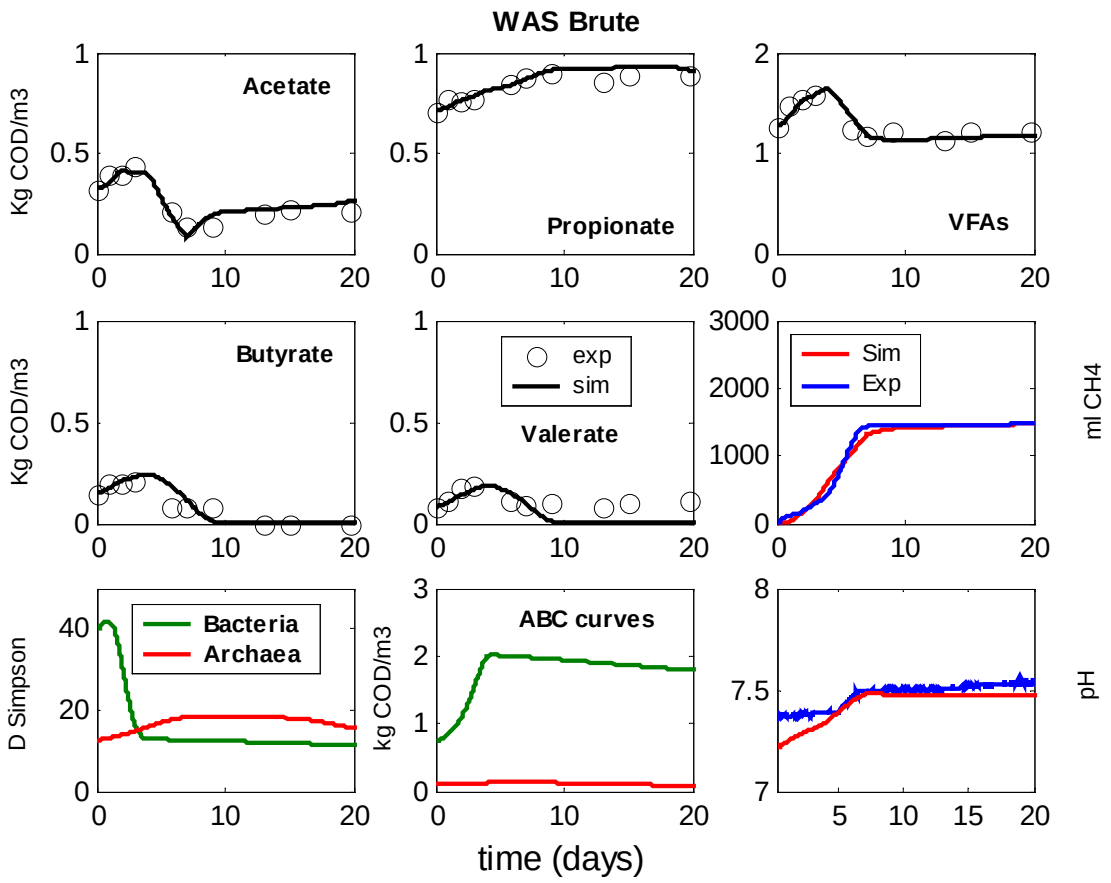

Figure III.17 Simulated pH, individual and total VFAs and cumulative CH_4 production vs experimental data for untreated WAS together with predicted Simpson's reciprocal diversity indices and ABC curves.

Since the propionate level persisted at relatively high levels during all the present experiments, it was suggested that propionate-degrading syntrophs could be present in low concentration in our inoculum. These syntrophs can indeed only use a very limited range of substrate (Schink, 1992) and have very low specific growth rate, so they need an extensive amount of time to reduce propionate concentrations. On the other hand, butyrate/valerate-degrading syntrophs could be present at high concentrations in our inoculum. Thus, while butyrate/valerate was consumed rapidly in the reactors, the propionate accumulated due to its slow conversion into acetate by propionate-degrading syntrophs.

Methane production, the major result of anaerobic digestion, was markedly increased by the 165°C pretreated WAS. Cumulative volumetric methane production values were 284 and

1432 ml CH_4 in the case of untreated WAS after 3 and 7 d, respectively. Cumulative volumetric methane production values were 353 and 1697 ml CH_4 in the case of 165°C (electric mode) pretreated WAS for the same periods, respectively. The differences in the amount of methane produced showed that the impact of the rate-limiting step could be reduced by pretreatment up to a maximum at 165°C, by increasing the avaibility of organic matter. At higher temperatures, the biodegradability decreased sharply.

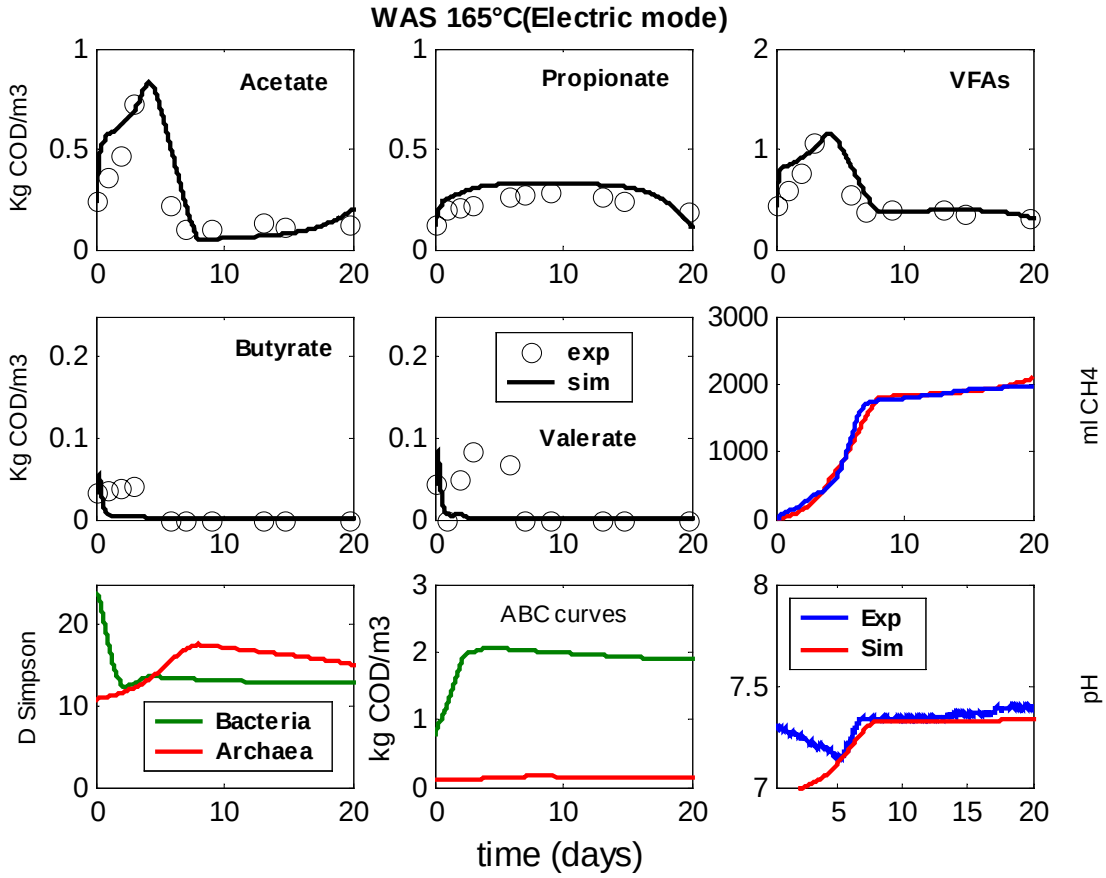

Figure III.18 Simulated pH, individual and total VFAs and cumulative CH_4 production vs experimental data for 165°C pretreated WAS. Predicted Simpson's reciprocal diversity indices and ABC curves.

It was presumed that formation of inhibitory compounds caused decreased biodegradability above 165°C, as suggested by others (Fisher and Swanick, 1977; Bougrier et al., 2007). This results are in agreement with the findings of Stuckey and McCarty (1984). They found that waste activated sludge biodegradability increased with increasing pretreatment temperature up to a maximum at 175°C where an increase in methane production of 27% was noticed.

In comparison to our untreated WAS, the highest enhancement of the cumulative volumetric methane production was achieved by steam mode pretreated WAS at 165°C.

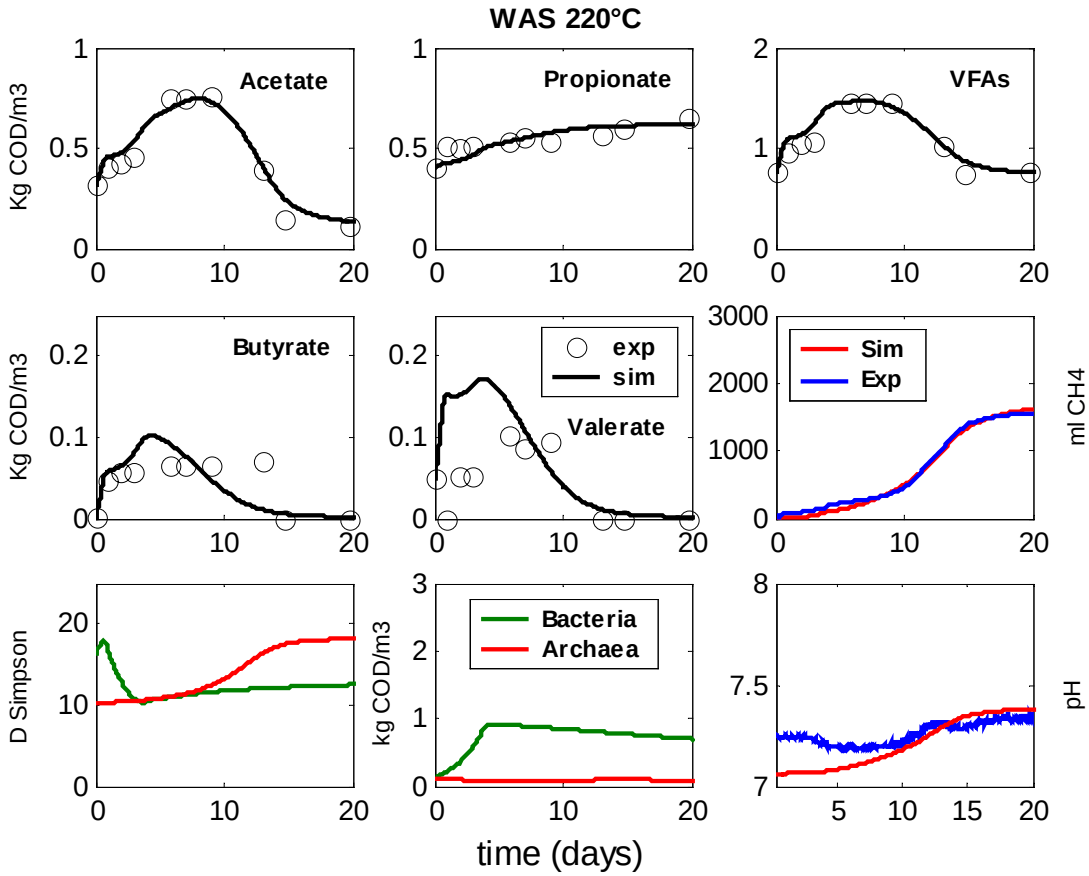

Figure III.19 Simulated pH, individual and total VFAs and cumulative CH_4 production vs experimental data for 220°C pretreated WAS. Predicted Simpson's reciprocal diversity indices and ABC curves.

III.3.3.2 Inoculum's MCS vs reactor performance

In all cases, the S_0/X_0 (substrate/inoculum) ratio is much larger than the value of K_S which, according with Liu *et al.*, (2005), implies that the kinetics observed would represent the maximum capabilities of the members of the microbial community with faster growth kinetics. The values of the main inoculum variables used for the simulations are shown in Table III.3.

Table III.3 Inoculum's characteristics of thefive thermophilic batch reactors.

	Raw Sludge	110°C	165°C(e)	165°C(v)	220°C
Total Biomass (kg COD/m3)	0,8375	1,1823	0,8904	0,7589	0,2249
Bacteria Biomass	0,7245	1,0798	0,7861	0,6424	0,1236
Archaea Biomass	0,1130	0,1025	0,1044	0,1165	0,1013
Diversity index Bacteria	39,4548	33,7743	23,8815	17,6790	16,4051
Diversity index Archaea	12,5568	10,4997	10,8733	13,3125	10,2600
Bacteria/Archaea	3,1421	3,2167	2,1963	1,3280	1,5989
VFA_in (kg COD/m3)	1,2508	0,5260	0,4079	0,2710	0,7669
pH	7,4100	7,4400	7,3100	7,4800	7,2700
V reactor (L)	2,7495	2,8227	2,7189	3,1289	2,7697

As can be noticed, inoculum's anaerobic microbial community structures are different between reactors. Anaerobic microbial communities can be placed into two domains: *Bacteria* and *Archaea.* Both domains are closely linked in the form of syntrophic associations between acetogenic bacteria (*Bacteria*) and methanogens (*Archaea*). As a result, the community structures of these two domains are closely related in an anaerobic ecosystem (Barlaz, 1997).

Performance of anaerobic digestion systems can be related to a number of different environmental parameters, but it is widely accepted that performance, based on BMP (or biodegradabily or volumetric methane production) is related to the microbial community structure (Chynoweth and Pullammanappallil 1996 ; Griffin et al. 1998 ; McMahon et al. 2001). However, changes in community structure may occur without detectable changes in performance (Fernandez et al. 2000). Thus, the link between community structure and performance is unclear, and more studies are needed (Bouallagui et al. 2005). Up to now, research on anaerobic microbial community structure has mainly focussed on the identification of organisms and their presence has been associated to prevailing environmental conditions and/or methane yields (Angenent et al. 2002; Fernandez et al. 1999, 2000; McMahon et al. 2001).

Table III.4 shows both input and output predicted VFAs, cumulative volumetric methane production, BMP and biodegradability of the simulated batch thermophilic anaerobic digestion of the untreated and thermally preteated WAS. The comparison of inoculum's parameters and model's prediction for BMP are summarized in Figure III.20.

As can be seen, inoculum's VFAs were significantly correlated to Simpson's reciprocal diversity index for *Bacteria*, suggesting a link between Simpson's reciprocal diversity index and function for *Bacteria.* This may be explained by VFAs being products of acidogenic

Bacteria and feedstock for acetogenic *Bacteria*. This suggest that different acidogenic *Bacteria* species produced different VFAs and that acetogenic *Bacteria* species differ in their capacity to utilize them. The solubilization and formation of recalcitrant or toxic compounds by Maillard reactions, which act as anaerobic digestion inhibitors, can explain the sudden VFAs increase in the 220°C pretreated WAS case. Simpson's reciprocal diversity index for *Archaea* was also significantly correlated with BMP (or Biodegradability or Cumulative methane production), suggesting a link between Simpson's reciprocal diversity index and function (based in BMP) for *Archaea*. This is due to the fact that methanogens (*Archaea*) species are the main responsible for the production of methane.

Table III.4 VFAs, cumulative volumetric methane production, BMP and Biodegradability of five thermophilic batch reactors.

	Raw Sludge	110°C	165°C(e)	165°C(v)	220°C
VFA_in (kg COD/m3)	1,25	0,53	0,41	0,27	0,77
VFA_out	1,21	0,54	0,31	0,08	0,76
VFA_net	0,04	-0,01	0,10	0,19	0,01
ml CH4	1473,08	1935,35	1966,61	2404,19	1536,05
BMP (ml CH4/g COD_in)	137,99	173,04	183,78	217,94	165,11
BD (%)	39,40	49,40	52,50	62,30	47,20

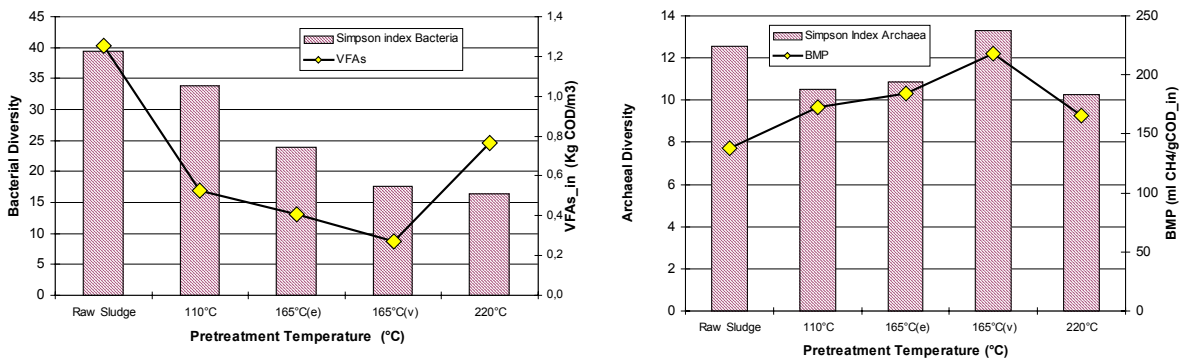

Figure III.20 Relationships between Inoculum's parameters and simulated BMP and VFAs.

The ratio *Archaea* biomass to VFAs in the inoculum are 0.09 and 0.42 for untreated WAS and 165°C (steam mode) respectively. This ratio reflects the concerted activity of acetogenic hydrogen-producing *Bacteria* and methanogenic *Archaea* required for anaerobic degradation of fermentable substrates, thus although archaeal biomass values are similars in both reactors, the amount of VFAs to be degraded in untreated WAS is much greater than in 165°C pretreated WAS. Furthermore, because the metabolic capacity of inoculum's methanogens in

the untreated WAS case was not sufficient to balance initial VFAs, acetate and hydrogen were not consumed at the same rate than the one at which they are produced. In this sense, even though significant levels of methanogens were present in the reactors, they were apparently not able to adjust to operational conditions, as demonstrate by the low levels in the methane produced. This could explain why, in the untretaed WAS reactor, the cumulative volumetric methane production is the smallest desspite its high Simpson's reciprocal diversity index value. These results corroborate the importance of obtaining a stable methanogenic community of archaea balanced to the bacterial community in order to maximize methane production and the substrate degradation.

Distribution of net VFA production and biomass composition is given in Figure III.21. The substrate was mainly composed of particulate and soluble macromolecules, that could explain a better development of *Bacteria* biomass against *Archaea* biomass in the reactor. Net VFA production (VFA_net) is the difference between influent and reactor effluent VFA samples at the end of the BMPs. In all cases, the biomass is mainly composed of *Bacteria*. Simpson's reciprocal diversity indices for *Archaea* were higher as the higher the inoculum's net VFAs concentration. This relationship reflects the related activity of acetogenic hydrogen-producing *Bacteria* and methanogen *Archaea* required for anaerobic degradation of fermentable substrates. Since the average growth rate of the methanogens is much lower than that of acetogens, the overall rate of the biomethanation process is therefore controlled by the methanogenic step. At low methanogenenic activity, the unstable response of anaerobic system is due to the reduced use by methanogens of acetic acid and H_2 generated by fermenting populations, causing accumulation of VFAs and a sharp decrease in the pH.

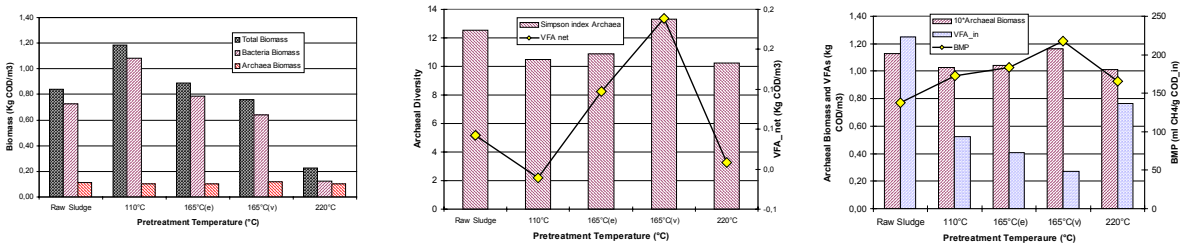

Figure III.21 Inoculum's composition and relationship between *Archaea* diversity-VFA_net.

III.3.3.3 <u>MCS vs reactor performance : Start-up and routine operation.</u>

In the following, the start-up will be denoted as the period during wich the methanogenesis step is initiated and the end of start-up is the beginning of routine operation period.

In all reactors, ABC curves show that *Archaea* biomass is stable over time whereas *Bacteria* biomass increases during start-up. However, diversity indices plot show that *Archaea* diversity increases while *Bacteria* diversity decreases. From our results, coexistence of the highest diversity in the *Bacteria* domain with the lowest diversity in the *Archaea* domain or vice versa argues against the notion that increased diversity at one trophic level necessarily favors increased diversity for a functionally linked trophic level. The observed decrease in *Bacteria* diversity suggests that the *Bacteria* community becomes specialized in degrading less deversified substrates through the complete process. Similar results were also reported by Briones et al., (2007). They found a clear trend towards increasing specialization of *Bacteria* involved in digesting swine manure. Moreover, the microbial community involved in a 30 days anaerobic digestion of two agricultural substrates (beets and grass) and its dynamics in a two-stage biogas production from energy crops was studied by Cirne et al., (2007) using FISH analysis. They found that *Archaea* started to appear in the hydrolytic stage between days 10 and 15 and that the fraction of *Bacteria* decreased accordingly.

If we consider the metabolic pathway of anaerobic digestion, this degradation can be divided into 5 steps: disintegration, hydrolysis, acidogenesis, acetogenesis and finally methanogenesis. The *Bacteria* microflora is responsible for the first four steps of the reaction while methanogenesis is performed by *Archaea*. During these first steps, *Bacteria* micro-organisms have to degrade a large panel of substrates – moreover, in our case, with high concentration of particulate organic matter. This variety of potential substrates can easily explain the large diversity of *Bacteria* organisms and also their versatility during the start-up phase. The hydrolytic and fermentative species indeed play a crucial role in the initial breakdown of the influent feed with the resulting by-products utilised by the underlying *Bacteria* (McLeod et al., 1990). These organisms have to develop specific degradation aptitudes in order to outcompete other *Bacteria* and to survive in this ecosystem which is always in equilibrium. Furthermore, during start-up, *Archaea* are less diversified than *Bacteria*. These results can be explained by the link between the variety of substrates and the biodiversity. The metabolic role of *Archaea* in the anaerobic digestion reaction is the last step of degradation (i.e., methanogenesis). Methanosarcinales are responsible for acetate degradation into methane whereas Methanomicrobiales and Methanobacteriales transform

hydrogen and carbon dioxide into methane. The reduced range of potential substrates induces a lower level of competition between micro-organisms leading to a more limited *Archaea* diversity.

On the basis of the patterns simulated, we can conclude that during routine operation, *Archaea* species were the most dominant group with a slight decrease at the end of the experiment. Decreases in the amount of both *Bacteria* and *Archaea* biomass could be attributed to reactor operation. The proportion between the bacterial species in the reactor treating thermally pretreated WAS was changed towards a reduced abundance of several representants, indicating that a limited number of *Bacteria* were present in the thermophilic reactors in the last stages of the digestion process.

Figures III.22 and III.23 display the *Archaea* diversity/cumulative volumetric methane production plot and *Bacteria* diversity/VFAs plot, respectively. These plots show clearly the link between community structure and function during routine operations. VFAs concentration was modulated by changes in Simpson's reciprocal diversity index for *Bacteria*, and cumulative volumetric methane production was modulated by changes in Simpson diversity index for *Archaea*. These last finding are similar with those obtained by Montero et al., (2008), altough they were obtained with different type of reactors treating other kinds of feedstock.

Once the VFAs degradation ends up (mainly acetate because the propionate accumulates), the cumulative volumetric methane production maximizes. Furthermore, the time at at which the Simpson's reciprocal diversity index for *Archaea* and the cumulative volumetric methane production maximizes are identical. It is important to notice that the time at which VFAs maximizes agrees with the time when the Simpson's reciprocal diversity index for *Bacteria* stabilizes. In the case of 220°C pretreated WAS (Figure III.19), the further increases in *Bacteria* diversity responds to slow VFAs degradation.

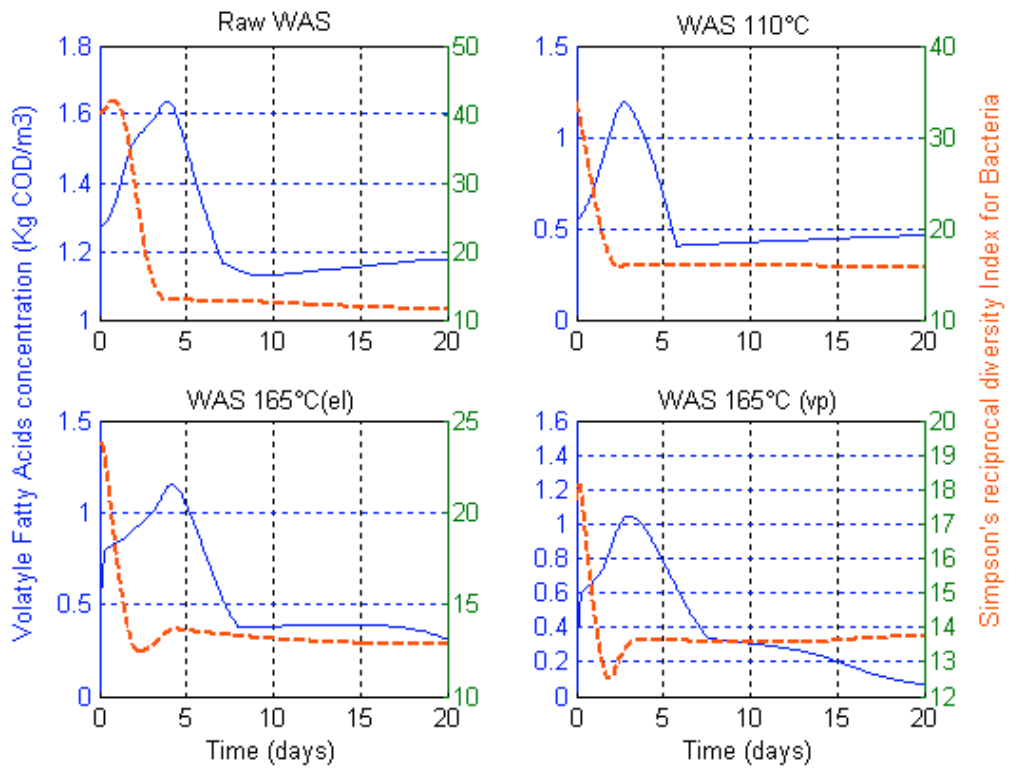

Figure III.22 *Bacterial* diversity (red dash line) vs VFA concentrations (blue continuous line) for untreated WAS and thermally 110°C and 165°C (both modes) pretreated WAS.

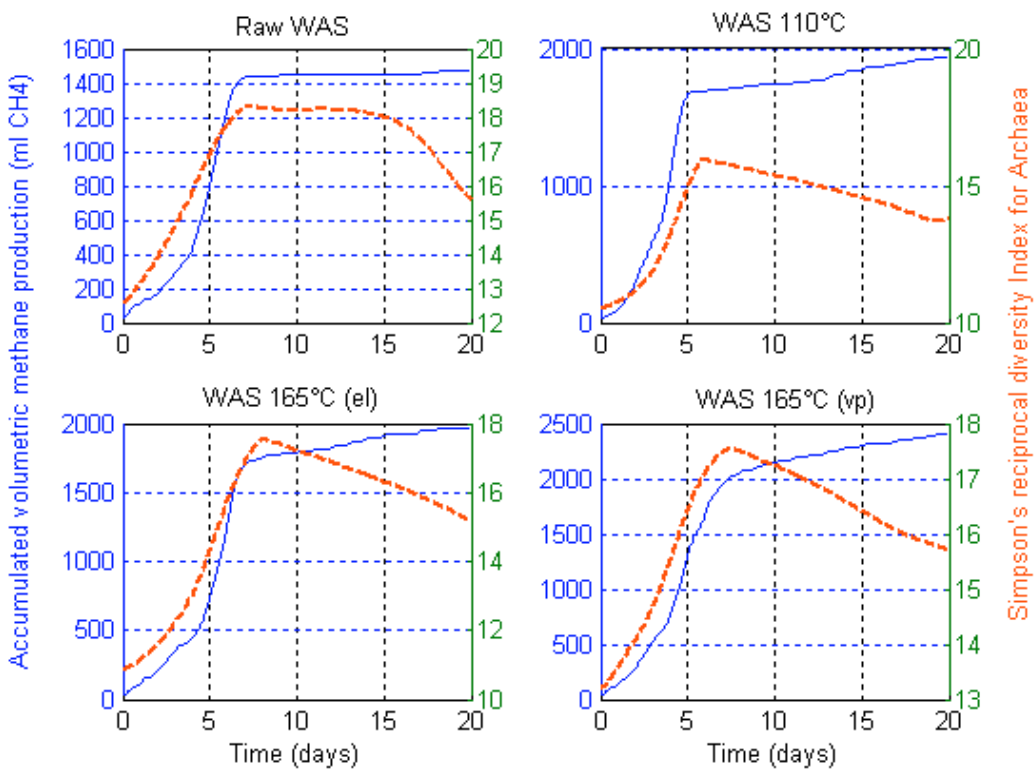

Figure III.23 Archaeal diversity (red dash line) vs VFAs concentration (blue continuous line) for untreated WAS and thermally 110°C and 165°C (both modes) pretreated WAS.

One of the recognized disadvantages of anaerobic treatment is the long start-up when inoculum with adequate microbial composition is not available. However, reports of reactors that started up successfully in the absence of highly active biomass (see e.g. Seghezzo et al., 1998) confirm that the actual effect of the type and quantity of the seed on the initial performance of anaerobic biological reactors has not been elucidated. Although the complexity of an open system precludes a rigorous quantitative analysis of microbial diversity (Godon et al., 1997), our work indicates that important changes in the structure of the microbial community may take place during the establishment of a functionally competent ecosystem. A decrease in *Bacterial* diversity during the reactor start-up suggests that the initial inoculum determines the structure of the microbial composition at later steps of operation. On the other hand, consistent performance can be achieved with a poor dynamic microbial structure on specific taxon at the *Archaeal* level. Therefore, these results do support the idea that many anaerobic thermally pretreated WAS systems fail due to the lack of an adequate inoculum.

In all cases, the Simpson's reciprocal diversity indices for both *Bacteria* and *Archaea* in steady state were low, which means that diversity per se may not be important for the ability to degrade complex substrates. Rather the structure of the community is of greater importance, although different community structures can result in similar methane productions. It has been shown by Haruta *et al.*,(2002) that a functionally stable system can be maintained by low diversity of microorganisms. This indicates that community diversity may not be as important for function as community flexibility, if a single species has the capacity to adapt to sub-optimal conditions; this can be as effective as the presence of many species with low adaptation capacity (Casserley and Erijman., 2003).

III.3.4 *CONCLUSIONS*

A modified ADM1_10 model for thermophilic anaerobic digestion of pretreated WAS was validated using batch experimental data. Predictions by the model using the parameters established in a previous study (Ramirez et al., 2008) agreed well with the data measured under different preteatment conditions. Relationships between MCS and reactor performance of anaerobic digestion of WAS with and without thermal pretreatment was investigated in a thermophilic batch process. The performances of the untreated and pretreated reactors were

followed during 20 days. Total and individual VFAs, pH, accumulated volumetric methane and carbon dioxide at the applied temperatures were monitored. The cumulative volumetric methane production was found to be the highest at 165°C vapour mode WAS reactor. With regard to the VFA levels, except for the previous reactor, all remining reactors exhibited significant and very similar concentrations of VFA in the effluents, with acetate and propionate being the most abundant acids. Finding of accumulated acids indicates a limited capability of the VFA-degrading microorganisms to play an active role in the methanogenesis.

The diversity of the *Bacterial* community was higher at the start-up of digestion than during routine operation where the maintenance of batch conditions seems to have caused the emergence of a few dominant microorganisms. This selection of a few *Bacterial* populations, compared to *Archaeal* populations which maintain higher microbial diversity, may result from batch conditions. A decrease in Simpson's reciprocal diversity index during the reactor start-up suggests that the initial inoculum is a key factor influencing the structure of the microbial composition at later steps of operation.

In all reactors, during the digestion, the microbial community structures present in the reactors in both domains *Bacteria* and *Archaea* changed over the time as consequence of the changes in the environmental conditions, such as substrate availability and/or pH. Up to a temperature of 165°C, changes in the structure and activity of the microbial community of the thermophilic batch treating thermally pre-treated WAS occurred and, under the operational conditions applied, the microbial community succeeded in maintaining the increased flow of carbon into methane as was reflected by the improved reactor performance.

The findings from the present study should be considered as a first step towards the development of strategies to further simulate hydrolysis and ultimately, to stimulate the methane production rates and yields from thermophilic anaerobic digestion of thermally pretretad WAS.

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CHAPITRE IV

DISCUSSION,
CONCLUSIONS GÉNÉRALES
ET PERSPECTIVES

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IV.1 DISCUSSION AND GENERAL CONCLUSIONS

The successful application of biological treatment to a wide variety of problems from domestic wastewater to acidic minewaters represents a tremendous achievement. This achievement is all the more remarkable because we have so little understanding of how such systems actually work. There has been therefore an understandable optimism and excitement that the application of new molecular tools might significantly improve design. However, these new tools have had relatively little impact in practice. Nevertheless, we believe that the power of these methods will not be realized in design until designers can call upon a complementary new generation in environmental engineering theory.

Traditional design of engineered biological treatment systems uses mass-balance concepts and Monod kinetics or rules of thumb (empirically derived loading rates) to ensure that a particular function is present in a particular reactor. In very basic terms, the designer ensures that, should an appropriate organism be present, the rate of growth of that organism will exceed the rate of loss. The approach often accurately predicts the biomass (and thus related engineering variables) because it is ultimately grounded in the thermodynamics of the process. However, these tools will not always predict the engineered reality. For example, they will not predict if a function will or will not appear in an engineered biological system and thus acclimatization, though vital, is a mystery. We have no way of knowing a priori if a system will acclimatize or not or how long the acclimatization will take and how easily it will be lost or what range of chemicals the system can be acclimatized to. The inherent "robustness" of the system is simply unknown, and certainly unpredictable, and thus a system's ability to resist to inhibition or shock loads or varying loads must typically be ascertained experimentally. Consequently, pilot plant work must precede all major process innovations and research is based on a deep seated culture of empiricism (though there are exceptions, see for example (Kreft et al., 2001)) and we are unable to transcend experience.

These deficiencies arise because, despite our ability to determine the volume of a reactor, we still cannot predict its microbial composition or community structure. Yet, it is the composition (i.e., the kind of micro-organisms present in the system) which ultimately defines many of its operational characteristics. It is clear that the development of a model to predict the composition or community structure would find wide range of application. A successful

stochastic approach would obviate the requirement for a detailed characterization of individual species that would be required to make a deterministic predictive model work. In ADM1_10, the only default ADM1 parameters needed are the yield coefficient Y, Monod maximum specific uptake rate μ_{max} and half saturation constant K_s, for each biomass. These three parameters are all we need to determine if a particular species arriving, the abundance it would attain, how long it would take to attain it and its stability within the system (for fuel cell work and all other questions of adaptation). Functional redundancy and thus system stability would be assessed by determining how many members of a functional group would survive.

This work developed a stochastic model in which data on micro-scale molecular diversity have been incorporated to more closely represent anaerobic wastewater treatment processes. The model, as it stands, offers significant insights into the development of wastewater treatment communities and highlights the important technical and theoretical developments that will be required. This includes the successful demonstration of the process of adaptation and a proper quantitative understanding of the relationship between reactor performance and microbial community structure. These developments could take much of the uncertainty out of the design of robust biological treatment systems and could possibly give environmental engineers wholly new technical abilities. In a later stage, this model can also be used to develop efficient control strategies adapted to model-based population optimization. For details about the suitability of a parameter as process state parameter we suggest the reader adressing to annexe II.

In chapter one, a bibliographical review about the diversity-stability debate and anaerobic digestion modeling was done. With regard to the diversity-stability debate, experiments have found that the positive diversity–stability correlation is not a pure species effect (that is, a diversity effect) and have indicated that ecosystem function and stability are more directly related to functional diversity. Population-level variation is relatively uninfluenced by diversity, whereas community-level variance tends to decrease with increased diversity.

Two main ideas have been advanced to explain of these findings. One is that increasing diversity increases the odds that at least some species will respond differentially to variable conditions and perturbations. The second is that greater diversity increases the odds that an ecosystem has functional redundancy by containing species that are capable of functionally

replacing important species. Within an ecosystem, diversity tends to be correlated positively with ecosystem stability. This correlation does not necessarily extend to population-level stability.

Much work is still required to determine the driver of the positive diversity–stability relationship. However, it seems that community level stability is dependent on the differential response of species or functional groups to variable conditions, as well as the functional redundancy of species that have important stabilizing roles. In an important theoretical contribution, Chesson and Huntley (1997) showed that diversity cannot be maintained by variation alone. Rather, maintenance of diversity requires the two following components: the existence of fluxes or variability in ecosystems; and populations capable of differentially exploiting these fluxes or variability. Regardless of the source of the variability (for example, whether spatially or temporally generated), their results indicate that coexistence requires that populations must be released, either directly or indirectly, from the limiting influences of species interactions such as predation and competition. Species interactions, therefore, must be important in maintaining and promoting persistence in diverse communities in spite of, and perhaps because of, the variability that underlines ecosystems.

Taken together, recent advances indicate that diversity can be expected, on average, to give rise to ecosystem stability. The evidence also indicates that diversity is not the driver of this relationship. Rather, ecosystem stability depends on the ability for communities to contain species, or functional groups, that are capable of differential response.

The ability to better define the behavior of influent substrate fractions and biomass fractions and their reaction stoichiometry and kinetics together with the power of the modern computer have made it possible to develop sophisticated dynamics models for the design, analysis, and optimization of complex biological treatment plants. The work of the International Water Association task group on Anaerobic Digestion Modeling is an excellent and continuous achievement of such modeling efforts that has resulted in the development, commercialization and widespread use of software packages such as WEST from the company MOSTforWATER in Belgium and SIMBA from Ifak System in Germany, among others. Publication of the ADM1 has largely addressed its primary objective of reducing duplicate published model structures. Indeed, no references to new, complex structural models were found in the last years. Of the four core limitations – regulation of glucose fermentation

products; physicochemical system modeling; input characterization and parameter variation – the last two are being well addressed by subsequent publications. Additional work is needed on physicochemical system modeling, particularly precipitates and specifically phosphorous modeling. However, the key limitation of glucose fermentation products needs model development and verification, given the high level of interest in carbohydrate fermentation technologies for hydrogen production, although this has decreased in importance due to possibility of thermally and electrochemically assisted hydrogen production directly from glucose and acetate.

In particular, Bio-Electrochemical Systems (BESs) have recently emerged as an exciting technology. In BESs, bacteria interact with electrodes using electrons, which are either removed or supplied through an electrical circuit. The most-described type of BESs is microbial fuel cells (MFCs) in wich useful power is generated from electron donors as, for example, those present in wastewater. This form of charge transport, known as extracellular electron transfer, was previously extensively described with respect to metals such as iron and manganese. In the near future, BESs will become an important tool in filling knowledge gaps in extracellular electrons transfer by microorganisms. BESs indeed allow a unique control mechanism on activity and redox potential in the bacterial environment. In the long term, the bio-electrochemical approach will strongly facilitate advanced interpretation of bio-geochemical cycles and the influence of changing environmental and antropogenic factors on the bacteria driving them.

One important step when we using a model like ADM1 is the selection of initial conditions because these need to guarantee the convergence of solutions of differential equations that compose the model. Another important criteria that we considered in this selection in each one of the simulations done in this work are:

- The initial state variables related to VFAs ionic forms must be calculated from their respective VFAs total forms using acid-base equilibrium equations.

- In all cases the initial anion concentration : San is equal to initial inorganic nitrogen concentration : S_{IN} (Rosen, C and Jeppsson, U., 2006).

- The initial cation concentration : *Scat* is based on the initial reactor pH as already mentioned in chapters II and III.

ADM1 is an excellent tool for both researchers and practitioners, which is able to simulate many different situations faced experimentally. However, ADM1 does not distinguish between microorganisms performing the same reaction, and can therefore not adequately represent or predict experimental results concerning this type of interspecies diversity, i.e., ADM1 cannot represent adequately experimental results that apparently seem to be obtained in similar conditions even though being driven by different microbial diversities present during the experiments.

A credible model for the prediction of community structure will allow us to explain how that composition will vary with environmental condition, because is the kind of microorganism that are present in the system (community structure) which ultimately defines many of its operational characteristics. So it is clear that the development of a model able to predict the composition or community structure would find wide applications.

ADM1_10 is a modeling approach we developed to account for microbial diversity in complex models such as ADM1. Chapter II & III present an overview of this model, its structure and assumptions and defines the important model parameters. It also contain the way to modeling the effect of non reactive toxicant affecting all species. It was emphasized that adding a stochastic component on top of the mass balance structure of the well defined and well structured model such as ADM1, could be a powerful approach to represent experimental data, both in normal and abnormal situations.

Experimental data from two different reactors (pilot scale up-flow anaerobic fixed bed and laboratory scale hybrid up-flow anaerobic sludge-filter bed reactors) treating wine distillery wastewater under different dynamic input regimes were provided to demonstrate the applicability of this approach. With regard to models behavior under pulse toxicant, simulations results demonstrated that ADM1_10 had higher robustness to the presence of toxicant than ADM1. The effect of the toxicant was indeed less pronounced in ADM1_10 than ADM1 and recovery was faster once the toxicant disappeared.

With regard to modeling the effect of non reactive toxicant affecting only methanogens group, discused in section III.2.3.2 : ADM1 vs ADM1_10 when facing a toxicant (TAN pulse), we concluded that the low diversity group is less resistant and recovers faster than the high diversity group. However, a question can arises : What happens if the toxicant pulse is applied before or after of the time in wich the reactor reached its steady state?

To analyze the model sensibiliy to the time of application of FAN pulse, other ADM1_10 simulations have been done with two identical pulses applied before and after day 150 as in Figure III.12. Simulation results are presented in Figure IV.1.

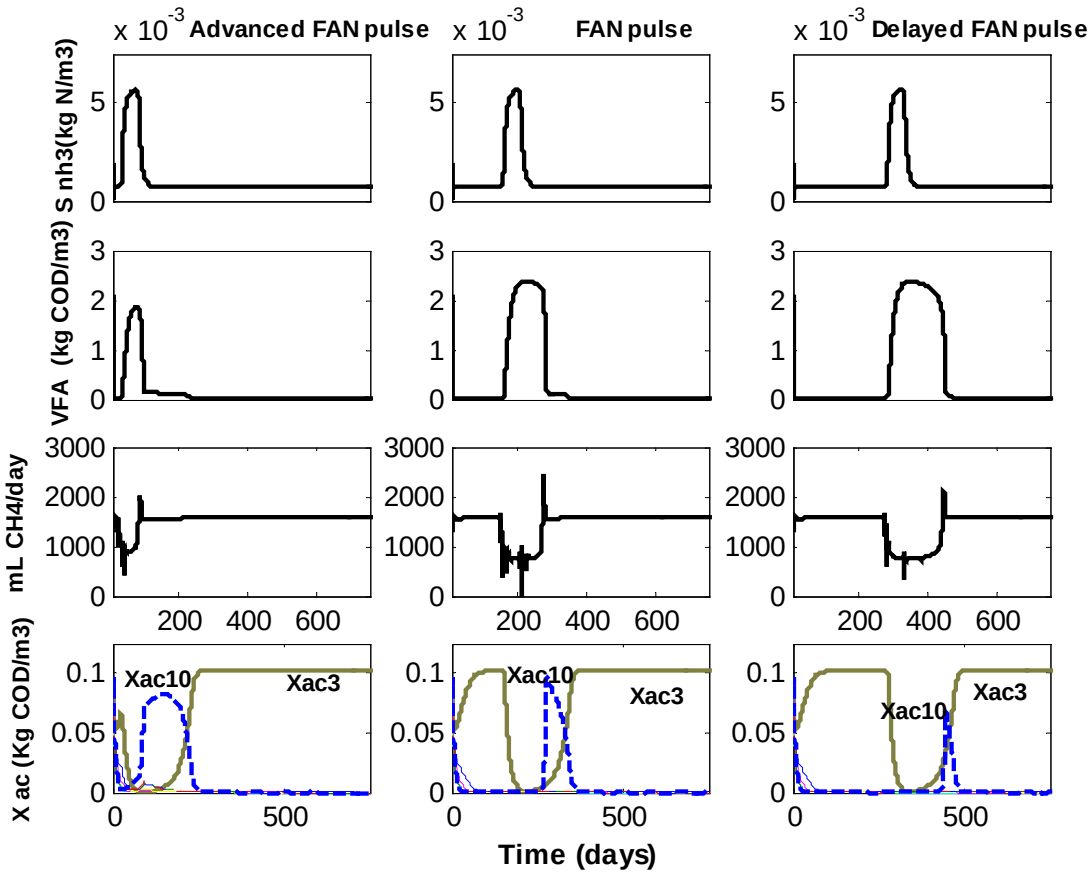


Figure IV.1 Model sensibility towards time of application of FAN pulse.

As it is seen both resilience and resistance are higher in the advanced FAN pulse than in the delayed one, however once the perturbation desapear the steady state values are the same. This is related to the concentration value of resistant acetoclastic mathanogens i.e. in the advanced FAN pulse case the acetate degrader concentration is higher than in the delayed one, altough the selected species in all cases is the same. However, from both cases the finding is

the same : low diversity group is less resistant and recovers faster than the high diversity group.

It was accentuated that the distribution type and their parameters values could be found following a curve-fitting process using experimental data from each reactor type i.e., with the experimental data and the model, we first select the distribution type (among normal, uniform or unimodal, bimodal), next their parameters (mean and standard deviation). We run the simulations and if the model does not fit experimental data, we change distribution parameters in first instance. If the misalignment persists, we change modal type and in last trial, we change the distribution type. As illustration Figure IV.2 displays the changes in some reactor performance parameters with the distribution type and Figure IV.3 shows the changes in some microbial community structure parameters with the distribution type. All other parameters used for these simulations are the same than thoseused in the hybrid up-flow anaerobic sludge-filter bed reactor described in Chapter II.

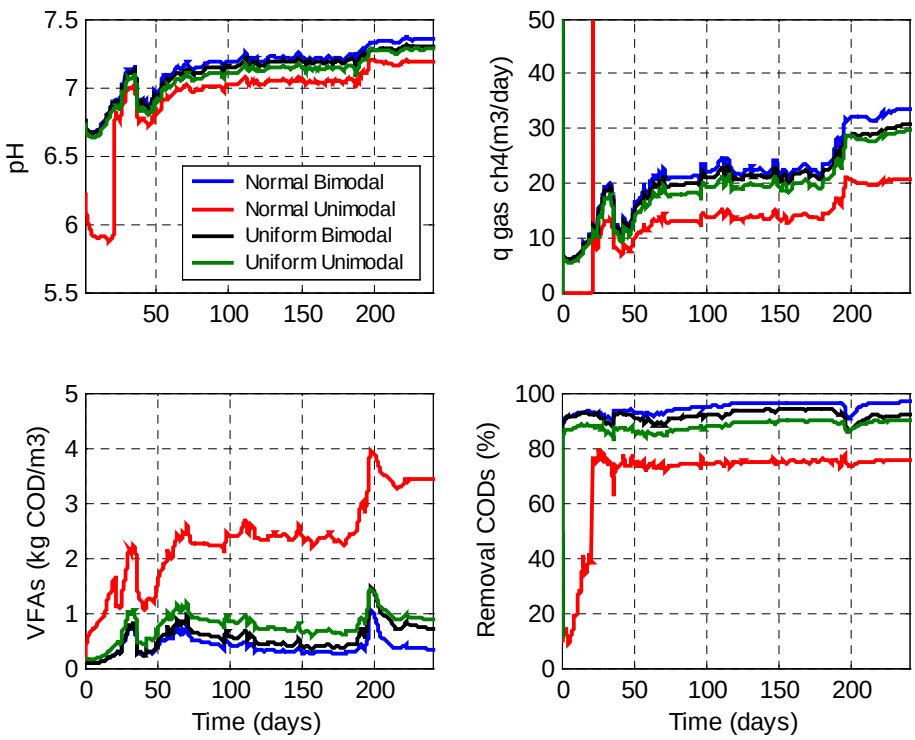


Figure IV.2 Changes in some reactor's performance parameters with the distribution type.

These plots are guidelines useful for model calibration because if too low VFAs are simulated for example, one needs to choose (after "playing" with some kinetic parameters) uniform

bimodal or unimodal distribution. However, as can be seen in Figure IV.3, at the beginning pH inhibition is present for aceticlastic methanogenesis in normal unimodal distribution case.

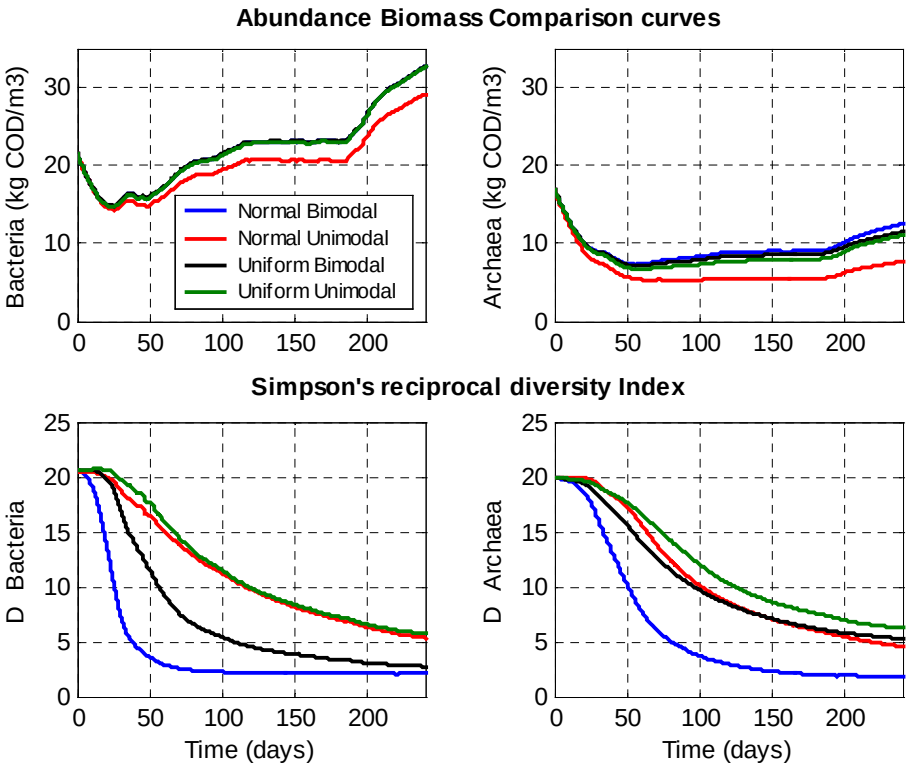


Figure IV.3 Changes in microbial community structure parameters with the distribution type.

As already mentioned in chapters II and III within each functional group, the kinetic parameters k_m and K_s, were randomly chosen from a normal bimodal distribution, with means of $\mu_1 = 0.6 * k$, $\mu_2 = 1.4 * k$, and standard deviations of $\sigma_{1,2} = 0.125 * k$ where k is the value of the corresponding standard ADM1 parameter. To analyze the sensibility of the proposed model towards chosen means, another two simulations were done. In the first one, the mean values were extended to $\mu_1 = 0.2 * k$ and $\mu_2 = 1.8 * k$, and in the second one, we chose a normal multimodale distribution with means $\mu_1 = 0.2 * k$, $\mu_2 = 0.6 * k$, $\mu_3 = 1.4 * k$ and $\mu_4 = 1.8 * k$. In the following, these models will be called "Extended Bimodal" and "Multimodal" respectively. Standard deviations were kept identical in all simulations, to avoid negative kinetic parameters values.

Figure IV.4 shows specific growth rates (Monod curves) characterizing both groups of acetate degrading biomass, where the different μ-strategists groups and K-strategists groups are indicated as legends. The $K_{I,NH3}$ values for all distributions are summaried in table IV.1.

Table IV.1 Inhibition constant values for acetate degraders

No species	Bimodal	Extended Bimodal	Multimodal
1	0.0013	0.0012	0.0012
2	0.0014	0.0012	0.0010
3	0.0008	0.0012	0.0013
4	0.0015	0.0008	0.0012
5	0.0011	0.0009	0.0006
6	0.0025	0.0024	0.0026
7	0.0029	0.0023	0.0026
8	0.0027	0.0025	0.0024
9	0.0024	0.0027	0.0025
10	0.0025	0.0028	0.0024

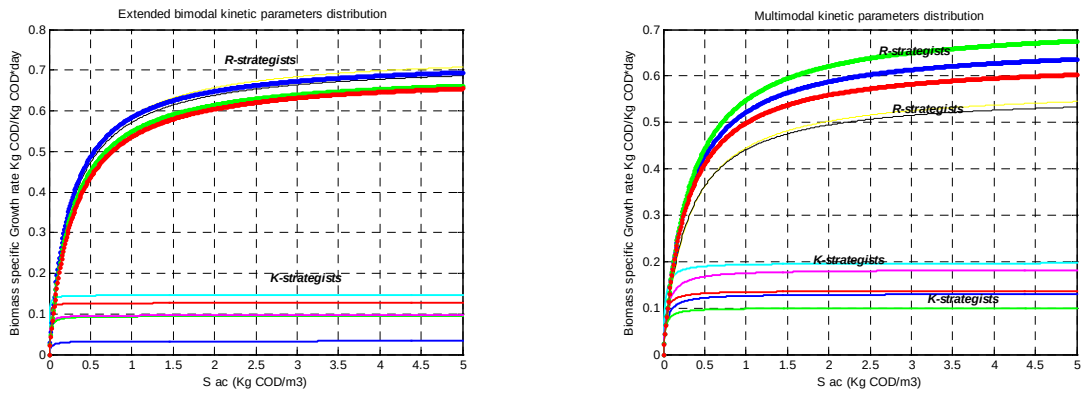


Figure IV.4 Specific growth rates for both groups of acetate degrading biomass distributions.

Figures IV.5 displays simulation results for reactor performance, dynamic evolution of acetate degraders and Simpson's diversity index for Bacteria and archaea.

As it is seen the microbial community archaea diversity from the new groups (ADM1_10 with extended mean values and multimodale) is higher than for old group (ADM_10 with Bimodal values) as reflcted through simpson's diversity index (Figure IV.5.b) and the news high diversity groups displayed more resistance (less accumulation of VFA, Figure)and recover faster (more resilience) than the low diversity group, maybe attributing to selection of more resistante acetoclastic methanogens. In fact, in the bimodal distribution case (Figure IV.5.c) there was a population shift induced by the ammonia pulse: whereas species 1 is initially dominating, it is replaced with species 8, which is less inhibited by ammonium (higher $K_{I,NH3}$ value, see Table IV.1); once the ammonium concentration has decreased again, species 1

again wins the competition. However, in the other two cases (Extended bimodal and Multimodal distributions) there was not a population shift. Mmoreover the species with higher $K_{I,NH3}$ value i.e. the species less inhibited by ammonium are dominating during all the simulation time.

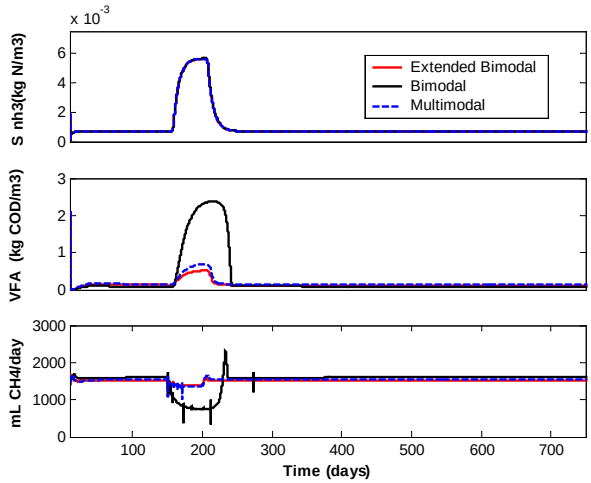
Summarizing, in chapter III, ADM1_10 was used to assess the relationships between reactor performance and microbial community structure by means of different case studies. Traditional reactor performance parameters such as Methane Production Rate (MPR), VFAs concentration and removal soluble COD were used to evaluate reactor's performance. Abundance Biomass Comparison (ABC) curves and Simpson's reciprocal diversity index for both *Bacteria* and *Archaea* domains, were used to describe the microbial community structure. To measure functional stability, we adopted parameters described in ecology in terms of the amplification envelope of key intermediate products in response to a perturbation. The two main parameters obtained from this envelope are resistance and resilience.

In a world that conforms to the model assumptions, some important findings of this chapter can be summarized as follows:

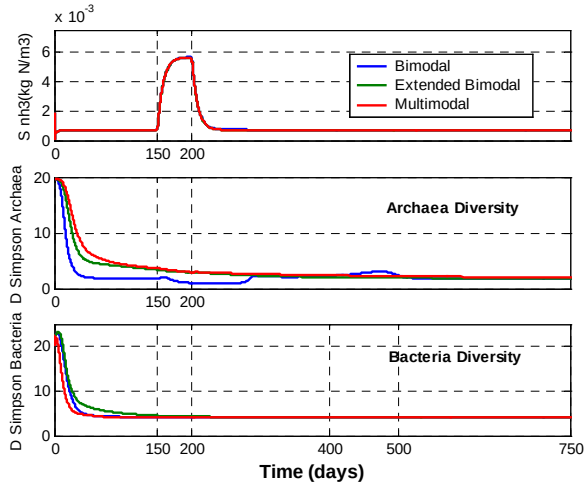
a) *Coexistence of the highest diversity in the Bacteria domain with the lowest diversity in the Archaea domain argues against the notion that increased diversity at one trophic level necessarily favors increased diversity for a functionally linked trophic level.*

b) *Different process conditions favour the selection of different types of bacteria, modifying the microbial diversity and consequently the behaviour of the ecosystem.*

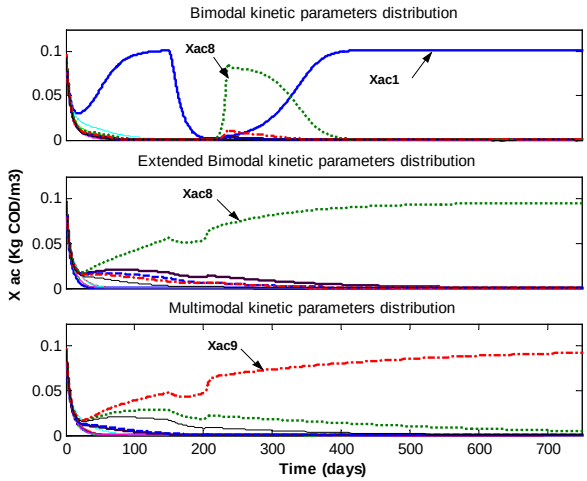
This was demonstrated in nitrification and anaerobic digestion processes. In the former case two Inverse Turbulent Bed Reactors (ITBRs) with different solid hold-up ratio were studied. The two ITBRs showed a different nitrifying performance, both from a macroscopic and microbiological point of view. The reactors' solid hold-up was the only operating parameter different between both reactors and it acted upon nitrifying activity and on the major ammonium oxidizer present.



(a)



(b)



(c)

Figure IV.5 Simulation results for reactor performance, Simpson's diversity index for Bacteria and archaea and dynamic evolution of acetate degraders. In both cases: Extended Bimodal and Multimodal distributions, a FAN pulse was applied at day 150 as in Figure III.12

In the later case, the response of two AD CSTRs with identical inoculums was simulated for constant and pulsed organic loading rate (OLR) operation, in such a way that the average organic loading rates for the perturbation cycle for both reactors were equal. The input OLR profile was the only operating parameter different between both reactors and it acted upon microbial community. In fact, pulsed OLR reactor with high diversity and less stable microbial community displays a better performance than the reactor with constant OLR. In other words, the less stable community was correlated to the more stable function. In this case, the less stable community was one that displayed the greater temporal variations of *Bacteria* and *Archaea* populations in response to substrate shocks.

c) *The biodiversity acts as insurance for CSTR functions (buffer) against temporal changes in environmental factors like pulsed toxicant.*

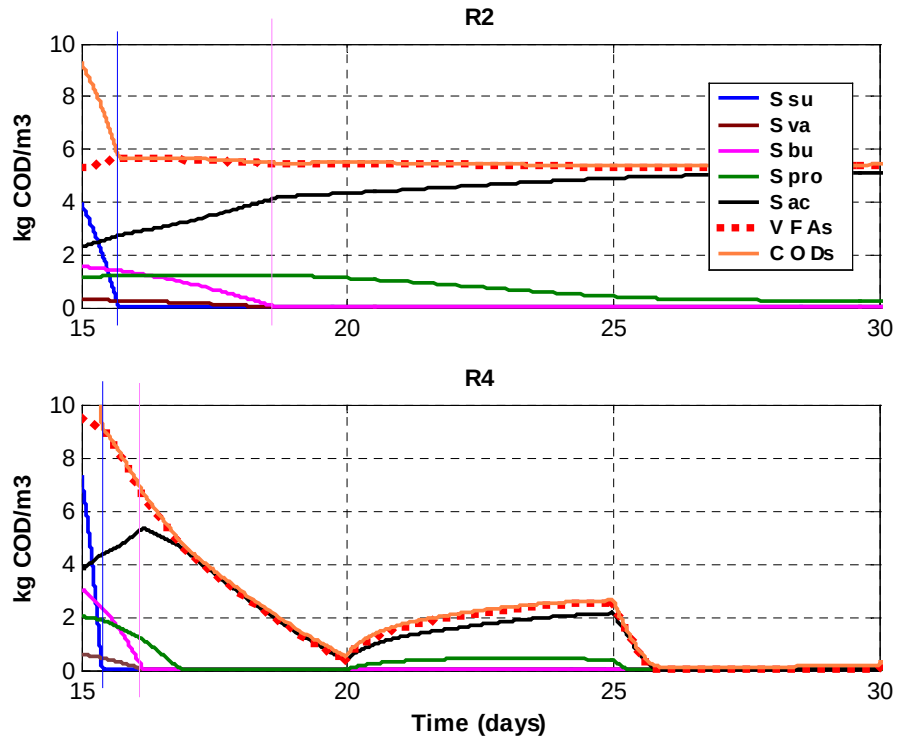
This was demonstrated through of the study of two reactors with different biodiversity under pulsed toxicant that affect all trophic levels or a specific trophic level such as methanogens. In both cases, the simulation results indicated that the reactor with a less stable community but with higher diversity was more functionally stable towards pulsed toxicant disturbances. In other words, the reactor with higher biodiversity displayed more resistance (less accumulation of VFA and CODs) and less resilience in response to toxicant shocks (reflected by a larger recovery period). The later finding is in agreement with Petchey and Gaston (2009). They found that variation in species richness and in functional diversity have a opposite effects on resiliencie.

In the first section of chapter III, we analyzed the behavior of two reactors with different biodiversity (R2: with constant loading rate operation and less diverse and R4: with pulsed loading rate and more diverse) under pulsed toxicant affecting all trophic levels. The main difference between these communities was that R4 microbial community was able to return to the pre-toxicant conditions, while this was not the case for the one in R2. The rapid accumulation of VFAs in both reactors reflected an apparent decrease in the activity of acetate-utilizing methanogens and acetogens. Fermentative bacteria were also affected by the toxicant substrate perturbation. The H_2-utilizing methanogens appeared to be less affected by the substrate perturbation; furthermore no significant accumulation of H_2 was observed during the entire experiment.

As we said, for a complete conversion of carbohydrates into methane, five groups of bacteria are required: acidogenic bacteria, propionate- and butyrate-utilizing acetogens, and H_2- and acetate-utilizing methanogens. These bacteria must work syntrophically, as they are linked physiologically, kinetically, and thermodynamically. Sudden environment changes can cause changes in individual groups that eventually affect the whole microbial community. Changes in the concentrations of intermediate volatile fatty acids observed in these simulations indicate that all the major groups were impacted by the toxicant perturbation. A change in the fermentation pattern was accompanied by a shift in the predominant microbial populations. Furthermore, a sugar accumulation pointed out that the fermentative bacteria were also affected by the toxic substrate perturbation. The most evident sign of this was the dramatic change in the products of sugar fermentation. For sugar fermentation under methanogenic conditions, there are two major complementary fermentation routes. Butyrate-type fermentations are characterized by production of acetate, butyrate, carbon dioxide, and hydrogen as the main fermentation products. The propionate fermentation route is characterized by the formation of acetate, propionate, and carbon dioxide, with much lower hydrogen concentrations. Under normal conditions (pH 7 and temperature 35°C), the butyrate-type fermentation is thermodynamically more favorable than propionate-type fermentation. Normally, the butyrate fermentation route is predominant in anaerobic reactors. This is indirectly supported by observations that anaerobic systems usually have limited ability to degrade propionate, but can metabolize butyrate at relatively high rates. This capability was also observed in reactors prior to the start of the toxicant substrate perturbation. After the toxicant perturbation period, sugar fermentation quickly shifted from the butyrate type to a mixed butyrate-propionate type of fermentation (*Cf.* Figure IV.6). The remarkable differences observed between R2 and R4 reactors suggest that the ability of the R4 reactor to adapt to the toxic substrate perturbation was most likely the result of a shift in the predominant species.

d) Methanogens may adapt to ammonia concentrations several times higher than the initial threshold level, i.e., the level beyond which methane production is possible only after a certain period of adaptation.

For this, two reactors were used to assess the effects caused by a one step increase (from 13 to 58 mM) and by a two step increase, (first from 13 to 40 mM and, once the reactor is stabilized, from 40 to 58 mM, this latter change was performed in order to compare the effects of switching ranges of 13 to 58 mM to 40 to 58 mM).



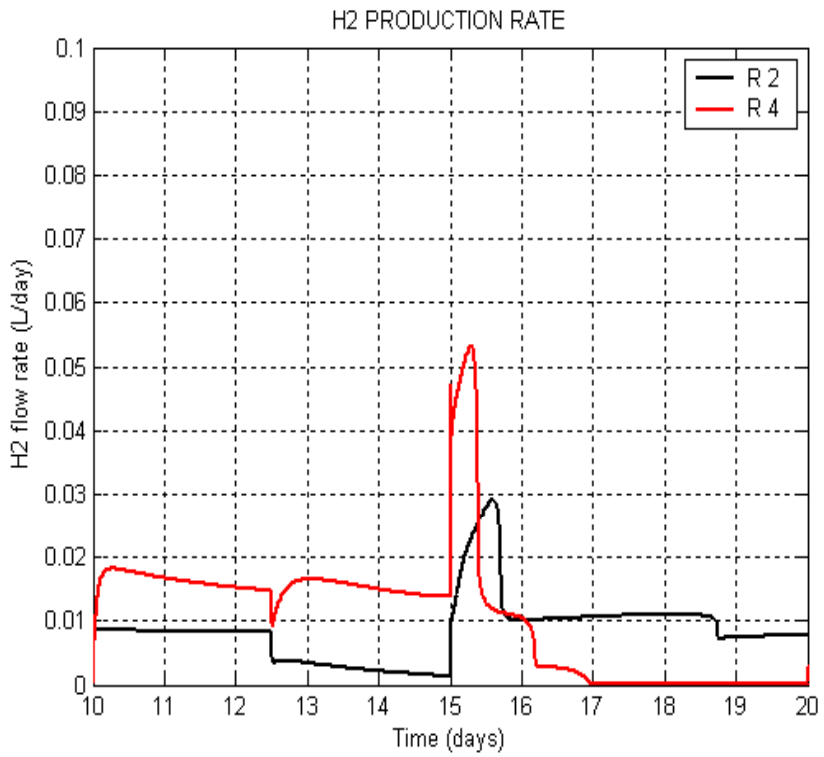


Figure IV.6. Sugar fermentation pathway in constant (R2) and pulsed (R4) OLR with pulsed toxicant affecting all trophic levels.

Simulation results showed the adaptation to increased TAN concentrations, indicated by the fact that when the TAN was changed from 40 to 58 mM, the reactor performance is not as disturbed as in the reactor where the TAN was changed from 13 to 58 mM. As a result, the time required for complete adaptation (i.e., return to steady state as noted by effluent VFAs concentration, soluble COD removal and MPR) was longer in one step increase reactor than in two step increase one. It was also clear from the simulation results that MPR and soluble COD removal efficiency decreased in the transition period (i.e., the time required for adaptation) and that these indices returned to lower levels than those obtained prior to the change in TAN concentration. Furthermore, a shift in the microbial communities (since one step increase reactor selects species 7, 8 and 9 while the other only the 10th one) suggests that adaptation to elevated ammonia concentrations results from the selection of resistant aceticlastic methanogens already present in the seeded sludge.

To study the influence of the number of resistant species (richness) at high TAN concentrations on the adaptation process, a CSTR where ammonia concentration was suddenly changed from 13 to 40 mM was simulated. We began with the reactor performance when all acetate degraders were present (ten species). Subsequently, the simulations were redone for a gradually restricted group of acetate degraders, successively elimitating the winning species from the previous simulation. The dynamic behaviour in terms of the lenght of the adaptation was significantly influenced by the microbial properties.

e) The adaptation period gets lower when the number of resistant species (richness) at high toxicant concentration levels increase.

The simulation also revealed that the adaptation period gets lower when the number of resistant species at high TAN concentration levels (richness) increases. Moreover, when all resistant species (6th to 10th sps) where supressed, the performance of the rector did not recover. Nevertheless, in such a case, the process ran in stable conditions but with high VFAs level, low MPR and low soluble COD removal, an operating point often encountered in practice and named “inhibited steady state”.

Combining the findings of Hsu *et al.* (1977) and Hsu (1980) about microbial competition for a single nutrient (in this case acetate), conditions for the outcome of microbial competition of X_{ac}(i) in a CSTR can be rigorously defined assuming that (i) input acetate concentration $S_{ac}(0)$

and dilution rate D are constant, (ii) the only competition between species is for the substrate, (iii) the mixing in the vessel is perfect and (iv) no or only low level inhibition occurs.

They have defined, for i=1:10: $$J_{ac}(i) = K_{S,ac}(i)\frac{D}{\mu_{\max,ac}(i) - D}$$

Then, if the number of competing species is such that their J_{ac}'s are ordered such as:

$$J_{ac}(1) < J_{ac}(2) < \ldots < J_{ac}(10)$$

all species die out if $S_{ac}(0) < J_{ac}(1)$.

On the other hand, if $S_{ac}(0) > J_{ac}(i)$ for all i, then only the first species (*i.e.,* the one associated to $J_{ac}(1)$) survives and outcompetes all rival species.

A priori, it might have been expected that the winner would always be the species with the highest affinity (*i.e.,* lowest *K_S*) for the substrate, or perhaps the species with the highest growth rate. A species with the highest affinity for the resource may nevertheless loose if it also has a low growth rate or a high decay rate.

It is important to notice that simulation results (selection of species in the order 10-9-6-8-7) do not agree with the ranking of the calculated J-values, which already indicates the findings of Hsu et al., (1977) are valid for one-step reactions without inhibition, while in our case the acetate degradation reaction is one step in a network and we have ammonia inhibition. So, it cannot be garanteed that the same results will hold. We have calculated *J*-values without inhibition and we would suggest to calculate alternative *J*-values introducing the inhibition term in the Hsu-expresion.

Let us define, for i=1:10: $$J_{ac}(i)^{*} = K_{S,ac}(i)\frac{D}{\mu_{\max,ac}(i) * Inhib_{ac}(i) - D}$$

The maximum growth rate has been corrected for inhibition effects through the same inhibition factors that we used for uptake of acetate in the model, i.e. $Inhib_{ac} = I_{pH}.I_{IN}.I_{nh3}$.

Note that the mathematical rigorousness of the Hsu-criterion valide for a single substrate, expires in our case, since NH_3 acts as an additional substrate during acetate degradation (even though not limiting).

Despite these uncertainties, the ranking of the *J*-values calculated according to definition J_{ac}^{*} values, agrees with the simulation results.

An analogous *J*-expression, including the decay coefficient for acetate ($k_{dec,ac} = 0.02$), has been defined for our case as follow:

$$J_{ac}(i)^{**} = K_{S,ac}(i)\frac{(D + k_{dec,ac})}{\mu_{\max,ac}(i) * Inhib_{ac}(i) - (D + k_{dec,ac})}$$

and the surprising thing is that the order still agree with the simulation results (*Cf.* Table IV.2). The results indicate the advantage of criteria to predict the outcome of interspecies competition.

Table IV.2 Biochemical parameters and *J, J***, J***-values for acetate degraders.

No specie(i)	Ks,ac(i)	mu_max,ac	Kl,nh3(i)	Jac(i)	J*ac(i)	J**ac(i)
1	0,0197	0,2892	0,0014	0,0150	0,2208	0,0917
2	0,0284	0,3281	0,0012	0,0175	0,7854	0,1679
3	0,0477	0,3114	0,0010	0,0320	0,2492	0,1574
4	0,0195	0,2856	0,0011	0,0152	0,0844	0,0579
5	0,0467	0,2563	0,0009	0,0444	0,1167	0,0967
6	0,2704	0,5635	0,0023	0,0771	0,2021	0,2663
7	0,2775	0,4850	0,0026	0,0964	0,2439	0,3292
8	0,2941	0,5391	0,0027	0,0888	0,2066	0,2700
9	0,2884	0,5590	0,0028	0,0831	0,1865	0,2413
10	0,2472	0,5714	0,0025	0,0692	0,1683	0,2191

Because we do not have mathematical proof of this criteria, further research in this direction for models involving multiple reactions in series and/or parallel and including inhibition needs to be developed.

In the last section of this chapter, ADM1_10 with modified disintegration/hydrolysis and Hill function for ammonia inhibition was used to analyze the relationship between batch reactor performance and microbial community structure (MCS) in thermophilic anaerobic digestion of thermally pretreated WAS. The dynamics of total and individual VFAs, pH and cumulative volumetric methane and carbon dioxide productions obtained from batch thermophilic anaerobic digestion of untreated and thermal pretreated sludges were used to validate the model.

Model predictions using the parameters established in annexe III, closely followed the temporal trend in the measured variables from thermophilic batch reactors. This agreement validated the modeling approach as well as the sixteen model parameters established. Since the propionate level persisted at relatively high levels during all the experiments, we suggested that propionate-degrading syntrophs was not present in high numbers in our inoculum. These syntrophs can only use a very limited range of substrate and have very low specific growth rate, so they need an extensive amount of time to reduce propionate concentrations. Methane produced, the major result of anaerobic digestion, was markedly increased by the 165°C steam mode pretreated WAS. The differences in the amount of methane produced showed that the impact of the rate-limiting step could be reduced by pretreatment up to a maximum at 165°C, by increasing the availability of organic matter. At higher temperatures, the biodegradability decreased sharply. It was presumed that formation of inhibitory compounds caused decreased biodegradability above 165°C, as suggested by others.

A link between Simpson's reciprocal diversity index and function, based in VFA concentrations for *Bacteria* and BMP for *Archaea,* was suggested according with the simulated results. As we know, the metabolic pathway of anaerobic digestion can be divided into five steps: disintegration, hydrolysis, acidogenesis, acetogenesis and finally methanogenesis. The *Bacteria* microflora is responsible for the first four steps of the overall reaction while methanogenesis is performed by *Archaea*. During these first steps, *Bacteria* micro-organisms have to degrade a large panel of substrates (in our case with high concentration of particulate organic matter) into the reactor. This variety of potential substrates can easily explain the large diversity of *Bacteria* organisms and also their versatility during the start-up phase. The hydrolytic and fermentative species play a crucial role in the initial breakdown of the influent feed with the resulting by-products utilised by the underlying *Bacteria*. These organisms have to develop specific degradation aptitudes in order to outcompete other *Bacteria* and to survive in this ecosystem which is always in equilibrium. Furthermore, during start-up, *Archaea* are less diversified than *Bacteria*. These results can be explained by the link between the variety of substrates and the biodiversity. The metabolic role of *Archaea* in the anaerobic digestion reaction is the last step of degradation, the methanogenesis. Methanosarcinales are responsible for acetate degradation into methane whereas Methanomicrobiales and MethanoBacteriales transform hydrogen and carbon dioxide

into methane. The reduced range of potential substrates induces a lower level of competition between micro-organisms leading to a more limited *Archaea* diversity.

During the thermophilic digestion of WAS, the microbial community structures in both *Bacteria* and *Archaea* domains changed over the time as consequence of the changes in the environment conditions, such as substrate availability and/or pH. Changes in the structure and activity of the microbial community of the thermophilic batch treating thermally pretreated WAS occurred, and under the applied operational conditions, except for 220°C pretreated WAS, the microbial community succeeded in maintaining the increased flow of carbon into methane as was reflected by the improved reactor performance.

The findings from this section could be considered as a first step towards the development of strategies to further stimulate hydrolysis and to ultimately increase the methane production rates and yields from thermophilic anaerobic digestion of thermally pretreated WAS.

Research effort has recently been directed towards particle size information for a better understanding of COD fractionation and related biodegradation patterns. Dulekgurgen et al (2006), in a recent study, proposed direct particle size measurement by sequential filtration and ultrafiltration as a convenient method for wastewater characterization for appropriate treatment technology. They also explored the correlation between particle size distribution (PSD) and chemical oxygen demand (COD) fractionation, as an index for biological treatability. In another study Karahan et al, (2008), the scientific link between particle size distribution (PSD) and biodegradability of different COD fractions of tannery wastewater, by means of sequential filtration/ultrafiltration, respirometric analysis and model evaluation was established.

To evaluate the effect that COD fractionation has on the diversity and reactor performance, another simulation was done. In this case the simulation results for the untreated WAS showed in chapter III (Figura III.17), were redone with another fractionation. Table IV.3 shows the modified disintegration stoichiometric coefficients that were stablished in the annexe III (Table 1). Figure IV.7 displays the simulation results.

Table IV.3 Fractionation values from composites for untreated WAS

Yiel of product on substrate ($kg_{COD}.kg_{COD}^{-1}$)	WAS Untreated	WAS Untreated modified
$f_{Si\ Xc}$	0.000	0.000
$f_{Xi\ Xc}$	0.606	0.506
$f_{ch\ Xc}$	0.096	0.134
$f_{pr\ Xc}$	0.160	0.164
$f_{li\ Xc}$	0.138	0.196

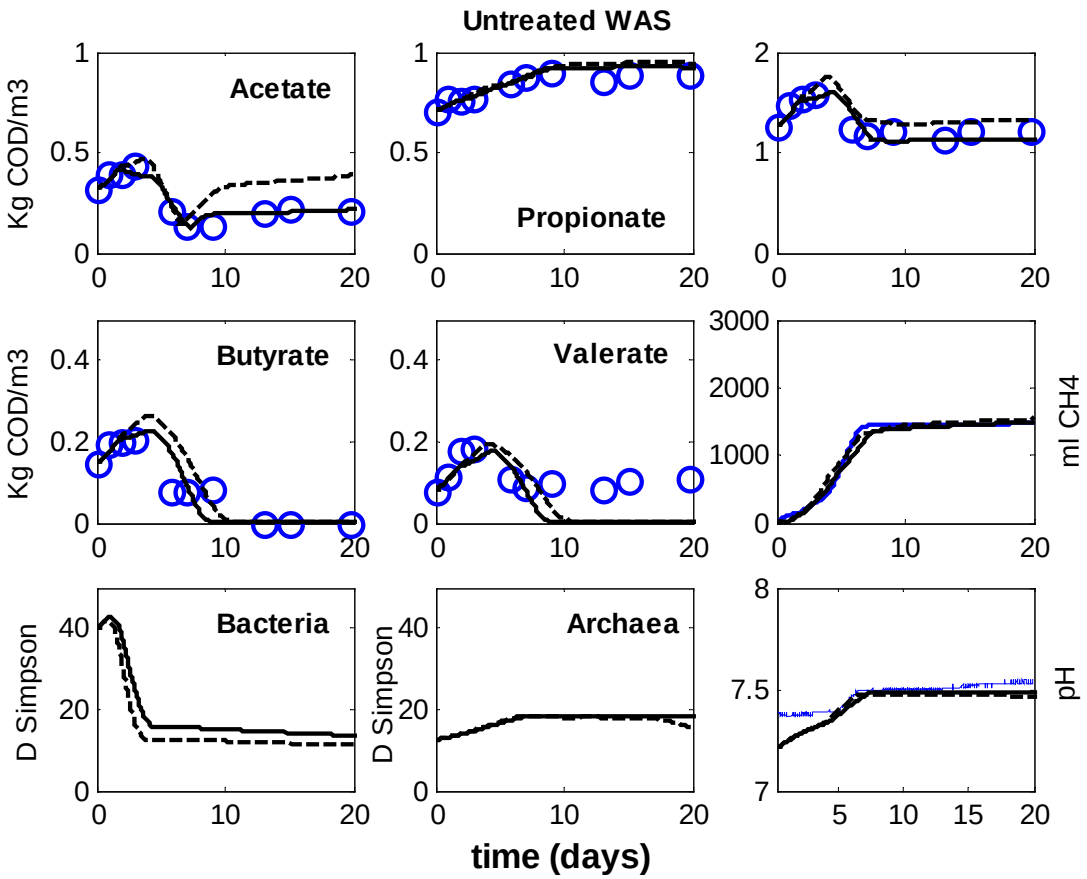


Figure IV.7 Simulated individual and total VFAs, pH and cumulative CH4 production vs experimental data for untreated WAS, togheter with predicted Simpson diversity index. (circles : experimental data points, solid line : simulated results with WAS untretaed parameters in tableX, dashed line : simulated results with WAS untretaed modified parameters in table IV.3).

Thus, we can conclude that due to the change in COD fractionation :

- VFAs accumulation increase due to mainly acetate accumulation increase. This is because carbohidrates production (fch_Xc) and lipids production (fli_Xc) have been increased and these are the metabolic pathway of acetate production.

- The high initial methane production is due to the high initial acetate accumulation.

- All decrease in fXi_Xc results in increase of methane production, however in our case this does not happen because the high final acetate accumulation inhibits the methane production.

- The increase in acetate accumulation is due to the decrease in acetogenic bacteria community and this explain the lower bacterial diversity index value

In annexes I and II, we assessed the issue of renewable energy sources balanced in CO_2. Linkages between different biological process to improve convertion efficiency to methane and hydrogen were showed in annexe I. Methane fermentation involves consortia of two major types of bacteria: the so-called acidogenic bacteria that break down the substrates into mainly H_2, acetic acid and CO_2, and the methanogenic bacteria, that convert acetic acid, H_2 and CO_2 to methane gas. These two reactions can, at least partially, be separated into separate bioreactors in series, in which the first, small, reactor produces organic acids, H_2 and CO_2, while the second, much larger, reactor produces CH_4 and CO_2. Such two-phase anaerobic digestions were proposed as a way to optimize the growth of each type of bacteria in the separated reactors, specifically by growing the acetogenic bacteria at a low pH (e.g., 5–6) and short hydraulic residence time (typically 1–2 days) in the first stage, while the slower growing methanogenic bacteria stage, requiring a more neutral pH, were preferentially cultured in the second stage with a much longer hydraulic residence time (typically 10–20 days), (Blonskaja et al., 2003). However, although the selection pressure of pH and dilution rate are sufficient to select for acetogenic and methanogenic bacteria in their respective first and second phases, separating these two basic processes will not generally significantly accelerate or increase overall methane-production, although it can be of some advantage in making the process more resistant to varying shock loads (Cooney et al., 2007).

Optimizing of biological production (biomethane and biohydrogen) from organic waste was emphasized in annexe II. However, economic viability of biogas production at industrial level

is still ensured by the government subsidies that vary from one country to another according to the policy applied. To reduce this dependence, we propose to look at them under an "optimization" point of view. We drew up there a state of the art of some aspects of biogas plants optimization. We indeed showed that many biogas plants are operating sub-optimally and 25 to 40% of the biogas potential of biomass is not utilized by the traditional operation of the plants. This is related to processes monitoring practices, which depend mainly on the know-how of the operators. In order to make biogas plants economically viable with decreased or no subsides, on-line acquisition data need to be implemented to follow process evolution. Although many parameters have been considered for their suitability as process state parameters, four of them are of particular interest: biogas production, hydrogen concentration, pH and volatile fatty acids concentration. The use of one individual parameter as the only control parameter is not recommended, although any of them may be suitable in connection with other parameters. Finally, we suggest to use closed loop control laws based on on-line measurements in order to optimize biogas processes.

The IWA Anaerobic Digestion Model N°1 (Batstone et al., 2002) is a powerful tool for predicting the behaviour of anaerobic digesters treating sewage sludges (Parker, 2005). This generalised model could take into account physical and chemical interactions between liquid, gas and biological phases. However, ADM1 uses some simplifications in reactions for particulate organic compounds, in particular the first-order kinetic may be inaccurate to describe the desintegration and hydrolysis steps. Fernandez et al. (2001) showed that the hydrolysis step might depend on biomass concentration or activity. Thus, it has been necessary to integrate an hydrolysis rate which takes into account the limitation by biomass concentration and substrate concentration. In such cases, first-order kinetics should be corrected by taking account the impact of accessibility and hardly biodegradable material. It has been shown that models in which hydrolysis is coupled to the growth of hydrolytic bacteria work well at high or at fluctuant organic loading. In particular, the surface-related two-phase and the Contois models showed good fits to experimental data from a wide range of organic waste. Both models tend to the first order kinetics at a high biomass-to-waste ratio and, for this reason, they can be considered as more general models. In actual digester conditions, firstly, it is not possible to know all the parameters to apply the surface-related hydrolysis kinetics model that takes into account the colonization of waste particles by hydrolytic bacteria and secondly, the applicability of the Contois kinetics for the description of continuous anaerobic digestion processes of particulate organic matter was demonstrated in

a number of papers (e.g. Chen and Hashimoto, 1980; Domenech and Flotats, 1997; Vavilin et al, 2008). Thus it may be assumed that the Contois model of hydrolysis is the best approximation of the actual processes.

For this, in annexe III, we modified the desintegration and hydrolysis steps in ADM1 in order to obtain a model able to predict and interpret results from thermophilic anaerobic digestion of thermal pretreated WAS. First, Contois models for desintegration and hydrolysis instead of first-order kinetics are included and second, the Hill function for modeling ammonia inhibition in aceticlastic methanogens was used instead of non-competitive function.

Anaerobic digestion of WAS consists of a complex series of interdependent reactions mediated by a diverse consortium of microbes. The stability and efficiency of the overall digestion process depends on the stability of the individual biochemical processes. Any significant increase in the concentration of intermediate substrates may inhibit directly, through toxicity and energetics, the kinetics of other biochemical processes and lead to digester instability. Transient peak loadings which produce substrate availability beyond the capacity of the degrading microbial consortium are one source of elevated substrate concentrations. The difference between the capacity of a consortium to degrade a substrate and the substrate production rate is a measure of stability. Improvements in the characterization of both the substrate production kinetics and the maximum substrate utilization capacity of the corresponding microbial consortium (i.e., predictive biodegradability of a substrate) will assist in the design and management of digesters.

Elevated VFAs concentrations (meanly as acetate) have been assessed as a key indicator of digester instability (Moesche and Joerdening, 1999). Acetate is a final product of both acidogenesis and acetogenesis, which in turn depend on hydrolysis for the production of soluble sugars, amino acids and long-chain fatty acids (LCFA). Because hydrolysis has been shown to be the rate-limiting reaction in the anaerobic digestion of WAS, any characterization of VFAs production rates is dependent on the hydrolysis kinetics of particulate organics.

Predictions by the model using the parameters established in this annexe agreed well with the data measured under different thermal pretreatment conditions. The resulting model was capable of explaining the dynamics of acetate accumulation obtained in some batch experiments, which were characterized by two peaks of acetate concentration, the result of

different hydrolysis rates for fats and proteins. However, methane production rate was not totally perfectly simulated and could be improved. Yasui et al. (2008) suggested to elaborate a more comprehensive degradation scheme considering the model structure limiting factor and readily and slowly fractions of WAS. So, dual-pathway disintegration structures were incorporated in the modified ADM1 (Figure IV.8).

In the dual pathway structure, the degradable particulate COD (i.e. pCOD) is solubilized in two parallel paths. The Monod maximum specific uptake rate (k_m) of the more readily hydrolyzed (Xc_{rh}) fraction exceeds the slower hydrolyzed (Xc_{sh}) rate by an order of magnitude, and the determination of the fractions Xc_{rh} and Xc_{sh}, were based on the volume of methane produced for each matter compartment compared to produced total volume (36% of pCOD is Xc_{rh} and 64% is Xc_{sh} in untreted WAS case, for example). Each produces soluble substrate with the same fractionalizations to amino acid, sugar and long-chain fatty acid. The biodegradable portion of endogenous biomass decay is assumed to be slowly hydrolyzed in the dual-pathway model.

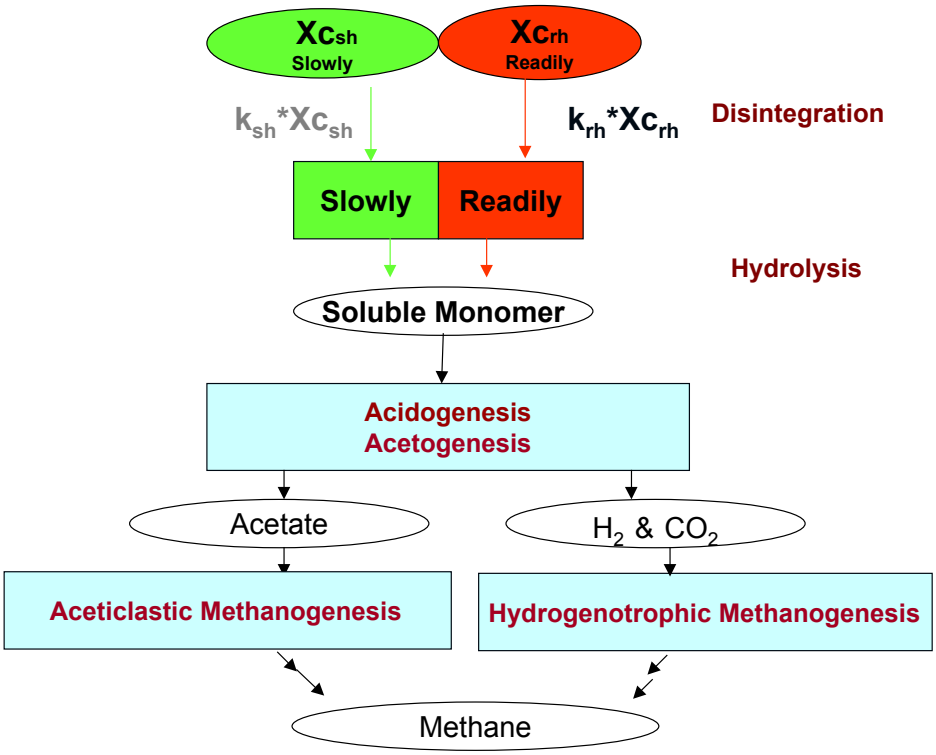


Figure IV.8 COD flux for a particulate composite in dual-pathway disintegration modified ADM1 model structure (from Yasui et al. 2008).

In our work, anaerobic thermophilic batch degradation of WAS showed a complex Methane Production Rate (MPR) curve marked with three well-defined temporal peaks. The first immediate peak could be associated with the degradation of soluble compound, the second delayed peak with the degradation of relatively readily hydrolysable substrates, while the third delayed peak could be associated with the degradation of slowly hydrolysable substrates. For simulating the last delayed peaks, it would be necessary to consider a more elaborate particle disintegration/hydrolysis model.

Figure IV.9 shows partial simulated results from the dual-pathway disintegration in modified ADM1 model. However, additional work is required before this new model can reflect the temporal trend of experimental data for untreated and thermally pretreated WAS.

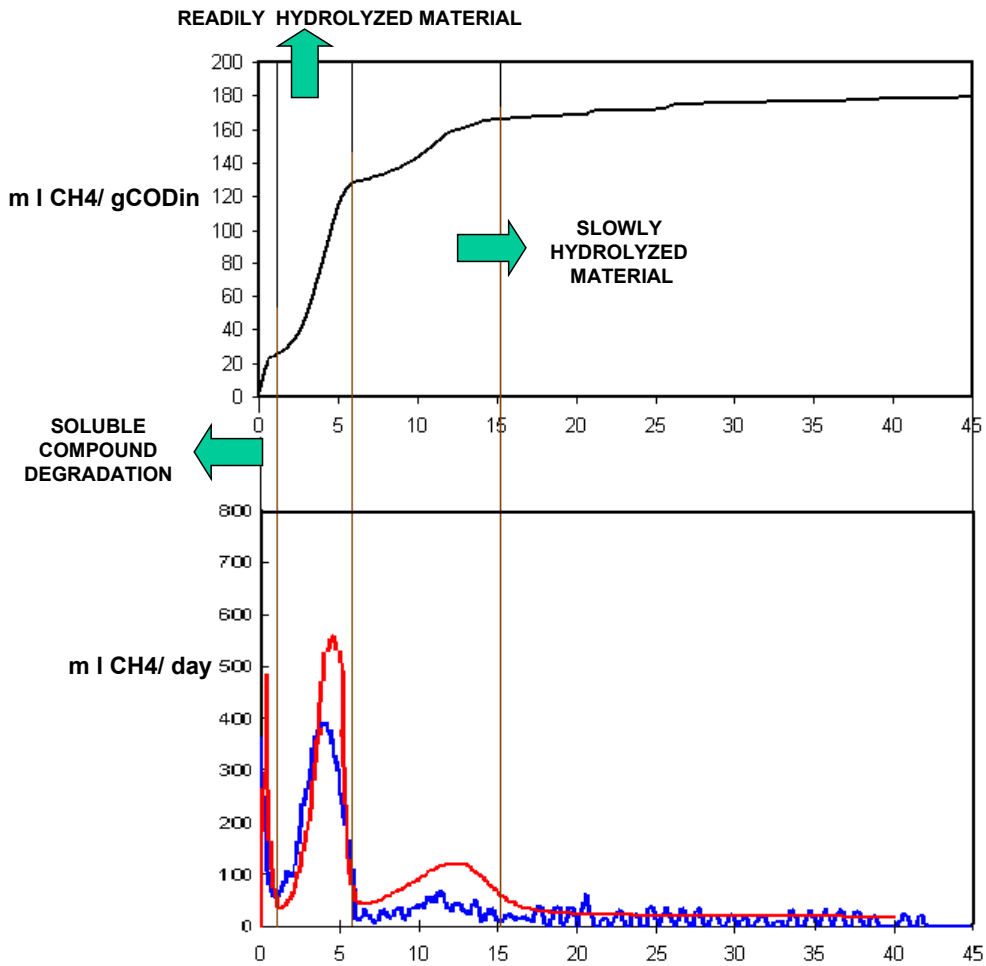


Figure IV.9 BMP and methane production relationships. Continuos red line are similated results from dual-pathway disintegration modified ADM1 model structure and continuos blue line are experimental results

IV.2 PERSPECTIVES

Finally, several perspectives can be imagined to this study and are presented in the following :

IV.2.1 *EXTRAPOLATING BMP PARAMETERS*

With a suitable inoculum, the BMP test is a very repeatable method of analyzing degradability rate and extent. The BMP test itself is relatively inexpensive in terms of analysis and chemicals, but is time-consuming, requires specialist expertise, and has substantial set-up costs. Therefore, only a limited number of laboratories can conduct a BMP test. Another concern is that the BMP test is done under different conditions to the continuous digesters. Generally, while temperature will be the same, it is impossible to maintain exactly the same buffer, pH, and gas phase conditions in the BMP test as in the continuous reactors.

In this work, we used iterative and sampling techniques to obtain biochemical parameters to fit the data set of batch thermophilic anaerobic digestion of thermally pretreated WAS from BMP test. The question is : Is it possible to use this model biochemical parameters set in order to model continuous reactors ? Further experiments should be performed in this direction.

IV.2.2 *HYDROLYSIS INHIBITION*

Inhibitory studies have mainly been focused on acetoclastic methanogens and acetogens,while less attention has been paid to the inhibition of hydrolysis. However hydrolysis can be inhibited by another amount of factors as :

<u>The accumulation of amino acids and sugars</u>

Kadam et al (2004) developed a multireaction kinetic model for closed-system enzymatic hydrolysis of lignocellulosic biomass such as corn stover. They modeled three hydrolysis reactions, two heterogeneous reactions for cellulose breakdown to cellobiose and glucose and one homogeneous reaction for hydrolyzing cellobiose to glucose. The sugar products of cellulose hydrolysis, cellobiose and glucose, as well as xylose, the dominant sugar prevalent in most hemicellulose hydrolyzates, were assumed to competitively inhibit the enzymatic hydrolysis reactions. Their model performed well in predicting cellulose hydrolysis trends at

experimental conditions both inside and outside the design space used for parameter estimation.

During cellulose degradation, cellobiose as the intermediate product may be a stronger inhibitor than glucose

Duff and Murray (1996) postulated that during enzymatic hydrolysis of lignocellulose, cellulases are very susceptible to end product inhibition by cellobiose and, to a lesser degree, by glucose. This severely limits the extent of saccharification that can be achieved in batch hydrolysis.

Non-ionized VFA are other possible inhibitors

Some controversy can be found in the literature about the inhibitory effect of VFA. Llabres Luengo and Mata-Alvarez (1988) proposed a kinetic model for MSW degradation where VFA acted as inhibitors, but they did not consider the effect of pH. With the same kind of substrate, Veeken and Hamelers (2000) used a Contois type of kinetics affected by a non-competitive inhibition term due to VFA, with an inhibition constant of 30 g VFAL-l, with satisfactory results. Veeken et al. (2000) designed a set of experiments to elucidate the mechanisms of VFA inhibition, concluding that no inhibition by VFA or by non-ionized VFA can be measured at pH values between 5 and 7, and that acidic pH was the inhibitor factor.

The effects of pH and acetate on the hydrolysis of carbohydrate differed from those on the hydrolysis of protein

Specifically for the hydrolysis of proteins, the study of the possible effect of VFA has received especial attention. While Breure et al. (1986) and Yu and Fang (2003) concluded that VFA do not inhibit protein degradation, using gelatine as substrate, Gonzales et al. (2005) clearly showed that acetic acid reduced the gelatine hydrolysis rate in a mesophilic saline environment, with 0.229 g COD-Ac L-1 as the inhibition constant for a noncompetitive inhibition affecting a first-order hydrolysis. In contrast, Flotats et al. (2006) showed that no inhibition by VFA occurred during gelatine hydrolysis.

Low pH and high lipid concentration can also affect the hydrolysis (Palenzuela-Rollon, 1999). It has been stated that lipid hydrolysis hardly occurs without methanogenic bacteria that keep pH at non-acidic levels and VFA at non-toxic concentrations. Lu et al. (2004) studied enzymatic activity during the start-up of dry anaerobic mesophilic and thermophilic digestion

of the organic fraction of MSW. It was shown that the low hydrolysing protease activity during the first 2–3 weeks was due to inhibition by low pH.

It is difficult to distinguish the inhibitory effects caused by pH or VFA. Previous works indicate that VFA accumulation induces a pH decrease, lowering the hydrolysis rate and making pH the effective inhibitor factor.

<u>The effects of pH and acetate on the hydrolysis of carbohydrate differed from those on the hydrolysis of protein</u>

In recent studies He at al (2006) found that hydrolysis of organic particulates in rapid fermentative processes can be inhibited. The volatile fatty acids (VFA) released during fermentation reduce pH. Whether VFA or the drop in pH inhibits hydrolysis is still unclear. They studied the effects of pH and acetate on the enzymatic hydrolysis of a potato sample that contains both carbohydrate and protein at fixed pH (5–9) in the presence/absence of 20 g/L of acetate. Their experimental results showed that the effects of pH and acetate on the hydrolysis of carbohydrate differed from those on the hydrolysis of protein. They used the Chen-Hashimoto model to fit the hydrolysis data obtained during 144 h of reaction. Finally they successfully described the inhibition of the hydrolysis of both carbohydrate and protein, using a non-competitive inhibition model of three inhibitors (H+, OH-, and total undissociated/dissociated acetate).

<u>High organic solids to inoculum ratio</u>.

Vavilin et al (2008) used experimental data from Angelidake et al (2006) to model methane accumulation curves during household solid waste anaerobic digestion in batch reactors for different volatile solids concentration. With the Contois kinetic of hydrolysis, they can not explained experimental data ontained with initial concentration of 96 gVS l-1 ,which is in the range of causing inhibition, by a high organic solids to inoculum ratio. They concluded that in general a balance between hydrolysis and methanogenesis during anaerobic digestion is very important as some of the intermediates are known to be inhibitors. In these cases, models must be adapted in order to account for inhibitory phenomena.

How do we include these inhibitions in ADM1 and in models such as ADM1_10 ? This is another direction for further research.

IV.2.3 *ADM1_10 AS A TOOL FOR PARAMETER ESTIMATION*

As already mentioned to deal with microbial diversity, the number of species considered for each biological reaction in the proposed model (ADM1_10) is arbitrary and in this study was set to 10, which is sufficient to demonstrate the potential of modelling microbial diversity. Even though we do not yet know exactly the diversity of the different functional groups nor how this diversity is sustained.

An interesting point for further research is to know **how do the simulation results change, if any, with different species number for each group ?**

Moreover, due to computer limitations, we could not handle more than 10 species for each biological reaction but it is our strong belief that more species (*i.e.,* ADM1_100 to ADM1_1000) should be accounted for to accurately handle microbial diversity. Moreover, by handling a much higher number of species per reaction, one would minimize the efforts for parameter estimation (*i.e.,* only a "global" value of the model parameters such as in ADM1 would be required, microbial diversity being later accounted for by the high number of species handled with random kinetic parameters centered around the average values found to fit ADM1).

IV.2.4 *MODEL DISTRIBUTION TYPE*

A question that the reader may ask is related to the parameters distribution type selection in the model. Although this was already discussed, we suggest to explore another approach : "to make emerge the distribution type from the same model". How can we do it? One way could be :

- to select other reactor performance parameters than COD or pH because these variables are inadequate to reveal community structure variations (Fernandez et al., 1999).
- to write a Nx3 matrix where the two first columns correspond to model biochemical parameters (k_m^* and K_s^*) and the third one to functional group (X_{su}, X_{aa}, $X_{fa,}$ X_{c4}, X_{pro}, X_{ac} or X_{h2}).
- for each functional group, to choose k_m^* and K_s^* as follow :

  if rand(1,2) = [a b], then $k_m^* = a.k_m$ and $K_s^* = b.K_s$

where k_m and K_s are the values of corresponding standard ADM1 biochemical parameters.

- to simulate the model with one functional group (X_{su} for example) while keeping biochemical parameters of the others functional groups as default ADM1 values. If the obtained model fits the experimental data for the performance parameter choosen, the values obtained for k_m^* and K_s^* are saved. Otherwise, the procedure is restart. In this way, after N simulations, we have a Nx3 matrix in the form of :

$$\begin{bmatrix} k_{m1}^* & K_{S1}^* & X_{su} \\ \\ k_{mN}^* & K_{SN}^* & X_{su} \end{bmatrix}$$

- with this matrix and a statistical software, the k_m^* and K_S^* values can be adjusted to one known statistic distribution (normal, lognormal, weibull, etc.).
- the procedure is repeated for each functional group and at the end, one biochemical parameter distribution is obtained for each functional group.

IV.2.5 *TEMPERATURE DEPENDENT MODEL*

Thermophilic anaerobic digestion is superior to mesophilic anaerobic digestion in terms of process efficiency and required reactor capacity since shorter HRT can be used in thermophilic digesters (Kim et al., 2002). It has also been reported that the diversity in thermophilic anaerobic digestion is lower than the diversity in mesophilic digesters (Hashimoto et al., 1981; Hill 1990). Changes in temperature have indeed a fundamental influence on the physico-chemical system, mainly because of changes in equilibrium coefficients. The overall effect on the system due to changes in physico-chemical parameters with temperature is generally more important than that those due to changes in biochemical parameters. The van't Hoff equation describes the variation of equilibria coefficients with temperature. This variation are already included in ADM1_10, but the relationships betwen temperature-microbial community structure and temperature-reactor performance need to be examined. This would allow us to make comparative studies on process stability and efficience of anaerobic digestion in differents temperature ranges: psycrophilic, mesophilic

and thermophilic. The hypothesis is that biokinetic rates would increase with temperature, but bacterial diversity would decline as temperature increase such that the treated wastewater quality and the overall metabolic ability of the bacterial community would decline as observed experimentally by Lapara et al. (2000).

IV.2.6 *SPECIES SELECTION ADAPTED TO A TECHNOLOGY*

Nowadays, the species selection adapted to a technology is carried out by several methods (Zhang et al., 2006) :

- **Rational design**: by DNA manipulation, one might hope to build the "ultimate bug". However, an enormous amount of knowledge on the relationships between DNA and phenotypes and this kind of knowledge in not fully available yet, not mentioning the DNA manipulation is not the best solution for environmental objectives.
- **Random screening:** the individuals are here randomly isolated and it is hoped to fall on the suitable ones but this solution might be time consuming.
- **Facilitated screening**: some artifice can be used to isolate interesting individuals but randomness can still be present. For example, to select noninflammable humans, we could burn all the humans and, at the end, we would obtain the set of noninflammable humans… if any.
- **Continuous selection** (in chemostat for example): a chemostat can be operated in conditions where the species of interest win the competition. Until today, this is the technique that seems the most succesfull, for example for biohydrogen production in dark fermentation: by running the procees at low pH (around 5.5) and low HRT (few hours), the methanogens are washed out and only the hydrogen producers remain in the reactor. The main difficulty is here to find the appropriate selection pressure to select the expected species.

In this last case, ADM1_10 can be of great interest to evaluate different control strategies and to analyse their effect on species selection.

IV.2.7 *J-FACTOR AS CONTROL PARAMETER*

In section III.2, we concluded that one way for enhancing system heterogeneity by fostering the right populations (i.e. to increase microbial diversity), can be achieved at temporal scale by means of providing substrate periodic pulses to encourage the growth of desired microorganisms (development of communities that are more resilient in the long term due to the pulse disturbances).

Moreover, in section III.3, we discussed the relationship between J-factor, microbial community structure and reactor's performance. This suggest to find a way to use this J-factor, to design the desired input periodic OLR's parameters (height, width and frequency), in order to maximize the microbial community in the reactor. Further research is necessary to include competition criteria as control objective.

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Publications dans des revues

- C.A. Aceves-Lara, E. Trably, J.R. Bastidas-Oyenadel, **I. Ramirez**, E. Latrille, J-Ph. Steyer : *"Production de bioénergies à partir de déchets: Exemples du biométhane et du biohydrogène",* Journal de la Société de Biologie, 202 (3), 177-189 (2008).

- **I. Ramirez**, J-Ph. Steyer: *"Modeling microbial diversity in anaerobic digestion"*, Water Science & Technology, vol, 57, n° 2, pp. 265-270 (2008).

- R. Rajinikanth, **I. Ramirez**, J-Ph. Steyer, R. Escudie, M. Torrijos, I. Mehrotra and P. Kumar : *"Experimental and modeling investigations of a hybrid upflow anaerobic sludge-filter bed (UASFB) reactor"*, Water Science & Technology, vol. 58 , n°1, pp. 109–117, (2008).

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- **I. Ramirez**, J-Ph. Steyer: *"Modeling microbial diversity in anaerobic digestion",* 11th IWA World Congress on Anaerobic Digestion; Brisbane, Australie; 23-27 Septembre 2007 (communication orale)

- R. Rajinikanth, **I. Ramirez**, J-Ph. Steyer, R. Escudie, M. Torrijos, I. Mehrotra and P. Kumar: *"Experimental and modeling investigations of a hybrid upflow anaerobic sludge-filter bed (UASFB) reactor",* 11th IWA World Congress on Anaerobic Digestion; Brisbane, Australie; 23-27 Septembre 2007 (communication orale)

- **I. Ramirez**, E.I.P. Volcke, J-Ph. Steyer: *"Modeling and Monitoring of Microbial Diversity in Ecosystems - Application to Biological Wastewater Treatment",* Conférence orale lors de l'IFAC World Congress 2008, Seoul, Corée (6 pages sur CDROM).

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ANNEXE I

PRODUCTION DE BIOENERGIES A PARTIR DE DECHETS : EXEMPLES DU BIOMETHANE ET DU BIOHYDROGENE

Production de bioénergies à partir de déchets: Exemples du biométhane et du biohydrogène

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Abstract: This new century addresses several environmental challenges among which distribution of drinking water, global warming and availability of new renewable energy sources in substitution of fossil fuels are of the most importance. The last two concerns are closely related because the major part of carbon dioxide (CO_2), considered as the main cause of the greenhouse effect, is widely produced from fossil fuel combustion. Renewable energy sources fully balanced in CO_2 are therefore of special interest, especially the issue of biological production from organic wastes. Among the possibilities of bioenergy production from wastes, two approaches are particularly interesting: The first one is relatively old and related to the production of biomethane by anaerobic digestion while the second one, more recent and innovative, relies on biohydrogen production by microbial ecosystems.

Résumé: Ce nouveau siècle présente plusieurs défis environnementaux: La distribution d'eau potable, le réchauffement planétaire et l'obtention de nouvelles sources d'énergie en substitution des combustibles fossiles en sont les plus cruciaux. Ces deux derniers sont intimement liés car la majeure partie du dioxyde de carbone (CO_2), considéré comme le principal responsable de l'effet de serre, est issu de l'utilisation de combustibles fossiles. Ainsi, les nouvelles sources d'énergie devront avoir comme caractéristique principale un bilan presque nul en CO_2. Parmi elles, les voies de production de bioénergies à partir de déchets sont tout particulièrement intéressantes: Notamment, une première voie, relativement ancienne, est liée à la production de méthane par digestion anaérobie. Une seconde possibilité, beaucoup plus récente et innovante, repose sur la production d'hydrogène par des écosystèmes microbiens.

Mots-clés: bioénergie, biogaz, méthane, hydrogène, déchets.

1 *LA PRODUCTION DE BIOMETHANE*

1.1 La Decouvert Du Biogaz

Les premières grandes découvertes sur le biogaz datent de 1630, avec Jan Baptist van Helmont, surnommé le "Leonard de Vinci Bruxellois", qui a découvert que la fermentation de la matière organique produisait un gaz inflammable. En 1776, Alessandro Volta passant ses vacances sur les rives du Lac Majeur, a remarqué qu'en remuant le fond du lac avec un bâton au cours d'une promenade en bateau, des bulles de gaz remontaient en surface, qu'il appellera gaz des marais. En collectant une partie de ce gaz, il a montré que ce gaz était un combustible. Il a également observé que le volume de gaz était proportionnel à la masse de matière en décomposition. Le terme de méthane ne sera proposé qu'en 1865 pour être définitivement accepté en 1892 lors d'un congrès international de nomenclature chimique.
Concernant l'utilisation du biométhane, des preuves historiques suggèrent que dix siècles av. J.C., les Assyriens s'en servaient pour chauffer l'eau de leur bain (Lusk, 1998). De nombreux pays ont très tôt saisi l'intérêt de produire du biogaz pour prévenir une trop forte dépendance aux hydrocarbures. Ainsi l'Inde commença dès le début du 19e siècle à produire du biogaz (mélange de biométhane et de dioxyde de carbone) et la première unité de traitement de déchets pour produire du biogaz aurait même été construite en 1859 à Bombay (Meynell, 1976). Vers 1890-1895, Donald Cameron construisit une fosse septique pour la ville d'Exeter au Royaume-Uni. Le gaz produit était alors collecté et servait à l'éclairage public.
Au 19s siècle, Mitscherlich suggéra le rôle des microorganismes dans les réactions de dégradation de la cellulose et la production de méthane (1939). Vers 1930, les travaux en microbiologie conduisirent plusieurs scientifiques, dont Arthur M. Buswell, à la découverte des bactéries anaérobies et au moyen de produire plus efficacement du biogaz. C'est également à cette époque que furent formulées les premières équations macroscopiques de dégradation de la matière organique par fermentation méthanogène.

1.2 Biochimie Du Procede

La digestion anaérobie n'est autre que l'exploitation par l'homme d'un processus de fermentation méthanogène de la matière organique. Les micro-organismes métabolisent la matière organique en absence d'oxygène et produisent du biogaz composé de méthane et de gaz carbonique. Ces transformations se trouvent intiment liées aux transformations énergétiques, représentées par l'énergie libre de Gibbs. Les énergies libérées lors des procédés anaérobies sont faibles, ce qui conduit à de faibles productions de biomasse, car la plupart de l'énergie est libérée sous forme de méthane (CH_4).
D'une façon générale, quatre étapes interviennent (Figure 1). Tout d'abord, la matière organique complexe est fractionnée en unités plus simples de sucres, lipides et protéines. Ceux-ci sont ensuite hydrolysés en acides aminés, monosaccharides et acides gras à longues chaînes à l'aide d'enzymes sécrétées par les micro-organismes, pour pouvoir ensuite être transportés au travers de la membrane cellulaire. Une fois dans la cellule, ces molécules simples peuvent être utilisées comme source d'énergie pour le métabolisme.
Dans une seconde étape, les monomères issus de l'hydrolyse, ainsi que les composés dissous, servent de substrats à des microorganismes fermentaires qui les transforment principalement en acides de faibles poids moléculaires comme les acides gras volatils (AGVs) tels que l'acétate, le propionate, le butyrate, le valérate, en alcools tels que le méthanol, l'éthanol et en gaz carbonique et hydrogène. Les microorganismes réalisant cette étape peuvent aussi bien être anaérobies facultatifs (du genre *Acetobacter* ou *Streptococcus*) que anaérobies stricts (*Clostridium* sp.). Leur taux de croissance, plus rapide que les autres microorganismes

anaérobies et de l'ordre de 6 h^{-1}, est responsable de l'accumulation de composés intermédiaires comme l'acétate ou l'hydrogène, qui peuvent inhiber l'ensemble de la chaîne trophique.

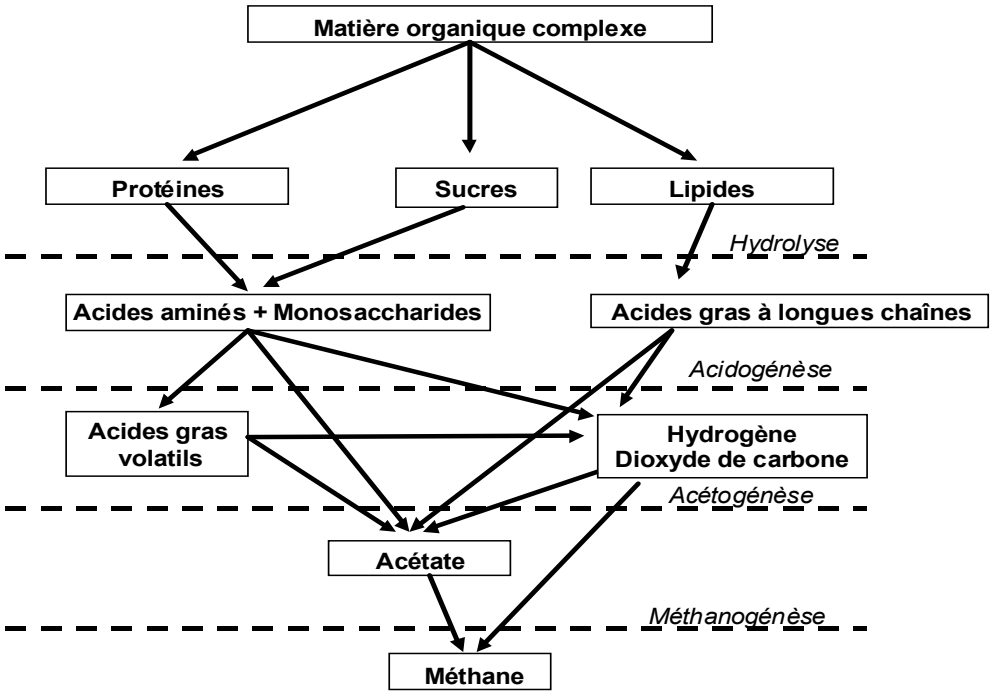

Figure 1 Schéma réactionnel général de la digestion anaérobie

Les produits de l'hydrolyse et de l'acidogénèse (acides, sucres, alcools,...) sont réduits en acétate, hydrogène et dioxyde de carbone par un groupe hétérogène de deux populations bactériennes: les bactéries acétogènes productrices d'hydrogène et syntrophes des méthanogènes, les acétogènes non-syntrophes. Les bactéries acétogènes productrices d'hydrogène produisent de l'acétate et de l'hydrogène à partir des acides qui contiennent trois atomes ou plus de carbone dans leur structure. Les réactions d'acétogénèse à partir du propionate, du butyrate et de l'éthanol sont thermodynamiquement défavorables dans les conditions standards ($\Delta G_o > 0$) et elles ne deviennent possibles que pour de très faibles pressions partielles en H_2 (inférieures à 10^{-4} ppm- Fukuzaki *et al.* 1990b); Lee & Zinder 1988). Ceci nécessite que les bactéries oxydant les acides fonctionnent en syntrophie avec des espèces hydrogénotrophes, comme par exemple les méthanogènes, qui en consommant le hydrogène, maintiennent une pression partielle faible et permettent à ces réactions de se produire (Ahring & Westermann, 1987).
Les acétogènes non-syntrophes produisent majoritairement de l'acétate et peuvent également utiliser le CO_2 comme accepteur final d'électron (Buschhorn *et al.* 1989). Ces bactéries sont anaérobies strictes et sont réparties en deux groupes: les fermentatives acétogènes (*Pseudomonas*, *Clostridium*, *Ruminococcus*...) et les acétogènes hydrogénotrophes ou homoacétogènes (*Acetogenium*, *Acetobacterium*, *Clostridium*) qui consomment le CO_2 et l'H_2. Finalement, l'acide acétique et le couple gazeux CO_2/H_2 sont convertis en CH_4 par des archaeas respectivement appelées méthanogènes acétoclastes (*Methanosarcina sp.* et *Methanotrix sp.*) et méthanogènes hydrogénophiles.

1.3 Facteurs Influençant La Digestion Anaerobie

1.3.1 pH et alcalinité

On considère habituellement que la gamme optimale de pH pour la digestion anaérobie se situe entre 6,7 et 7,3 (Barker, 1943; Braun, 2007). Un écart du pH de cette gamme est en général le signe d'un mauvais fonctionnement du digesteur et d'une accumulation d'acides ou de composés alcalins. Un procédé opérant à un pH trop faible (en dessous de pH 4) ou trop élevé (au dessus de pH 9) aura tendance à inhiber l'activité microbienne
Certains auteurs ont mis en évidence l'adaptation de populations anaérobies à des pH inférieurs à 5 (Goodwin & Zeikus, 1987). Jain & Mattiasson (1998) ont étudié l'adaptation d'une culture de bactéries méthanogènes à des pH extrêmes de l'ordre de 4. Ils ont montré que la production de méthane était fortement ralentie mais pas entièrement stoppée.

1.3.2 Composition du milieu

Hall *et al.* (1992) rapportent que des rapports C/N de 400/7 et 1000/7 sont suffisants pour le traitement de faibles et fortes charges, et que le rapport N/P doit être supérieur à 7. Moletta (2005) et Braun (2007) évoquent quant à eux un rapport C/N/P minimal de 800/5/1. En dehors de ces macro-nutriments, les bactéries anaérobies ont besoin d'un grand nombre de micronutriments tels que des vitamines, des acides aminés et des éléments traces métalliques (cuivre et nickel en particulier – Cresson *et al.,* 2006). Certains microorganismes méthanogènes comme *Methanobacterium thermoautotrophicum* sont toutefois capables de synthétiser l'ensemble de leurs composants cellulaires uniquement à partir d'un milieu inorganique (Murray & Zinder, 1985).

1.3.3 Composés toxiques ou inhibants la digestion anaérobie

Des composés toxiques et inhibiteurs peuvent être responsables du mauvais déroulement de la méthanisation au sein d'un réacteur. Un excès d'AGVs sous leur forme non dissociée (-COOH) accélère leur entrée dans les cellules, ce qui provoque une baisse du pH intra-cellulaire (Boe, 2006). Dans ces conditions, une partie de l'ATP servant habituellement à la croissance, est hydrolysée pour libérer des protons, contrebalancer cet apport supplémentaire d'anions et assurer le maintien de l'homéostasie. En conséquence, moins d'ATP sera disponible pour la croissance bactérienne (Fukuzaki *et al.* 1990a). La concentration seuil à partir de laquelle les AGVs sont inhibiteurs dépend alors du pouvoir tampon du milieu.
D'autres composés comme l'acide 2-bromoéthane-sulfonique (BES) ou le chloroforme inhibent également la méthanogénèse (Ahring & Westermann, 1987). Ces mêmes auteurs ont montré que l'ajout d'oxygène (O_2) dans le ciel gazeux d'un digesteur dégradant en co-culture du butyrate, stoppe la dégradation du butyrate.

1.3.4 Température

Dans le domaine de la digestion anaérobie, les microorganismes sont répartis en trois grandes catégories selon la plage des températures auxquelles leur croissance est optimale :
- les psychrophiles qui croissent entre 4-20°C, avec un optimum vers 15°C,
- les sub-mésophiles et mésophiles qui croissent entre 20-45°C avec un optimum vers 37°C,
- les thermophiles qui croissent entre 55-70°C, avec un optimum vers 60°C.

La figure 2 montre les plages de températures où la croissance de ces trois populations est possible. La dépendance des taux de croissance à la température peut être décrit pour une loi d'Arrhenius (Batstone *et al.* 2002). Karakashev *et al.* (2005) ont par ailleurs montré que les réacteurs mésophiles présentaient une diversité bactérienne supérieure à celle des réacteurs thermophiles.

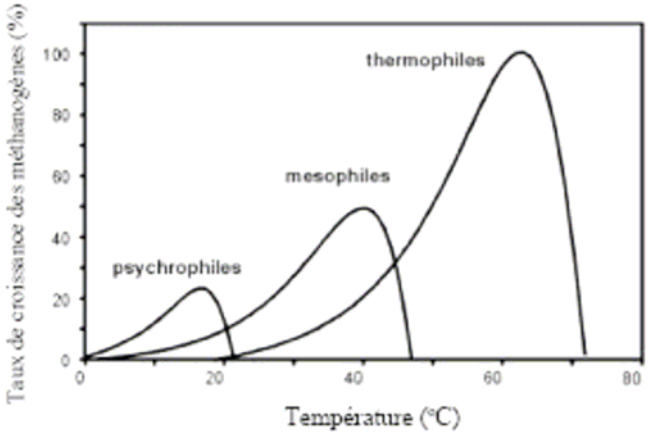

Figure 2 Effet de la température sur les taux de croissance des méthanogènes (van Lier *et al.*, 1997; Batstone *et al.*, 2002)

2 PRODUCTION ANAEROBIE DE BIOHYDROGENE

L'hydrogène a été découvert par Henry Cavendish en 1766 qui le dénommait « air inflammable ». Il a montré que l'hydrogène combiné avec de l'oxygène produisait de l'eau. Cette caractéristique amena Lavoisier à proposer le nom d'hydrogène (Piera *et al.* 2006). Le rêve de l'utilisation de l'hydrogène comme source inépuisable d'énergie a en fait commencé avec Jules Verne. Dans son roman L'Île Mystérieuse (1874), il imagina en effet l'hydrogène comme un substitut du charbon. Bien plus tard, l'hydrogène a été utilisé comme combustible, entrant à 50 % dans le mélange de gaz de ville fourni aux grandes métropoles jusque dans les années 1950 (Maddy *et al.* 2003). Ce gaz est d'ailleurs toujours utilisé en Chine, en Afrique du Sud et dans tous les lieux où le gaz naturel est cher (Bjørnar *et al.* 2002).

2.1 Principes généraux de production de biohydrogène par voie anaérobie

Dans les procédés anaérobies, l'hydrogène est produit essentiellement pendant l'acidogénèse des sucres par des espèces des genres bactériens, entre autres, *Enterobacter, Bacillus* et *Clostridium* sp. (Hawkes *et al.* 2002; Iyer *et al.* 2004). Les voies biochimiques qui décrivent l'acidogénèse montrent que les réactions qui produisent de l'éthanol, du lactate et du propionate ne peuvent produire de l'hydrogène car elles sont en compétition pour l'élément réducteur NADH (Figure 3). Par contre, celles qui produisent de l'acétate et du butyrate produisent de l'hydrogène (Vavilin *et al.* 1995; Hawkes *et al.* 2002). Les limitations énergétiques revêtent beaucoup d'importance dans ce cas. En effet, d'un point de vue stœchiométrique, il est possible de produire jusqu'à 4 moles d'hydrogène via l'acétate mais avec une limitation thermodynamique forte. Cette limite est connue sous le nom de *limite de Thauer* (Hallenbeck, 2005). Elle établit que l'oxydation enzymatique du NADH, réalisée par les hydrogénases et associée à la production d'hydrogène, a besoin d'une pression partielle en hydrogène inférieure à 10^{-3} atm. En pratique, cela signifie que la limite supérieure de la production anaérobie d'hydrogène est de 4 moles par mole de glucose avec de faibles débits

de gaz. Toutefois, dans la littérature, les rendements d'hydrogène obtenus en cultures complexes sont proches de 2 moles/mole de glucose due à une production combinée d'acétate et de butyrate (Hallenbeck, 2005).

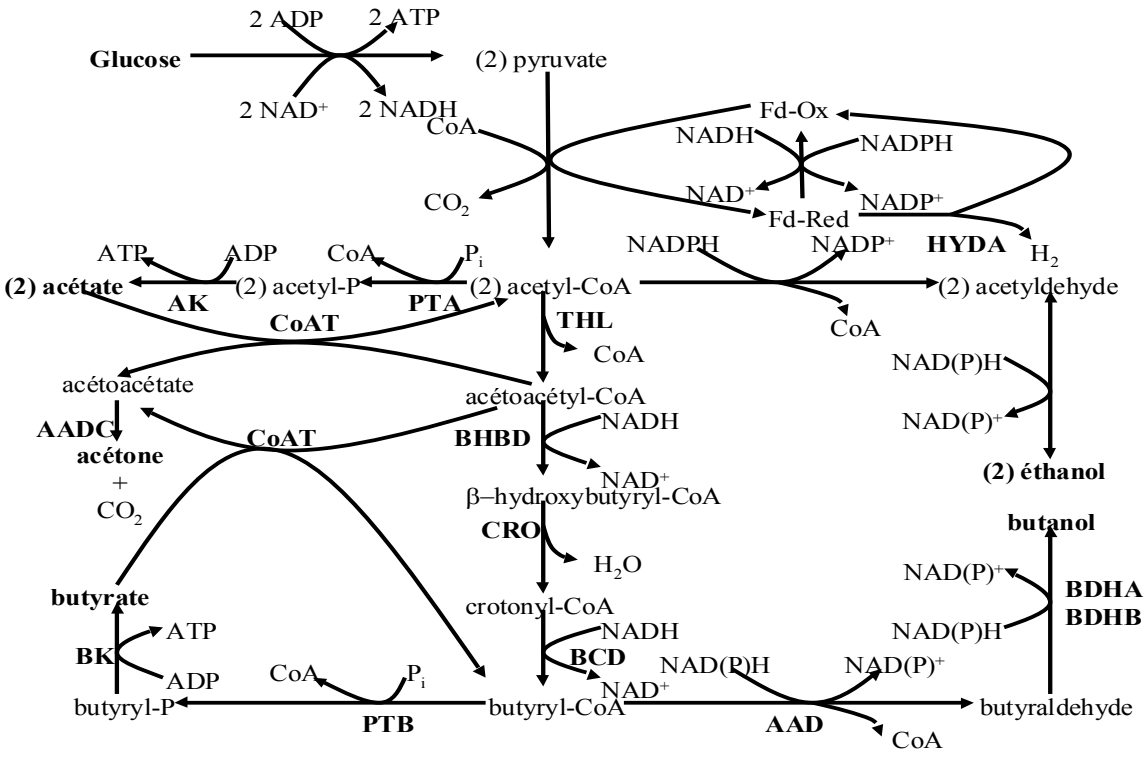

Figure 3 Principales voies biochimiques de l'acidogénèse (Desai *et al.*, 1999).

(HYDA) hydrogénase, (PTA) phosphotransacétylase, (AK) acétate kinase, (THL) thiolase, (CoAT) acétoacétyl-CoA:acétatebutyrate: CoA transférase, (AADC) acétoacétate decarboxylase, (BHBD) b-hydroxybutyryl-CoA dehydrogénase, (CRO) crotonase, (BCD) butyryl-CoA dehydrogénase, (PTB) phosphotransbutyrylase, (BK) butyrate kinase, (AAD) aldehyde:alcool dehydrogénase, (BDHA&B) butanol dehydrogénase A & B.

2.2 Facteurs influençant la production de biohydrogène

2.2.1 Le type d'inoculum

Des inocula de souches pures ou de cultures mixtes ont été utilisés pour la production d'hydrogène. *Clostridium butyricum* a été l'espèce la plus utilisée en culture pure (Yokoi *et al.* 1998a; Yokoi *et al.* 1998b; Yokoi *et al.* 2001; Yokoi *et al.* 2002; Chen *et al.* 2005; Zhang *et al.* 2006a; Lin *et al.* 2007), mais d'autres espèces telles qu'*Enterobacter* ont aussi été largement étudiées (Fabiano & Perego, 2002). Les cultures mixtes sont quant à elles issues de plusieurs sources dont le plus fréquemment des boues de stations d'épuration (Chen *et al.* 2002), des boues de compostage (Ueno *et al.* 1996), des décanteurs (Fang *et al.* 2002b) et des boues des digesteurs mésophiles (Lin & Chang, 1999; Zhang *et al.* 2006b).
Trois méthodes sont généralement utilisées afin de sélectionner les espèces productrices d'hydrogène à partir de cultures mixtes. Ces méthodes mettent à profit la capacité de sporulation présente chez des espèces telles que *Clostridium* sp.. Le traitement thermique des boues est la méthode la plus couramment utilisée (Li & Fang, 2007). Elle consiste en un

chauffage de l'inoculum, typiquement une centaine de degrés Celsius pendant quelques dizaines de minutes (Lay, 2000; Van Ginkel & Sung, 2001; Iyer *et al.* 2004; Zhang *et al.* 2006b; Mu *et al.* 2007). La deuxième méthode consiste à appliquer, lors du fonctionnement en continu des réacteurs, des temps de séjour hydrauliques courts afin de lessiver les bactéries les plus lentes et consommatrices d'hydrogène, comme les méthanogènes (Lee *et al.* 2002; Zhang *et al.* 2006b). Cette méthode est basée sur le fait que les micro-organismes producteurs d'hydrogène présentent des taux de croissances supérieurs à ceux des archaea. Des chocs de pH, inférieurs à 4 ou supérieurs à 10, sont la dernière méthode possible pour enrichir en producteurs d'hydrogène une culture mixte (Chen *et al.* 2002; Zhang *et al.* 2006b). Certains auteurs utilisent une combinaison de ces diverses méthodes afin d'obtenir une bonne sélection des micro-organismes. Ainsi, Zhang *et al.* (2006b) proposent d'utiliser un choc thermique associé à un choc de pH. D'autres auteurs ont préféré travailler avec de faibles temps de séjour associés à un pH bas (Fang & Liu, 2002; Lee *et al.* 2002) ou avec un prétraitement thermique (Iyer *et al.* 2004; Zhang *et al.* 2006b).

2.2.2 Composition du milieu en minéraux et en métaux

De nombreuses références montrent l'importance de la composition du milieu pour les bactéries productrices d'hydrogène (Lin & Lay, 2005; Zhang & Shen, 2006). La concentration en ions Fe^{2+} dans le milieu est très importante car il s'agit d'un constituant essentiel du site actif des hydrogénases (Das & Veziroglu, 2001). La quantité nécessaire d'ions Fe^{2+} varie en fonction de la température, toute augmentation de température conduisant à une meilleure activité des enzymes (Zhang & Shen, 2006). Zhang & Shen (2006) ont trouvé que la concentration optimale en ions Fe^{2+} était de l'ordre de 200 mg/L à une température proche de 35 °C.
Les concentrations en carbone et en azote sont également intimement liées. Lin et Lay (2004) ont montré que le ratio C/N optimal était de 47 en utilisant le saccharose comme substrat carboné. De plus, ils ont souligné une dérive métabolique de la production d'acétate vers celle de l'éthanol quand le ratio C/N diminue. Ils ont démontré qu'il était possible de limiter la concentration en carbone en utilisant des phosphates. Enfin, une étude générale des minéraux utilisés pour la production d'hydrogène a été faite par Lin and Lay (2005) en utilisant le saccharose comme substrat. Ils ont trouvé que les minéraux les plus importants étaient le magnésium, le sodium, le zinc et le fer.

2.2.3 Température

Comme pour tous les procédés biologiques, la température est un facteur à contrôler car elle réduit l'énergie d'activation des enzymes. Bien que pour les hydrogénases, la température optimale soit de l'ordre de 50 °C (Adams & Mortenson, 1984), des températures mésophiles comprises entre 35 et 37 °C ont été utilisées (Kraemer, 2004; Li & Fang, 2007). La principale raison est liée à une instabilité plus grande des procédés thermophiles par rapport aux procédés mésophiles (Hawkes *et al.* 2002). Lin & Chang (2004) ont également montré qu'une température inférieure à 35 °C nécessitait des temps de séjour plus longs afin d'avoir une bonne production d'hydrogène. Mu *et al.* (2006) ont, quant à eux, étudié les variations du rendement de conversion du substrat en hydrogène pour une gamme de températures allant de 33º à 41 °C. Ils ont observé que l'augmentation de la température orientait le métabolisme de l'éthanol vers le butyrate et augmentait aussi le rendement de conversion. Ils ont également estimé l'énergie d'activation pour la production d'hydrogène à environ 107,66 kJ/mol. Finalement, Li & Fang (2007) ont montré, au travers d'une étude bibliographique, que les rendements et les taux de production d'hydrogène étaient similaires pour les températures

mésophiles et thermophiles mais que les rendements étaient plus faibles pour des températures ambiantes (Lin & Chang, 2004; Li & Fang, 2007).

2.2.4 Nature du substrat

Les espèces de type *Clostridium* sp. ont une prédilection pour les sucres et particulièrement pour le glucose (Mitchell *et al.* 1995). Ainsi, les substrats synthétiques les plus utilisés pour produire de l'hydrogène sont le glucose (Lin & Chang, 2004; Van Ginkel & Logan, 2005; Mu *et al.* 2006; Li & Fang, 2007) et le saccharose (Hussy *et al.* 2005; Zhang *et al.* 2005; Chen *et al.* 2006a; Lin & Chen, 2006; Mu *et al.* 2007). Des sucres plus complexes ont toutefois été utilisés comme la cellulose (Ueno *et al.* 1995; Lay, 2001), les effluents de levureries (Guwy *et al.* 1997), les effluents d'une distillerie de riz (Yu *et al.* 2002), les déchets alimentaires (Han & Shin, 2004; Shin *et al.* 2004), les déchets de la production du biodiesel (Nishio & Nakashimada, 2007), les déchets ménagers (Lay *et al.* 1999; Nielsen *et al.* 2001) et les mélasses (Tanisho & Ishiwata, 1995; Ueno *et al.* 1996).

2.2.5 pH

Li et Fang (2007) ont répertorié plusieurs pH optimaux dans la littérature; Pour les études comportant une régulation du pH, les pH optimaux sont compris entre 5,2 et 7,0 sur substrats synthétiques en utilisant de cultures mixtes. Par contre, lorsque le substrat est un effluent ou un déchet, la gamme de pH optimal est plus réduite et est comprise entre 5,2 et 5,6.
Le pH modifie en effet le métabolisme de la production d'hydrogène. Fang et Liu (2002) ont par exemple montré que l'acétate était favorisé à un pH de 6,5 alors que le butyrate est favorisé à un pH inférieur à 6,0. Plus précisément, des pH optimaux ont été déterminés pour la production d'autres produits de fermentation, comme un pH de 4,3 pour le butanol (Kim *et al.* 2004), un pH compris entre 4,5 et 6,0 pour l'éthanol et entre 5,0 et 6,0 pour le propionate (Hwang *et al.* 2004).

2.2.6 Inhibitions

La production d'hydrogène au sein d'un réacteur anaérobie peut être perturbée par divers facteurs avec, entre autres, la sporulation des producteurs d'hydrogène et les problèmes de transfert de matière entre la phase liquide et la phase gazeuse.
En effet, Zheng et Yu (2005) ont décrit l'inhibition de la production de l'hydrogène par le butyrate par un mécanisme non-compétitif. La sporulation est également un phénomène qui peut se produire avec les micro-organismes producteurs d'hydrogène, essentiellement chez *Clostridium* sp. Elle est le résultat de la mise en place d'un système de protection lorsque les conditions ne sont pas favorables, comme un excès ou une carence en nutriments, la présence d'oxygène, ou une baisse du pH (Sauer *et al.* 1995). L'accumulation toxique de substrats au démarrage du réacteur (Yu *et al.* 2002; Hussy *et al.* 2005) ou des acides à un pH inférieur à 5 (Sauer *et al.* 1995; Hawkes *et al.* 2002) conduit à la production des sous-produits tels que, entre autres, l'éthanol, le propanol, ou le butanol.
Les réacteurs anaérobies présentent également une sursaturation des gaz dans la phase liquide (Pauss *et al.* 1990; Kraemer, 2004) qui favorise la formation de bulles (Pauss *et al.* 1990; Merkel & Krauth, 1999). Cette sursaturation peut thermodynamiquement empêcher les réactions productrices d'hydrogène et la conversion du NADH en hydrogène par les hydrogénases (Tanisho *et al.* 1998; Mizuno *et al.* 2000; Hallenbeck, 2005), voire en faciliter sa consommation (Kim *et al.* 2006). C'est pourquoi, il est important d'avoir un bon contrôle de la concentration en gaz dissous. Actuellement, seulement deux méthodes ont été proposées

pour extraire l'hydrogène sous forme de gaz dissous: l'utilisation d'un gaz inerte, comme l'azote ou le dioxyde de carbone, et l'augmentation de la vitesse d'agitation (Aceves-Lara *et al.* 2008). Le balayage avec un gaz inerte comme l'azote (Tanisho *et al.* 1998; Mizuno *et al.* 2000; Hussy *et al.* 2005) a parfois conduit à des résultats spectaculaires, comme une augmentation de 80 % du rendement de la production d'hydrogène (Hussy *et al.* 2005). Ceci étant, dans cette étude, le rendement était relativement faible puisque inférieur à 0,85 moles-H_2/mol-hexose sur du saccharose. Kim *et al.* (2006) ont également démontré que l'usage du dioxyde de carbone permettait d'avoir de meilleurs rendements que celui de l'azote car ce dernier inhibe les micro-organismes qui consomment l'hydrogène. Finalement, l'augmentation de la vitesse d'agitation de 100 à 500 tr/min a permis à Lay (2000) de doubler les rendements en hydrogène.

2.2.7 Rendements de production

Le tableau 1 présente un résumé de la littérature des différents rendements obtenus en utilisant des réacteurs continus. Ce tableau prend en compte le type de réacteur, le pH, la température, le temps de séjour hydraulique (TSH), la charge volumique appliquée (CVA) et le type d'inoculum. En général, à de bons rendements correspondent de faibles débits en hydrogène. Cette relation n'empêche pas d'obtenir des débits d'hydrogène élevés en augmentant la charge volumique appliquée. Les meilleurs rendements ont, par ailleurs, été obtenus en utilisant des réacteurs infiniment mélangés avec comme substrat du saccharose (2,26 mol d'H_2/mol-hexose – (Chen & Lin, 2003)) et du glucose (2,8 mol d'H_2/mol-hexose – (Van Ginkel & Logan, 2005)). Ces résultats sont très intéressants puisque le rendement théorique maximal estimé en conditions anaérobies à partir du glucose est de 3,26 mol-H_2/mol-hexose (Chen *et al.* 2006b). Ce rendement a pris en compte la formation de la biomasse par *Clostridium butyricum*. De plus, dans le cas du glucose, les rendements élevés sont aussi liés à la sélection de l'inoculum. Finalement, il est important de souligner que des espèces telles que *Klebsiella pneumoniae* (anaérobie facultative) permettent d'atteindre des rendements théoriques plus élevés allant jusqu'à 6,68 mol-H_2/mol-hexose en conditions microaérobies qui restent toutefois des conditions très difficiles à maintenir en pratique (Chen *et al.* 2006b).

Tableau 1 Meilleurs rendements obtenus dans la littérature pour des réacteurs continus

Alimentation	Type de réacteur	pH	Température (°C)	TSH (h)	CVA ($g_{DCO}.L^{-1}j^{-1}$)	Rendement ($molH_2.mol\text{-}hexose^{-1}$)	Débit H_2 ($mLH_2.h^{-1}$)	Inoculum	Référence
Glucose	Infiniment mélangé	5,7	35	6	80	1,7	209	Boues de digesteur	(Lin & Chang, 1999)
Glucose	Infiniment mélangé	5,5	36	6	30	2,1	97	Boues de réacteur d'hydrogène (choc pH)	(Fang & Liu, 2002)
Glucose	Semi-continu	5,0	35	72	5	1,4	43	Boues de digesteur	(Hwang *et al.* 2004)
Glucose	Infiniment mélangé	5,5	37	10	24	1,8	436	Sol (choc thermique)	(Iyer *et al.* 2004)
Glucose	Lit fixe	6,7	35	1	480	0,5	1210	Boues de digesteur (choc thermique)	(Chang *et al.* 2002)
Glucose	Bioréacteur à membrane	5,5	-	3,3	77	1,0	640	Boues de digesteur (choc thermique)	(Oh *et al.* 2004)
Glucose	Infiniment mélangé	-	30	10	6	2,8	65	Sol (choc thermique)	(Van Ginkel & Logan, 2005)
Glucose	Infiniment mélangé	-	30	1	240	2,8	65	Sol (choc thermique)	(Van Ginkel & Logan, 2005)
Saccharose	Infiniment mélangé	6,7	35	13,3	40	2,23	2500	Boues de décanteurs aérobie	(Chen & Lin, 2001)
Saccharose	Infiniment mélangé	7,0	35	8	67	2,26	662	Boues de décanteurs aérobie	(Chen & Lin, 2003)
Saccharose	Infiniment mélangé	7,0	35	179	67	1,44	1121	Boues de décanteurs aérobie	(Chen & Lin, 2003)
Saccharose	Infiniment mélangé avec des flocs	5,5	26	6	52	1,95	541	Boues de décanteurs aérobie	(Fang *et al.* 2002a)
Saccharose	Infiniment mélangé avec des flocs	6,0	35	6	80	1	1810	Boues de décanteurs aérobie (choc thermique)	(Wu & Chang, 2007)
Saccharose	Infiniment mélangé (balayage au CO_2)	5,3	35	12	40	1,68	660	Boues anaérobies (choc thermique)	(Kim *et al.* 2006)
Distillerie de riz	Flux ascendant	5,5	55	2	408	2,14	159	Boues de décanteurs aérobie	(Yu *et al.* 2002)
Amidon	Infiniment mélangé (balayage à N_2)	5,2	35	12	21	1,87	123	Boues anaérobies (choc thermique)	(Hussy *et al.* 2003)

3 *LA VALORISATION DU BIOGAZ*

3.1 Valorisation du méthane

La digestion anaérobie est intéressante car le biogaz produit peut-être valorisé. Le choix d'un mode de valorisation du biogaz découle principalement d'un calcul de rentabilité. De nombreux critères comme la proximité d'utilisateurs finaux pour l'électricité et/ou la chaleur produite(s), ou encore de la composition du biogaz brut entrent en ligne de compte. Le biogaz doit être purifié avant son utilisation pour en retirer les composés toxiques et corrosifs comme H_2S. Il peut également être nécessaire d'enrichir le biogaz en méthane pour accroître son pouvoir calorifique.
Les caractéristiques physico-chimiques du biogaz sont proches de celles du gaz naturel, ce qui permet sa valorisation sous diverses formes:
– énergie thermique (chaudière ou groupe frigorifique),
– énergie mécanique (moteur à gaz, turbine à vapeur, turbine à gaz),
– production simultanée d'énergie thermique (chaleur ou froid) et de travail par cogénération,
– production simultanée de chaleur, de travail et de froid par trigénération,
– carburant automobile (après purification poussée, compression et stockage),
– injection dans le réseau de gaz de ville.
Le méthane est le seul composé du biogaz énergétiquement intéressant et le pouvoir calorifique du mélange dépend uniquement de la pression partielle en méthane. Pour du méthane pur, le pouvoir calorifique est de 12,67 $kWh.m^{-3}$ et pour un biogaz contenant 70% de CH_4, il sera donc de 8,87 $kWh.m^{-3}$, soit 32 $MJ.m^{-3}$.

3.2 Valorisation de l'hydrogène

Ces dernières années, l'hydrogène a reçu une attention particulière car il présente un grand pouvoir calorifique (*i.e.,* 122 kJ/g) et est utilisé en combustion propre en produisant uniquement de l'eau. Il est ainsi considéré comme étant le vecteur énergétique idéal car il permet le stockage et le transport d'énergie sur de courtes, moyennes et longues durées (Berry & Aceves, 2005; Orecchini, 2006). De plus, toutes les énergies renouvelables sont transitoirement transformables en hydrogène en tant que forme de stockage, ce qui renforce d'autant plus son attrait.
Entre autres, la pile à combustible permet de convertir l'hydrogène stocké en électricité. Il est à noter que le principe de la pile à combustible a été inventé dans les années 1800 et ce, bien avant le moteur à combustion interne. Son développement s'est ensuite accéléré à partir des années 1960, principalement sous l'impulsion des programmes spatiaux de la NASA (Bjørnar *et al.* 2002; Maddy *et al.* 2003; Berry & Aceves, 2005). Aujourd'hui, les piles à combustibles sont utilisables pour des voitures particulières mais avec des rendements encore faibles, proches de 40 % (Hetland & Mulder, 2007). La pile à combustible fonctionne comme une batterie, dans laquelle une membrane anodique et une membrane cathodique sont séparées par un électrolyte (Fuel Cell Handbook, 2000). L'anode et la cathode sont alimentées en continu par de l'hydrogène et de l'oxygène, respectivement, en produisant du courant électrique et de l'eau (Figure 4). La principale différence entre une batterie et une pile à combustible est que cette dernière ne consomme pas d'électrolyte mais ne fonctionne qu'en présence de débits continus en hydrogène et en oxygène. L'inconvénient majeur des piles à combustible réside dans la nécessité de l'utilisation d'un catalyseur en platine qui est relativement coûteux et sensible à l'empoisonnement par des impuretés.

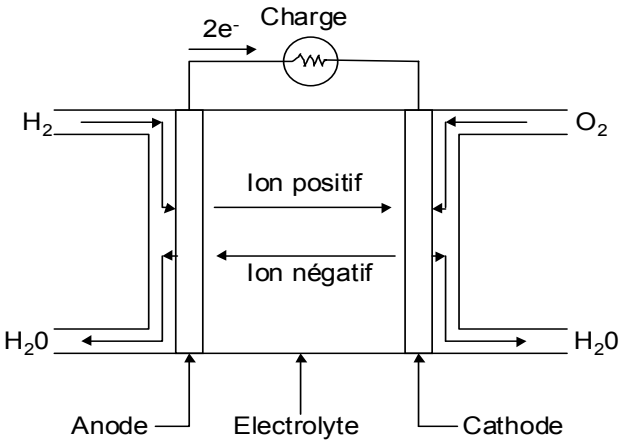

Figure 4 Principe de fonctionnement d'une pile à combustible à H_2

Outre le fait que l'hydrogène est un excellent vecteur énergétique, le principal inconvénient de son utilisation est que, d'un point de vue volumique, il n'est pas très attractif puisque son pouvoir calorifique est quasiment divisé par un facteur 10 (i.e., 1 L de H_2 est équivalent au 0,27 L d'essence). Il est donc nécessaire de le comprimer fortement ou de le liquéfier, la liquéfaction nécessitant des températures très basses consommant de 30 à 40% de l'énergie contenue dans l'hydrogène (Berry & Aceves, 2005). La compression implique également que, pour son transport, il faille utiliser des conteneurs résistants à des hautes pressions qui ne sont pas actuellement disponibles dans le commerce. L'autre inconvénient majeur est lié à sa détonabilité avec une limite de détonation dans l'air de 18,3 à 59 % (vol/vol) et à son inflammabilité à l'état gazeux ou liquide avec une limite d'inflammabilité dans l'air (4,1 à 75 % (vol/vol) (Piera et al. *2006).*

3.3 Couplage des procédés

Le couplage de différents procédés biologiques a été imaginé afin d'augmenter les rendements de conversion en hydrogène et en méthane. Ainsi, plusieurs configurations ont été imaginées et testées expérimentalement.

Un exemple est le couplage d'un réacteur de production d'hydrogène avec un réacteur de méthanisation (Figure 5). Le méthane ayant un apport énergétique égal à 800 kJ/mole, la production d'hydrogène dans une première étape et de méthane dans une deuxième, permet d'obtenir une distribution des productions d'énergie de 37,5 % et 62,5 % pour chacun des gaz respectifs. Liu *et al.* (2006) ont par ailleurs montré qu'une telle configuration était capable d'améliorer les rendements de production du méthane. Cette configuration peut toutefois présenter des problèmes de contamination si la sortie du deuxième réacteur est recyclé dans le premier (Kraemer & Bagley, 2005).

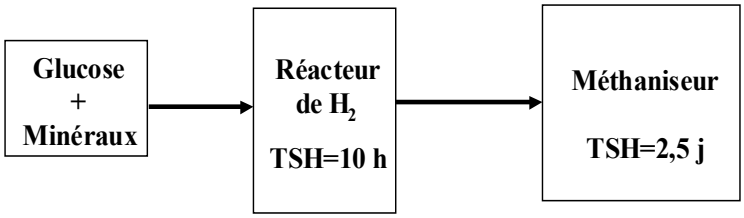

Figure 5 Couplage d'un réacteur produisant de l'hydrogène avec un méthaniseur

A partir d'une analyse technico-économique des filières de production du biogaz et du biohydrogène, Elias *et al.* (2007) ont comparé plusieurs configurations de procédés à une ou deux étapes. Ils ont envisagé des configurations qui prendraient en compte la valorisation à des degrés variables du biogaz et du biohydrogène en co-génération. Suivant les hypothèses faites en termes de coûts d'investissement et de fonctionnement, ces configurations présentent finalement des temps d'amortissement assez proches compris entre 5 et 6 ans. La réduction de ce temps est fortement liée au prix de rachat de l'électricité, qui est essentiellement fixé par

les politiques publiques. En France, dans ce contexte – et afin d'encourager cette technologie de production d'énergies renouvelables et d'assurer un seuil de rentabilité suffisant – de nouvelles conditions tarifaires de rachat de l'électricité produite à partir du biogaz issu d'une unité de méthanisation ont été fixées et publiées dans l'arrêté de 10 Juillet (2006).

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ANNEXE II

OPTIMIZING BIOGAS PRODUCTION FROM ANAEROBIC DIGESTION

OPTIMIZING BIOGAS PRODUCTION FROM ANAEROBIC DIGESTION[1]

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ABSTRACT

Anaerobic treatment of organic wastes for biogas production is a way to substitute fossil fuels and to reduce disposal of waste in landfills. Economic evaluation of biogas plants has revealed that many plants can only survive economically if special incentives are applied. Moreover, recent findings indicate that many biogas plants are operating sub-optimal and 25 to 40% of the biogas potential of biomass is not utilized by the traditional operation of the plants. It is therefore necessary to find ways to optimize the biogas production in order to make biogas plants economically viable with decreased or no subsidies. Optimization of the biogas process can undoubtedly lead to better economy. This optimization can be achieved by advanced monitoring and control of the biogas process.

KEYWORDS

Anaerobic digestion, biogas, optimization, modeling, control

INTRODUCTION

The world presently derives some 60% of its energy from fossil fuels. It is however widely recognized that the supplies of these are limited and, at projected future rates of consumption, are likely to be depleted well before the end of this century (ASPO 2002). One of the great challenges of the new century is therefore to obtain new sources of renewable energy, capable of replacing fossil fuels.

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In addition to renewable sources of energy such as solar, wind or hydroelectric energy, the use of solid, liquid and gaseous fuels from biomass-based raw materials is of importance. Biomass includes a broad range of materials (agriculture and forestry products and residues, fast-growing trees and grasses, farm and food wastes, municipal sludge and solid wastes, animal manure, marine and aquatic plants, industrial and manufacturing wastes) which are biological in nature and can be used to generate various forms of bioenergy. As such, biomass is a desirable source of renewable energy which can be converted by direct combustion or biological and/or thermochemical liquefaction or gasification into a variety of biofuels.

Among these biofuels, biogas produced from anaerobic digestion of biomass is potentially a very important one.

Anaerobic Digestion (AD) is a complex series of biological processes that take place in the absence of oxygen and by which organic matter is decomposed and bioconverted on one hand into biogas (*i.e.,* a mixture of mainly carbon dioxide and methane) and, on the other hand, into microbial biomass and residual organic matter.

AD process is naturally present in many ecosystems such as the digestive tract of insects (*e.g.,* termite) and mammalians (*e.g.,* cows, pigs, sheep, rats) and human beings as well as in natural and cultivated ecosystems like wetlands, marine sediments and rice fields where it is actively involved in biogeochemical cycles of matter.

Uncontrolled anthropogenic emission of methane into atmosphere from man-made sources (domestic animal wastes, landfills, rice fields, agricultural and forestry residues, industrial wastes, lagoons, …) is approximately 250 millions metric tons/yr worldwide. The energy value of this biomethane is about 14.2×10^{18} joules/yr which is equivalent to approximately 15 percent of the energy consumed in the United States. Recovery of this energy as biofuels is the first step towards optimization of biogas plants. It would first reduce global warming (methane is indeed an important greenhouse gas because of its capacity to trap heat) and second, avoid use of fossil fuels. All told, a 20 percent reduction in global warming may be achieved by utilization of organic wastes and residues for the production of biofuels and chemicals (Ghosh, 1997).

AD is also considered as one of the oldest technologies for waste and wastewater treatment. It has been indeed applied since the end of the 19th century for the treatment of household waste(water)s in septic tanks, of slurries in digesters and of sewage sludge in municipal treatment plants. It is also probably the major biological process involved in landfill wastes decomposition.

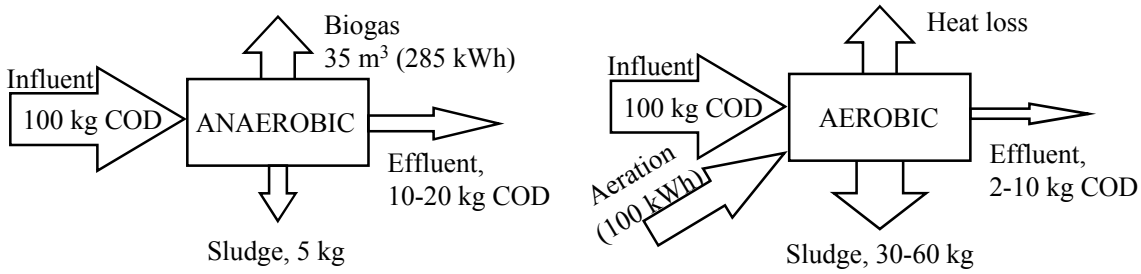
Several advantages are recognized to AD processes when used for waste and wastewater treatment: high capacity to treat slowly degradable substrates at high concentrations, very low sludge production (5 to 10 times less than in aerobic processes), potentiality for valuable intermediate metabolites production, low energy requirements (no aeration is required), reduction of odors in a closed system, pathogens reduction and possibility for energy recovery through methane combustion. As a consequence, AD compares very favorably with activated sludge processes in terms of energy balance and sludge production (*Cf.* Figure 1) and this makes it very well adapted to highly concentrated wastewater and solid wastes. Last but not least, when carried out properly and thoroughly, the digestion process will transform toxic organic materials into clean fertilizers which are free of pathogens and weed seeds.

As an illustration, Cecchi and Bolzonella (2005) reviewed the full scale experiences of AD of the organic fraction of municipal solid waste in Italy and they showed that 3.9 to 4.4 kWh could be produced for 1 KWh consumed when treating source sorted municipal waste.

AD is also used in different country worldwide. For example, 85 full-scale reactors were in operation in 1998 for wastewater treatment in Mexico (Monroy et al., 2000). Their total installed volume was 228,551 m^3 and they were treating 216,295 m^3 d^{-1} wastewater and 590 tons COD per day, which is equivalent to a population of 12.3 million.

AD has been also applied in China for the control of pollution and production of energy for more than 80 years and more than one million small scale digestion tanks have been built in farmers household in South East of China since 1950's (Yi, 1997).

Figure 1: Comparison of anaerobic digestion with activated sludge processes



SITUATION IN EUROPE

It has been demonstrated that a systematic approach to anaerobic treatment offers promise as the single most cost effective means of reducing direct and indirect greenhouse gas and nutrient emissions, and hence make a substantial contribution to meeting EU common environmental, economic and agricultural policies (van Lier *et al.,* 2001).

Europe is thus very active in conducting research on AD process. For example, anaerobic treatment of organic wastes in biogas plants has been promoted for the last two decades in many European countries, both as a way to substitute fossil fuels (reducing global emissions) and to reduce disposal of waste in landfills (*Cf.* Figure 2). Indications are indeed that anaerobic treatment can provide a cost effective solution to municipal organic waste problem while at the same time providing renewable energy (Murphy and Power, 2006).

As an illustration of this European increasing interest for AD technology, there are presently about 2700 biogas plants at the farm scale in Germany with an installed total electrical output of approximately 665 MW (*Cf.* Figure 3). Moreover, the increasing number of biogas plants is not only accompanied by an improvement of the plants, but also by an increasing number of different procedures and equipment suppliers.

Figure 2: Installed capacity for anaerobic treatment of municipal solid wastes in Europe (from De Baere, 2005)

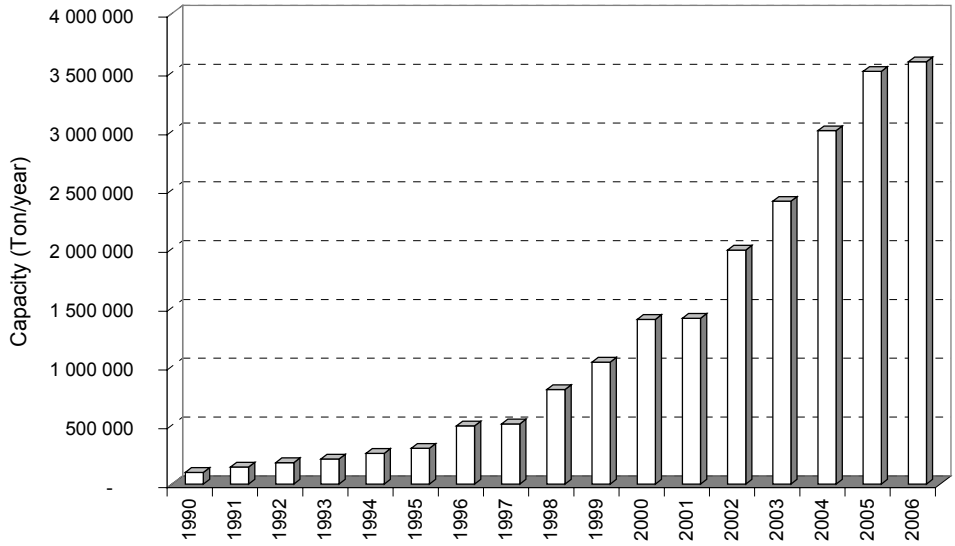
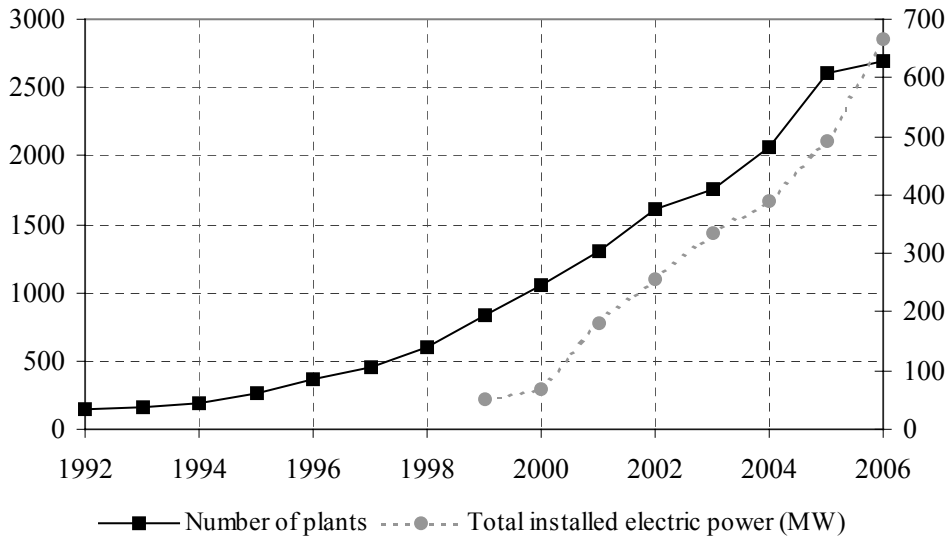


Figure 3: Development of farm scale biogas plants in Germany



In Sweden, several study were conducted to evaluate economical feasibility of biogas plants from crop residues on a farm-scale level. It appeared that simple but effective high-solids reactor systems have a better chance of being economically viable and that the methane yield, the degree of gas utilization and operational costs have the strongest impact on the financial success of the process (Svensson *et al.*, 2005; 2006)

The situation is a little bit different in Denmark where "only" 22 full scale biogas plants are in operation but these plants are centralized processes. Each plant is indeed shared by several farms or owned by a municipality and their main purpose is to treat livestock manure and reuse the material as fertilizer. The plants range in size from 550 m^3 to 8500 m^3 with a conversion capacity of 25 to 700 tons biomass per day. In 2001, these centralized biogas plants treated approximately 1.2 million tons of manure. The methane yield of manure typically ranges from 10 to 20 m^3 CH_4/t of manure treated, which unfortunately is today inadequate to obtain an economically profitable result (Angelidaki and Ellegaard 2003). As a consequence and in order to increase the biogas production of the plants, the manure is often co-digested with organic waste from food industries and municipalities that is rich in fat and protein. The use of these substrates can improve the economy of the plants but may also, if not handled properly, lead to inhibitions of the process. At various intervals, the plants are indeed suffering severe process imbalance and it often takes months to recover with serious economic consequences.

As an illustration, in the autumn of 2004, the biogas plant codigesting pig manure and industrial wastewater in Blaabjerg, Denmark, experienced a serious accident due to unintentional overdose of industrial waste. It led to a reduced gas production and periodically, the gas quality was so poor that it could not be used in the engines for cogeneration but had to be burned in the torch, serving no useful purpose. Following this overload, it took about three months before the biological process was stable again and the gas production returned to its normal level. The total operational loss was subsequently calculated as just under one million DKK (approximately 150,000 US$).

The most frequent process imbalances in these centralized Danish biogas plants are due to increasing concentrations of free ammonia or long chain fatty acids, which is a result of:

a) Inexpedient mixing of the different waste products in pre-storage tanks, hindering exact feeding of specific waste to the reactors.
b) Inadequate knowledge about the substrate composition.
c) Inadequate knowledge about the degradation characteristics of the waste, with regard to inhibition level and biogas potential.
d) Inadequate process surveillance, especially with regard to volatile fatty acids.

Moreover, economic evaluation of biogas plants has revealed that small scale plants can only survive economically if special incentives favor energy from biogas compared to energy from conventional fossil fuels. As a consequence, in France, since energy from biogas plants was rather cheap (4 to 7 cents per kWh), AD was not as popular at the farm level as in Germany. Only 3 plants were indeed in operation in France in 2005 (Ragonnaud, 2005), a rather small number compared to the 2700 in German farms ! But the increase of the price of energy from biogas decided in July 2006 by the French Ministry of Industry (9 to 14 cents per kWh – JORF, 2006) should hopefully change these numbers in the near future.

Nevertheless, in France, AD is rather popular for the treatment of industrial wastewater and especially in the food, chemical and paper industries. Hundred thirteen (130) digesters were indeed constructed in 2003 for industrial wastewater (*Cf.* Figure 4) and previsions are that this number should be doubled within 10 years (AND, 2003). The exact same trend can be noticed worldwide (*Cf.* Figure 5) with about 1350 plants referenced in 1999 (Totzke, 1999) and this trend is confirmed since more than 2000 industrial plants were installed in 2004.

Figure 4: Number of industrial biogas plants in France

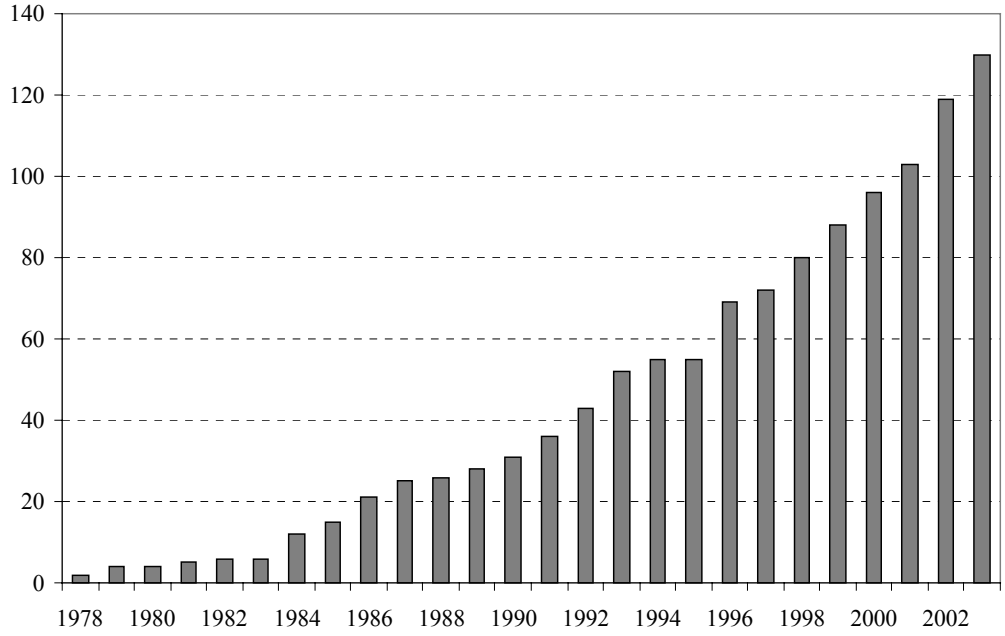
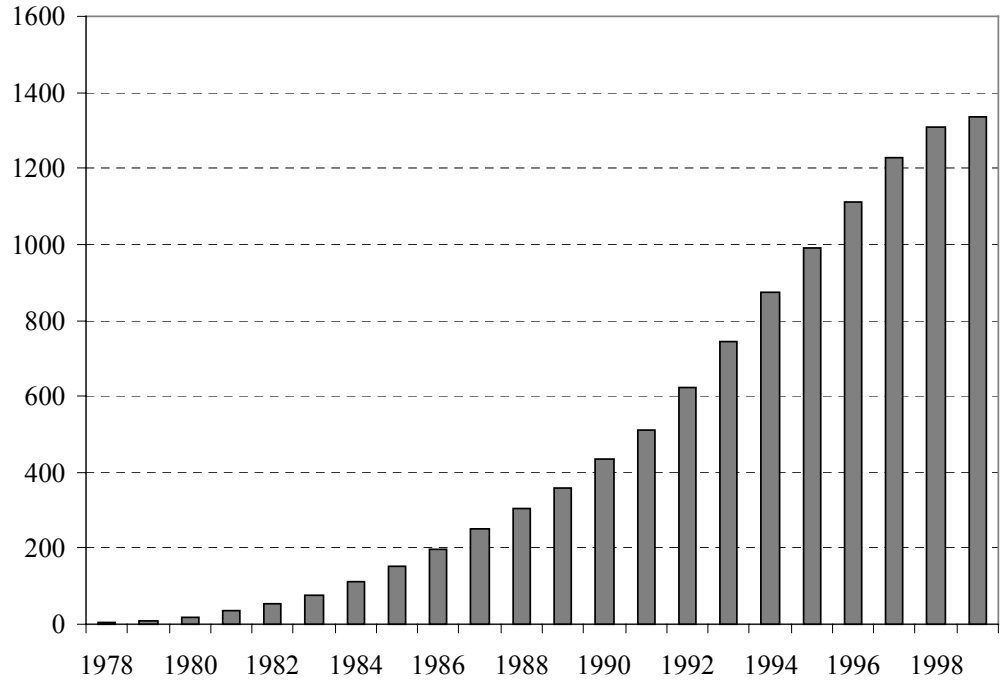


Figure 5: Number of industrial biogas plants worldwide



OPTIMIZATION OF BIOGAS PLANTS

Despite this already high interest for biogas plants worldwide and to significantly further expand anaerobic waste treatment, it is necessary to find ways to optimize the biogas

production. This will make biogas plants economically viable without, or with fewer subsidies. Indeed, optimization of the biogas process will undoubtedly lead to better economy and this can be achieved with better monitoring and control of the biogas processes.

As an illustration, in Denmark, it is recognized that on-line sensors and efficient monitoring of the process could give early warnings which are valuable information for control and optimization of the biogas process: 10% more biogas can thus be obtained due to better stability of the plants, which corresponds to 5 million m^3 biogas per year.

Nevertheless, the monitoring of biogas plants is currently rudimentary, mainly based on observation of the biogas production. Evaluation of the plant by the operator is generally subjective, and by change in influent flow. Two types of operation strategies can result from this type of control. The "cautious operator" keeps the loading low to be sure to avoid overloading and unpleasant surprises. However, the process will run at a sub-optimal level and the microbial populations will be present in a slow and un-dynamic state, as often it is the case today. The result is low plant productivity, with stable but relative low operating profit.

The second type of strategy is chosen by the "brave operator", who keeps increasing the loading resulting in an increased production of biogas, until the point where the process shows signs of overloading. Since overloading may be difficult to detect in time without real process monitoring, this strategy may lead to occasional long lasting process failures. Often these failures have serious economical consequences. The operating profit might be relatively high for periods, but the long-term result is risky.

In order to obtain an optimal (*i.e.,* brave but safe) operation of anaerobic digestion processes, the missing link is reliable information of the true biological state of the process. This indication can be supplied by monitoring specific intermediates formed during the anaerobic digestion process. Development of robust on-line process indicators can indeed lead to proper control strategies that will allow optimization of the process, without undue risk of process failures.

Many parameters have been considered for their suitability as process state parameters (See for example Smith and Mc Carthy, 1990; Moletta *et al.,* 1995, Vanrolleghem 1995; Bjornsson *et al.*, 2001; Steyer *et al.*, 2002a; Liu, 2003; Premier, 2003; Angelidaki and Pind, 2003, Morel *et al.,* 2004; Boe, 2006 and related references). As a summary of these studies and from the point of view of a plant manager, a control parameter must:

- give clear warning of a potential collapse but, on the other hand, not give unnecessary warning (*i.e.,* minimize the false alarms and avoid the "Christmas tree" syndrome for plant operators),
- early warn of an unstable process, not just a total collapse since it is then too late to react on the process,
- reflect when the process has re-established itself after a collapse,
- be easy to measure and preferably on-line.

If the above-mentioned guidelines are taken into account, four parameters are of particular interest: the methane production, the concentration of hydrogen, pH and the concentration of volatile fatty acids.

The methane production

As the end products of the digestion process mainly consist of biogas, a registration of the gas production is today the most commonly used control parameter. Usually, a distinction is made between the total gas production and the relative production, which indicates the amount of methane in relation to the amount of waste or wastewater added.

The total gas production may be used to gain an impression of the activity of the methane bacteria, but it does not give a precise impression of the process situation. Indeed, a decline in the gas production may be related to the methane bacteria being inhibited, but it may also be due to failure to add enough substrate to be degraded. Moreover, overloading of the reactors will often result in increased biogas production followed by a sudden drop and by then, it may be too late to intervene. Use of the total gas production as the only control parameter is therefore not recommended.

On the other hand, the relative gas production reflects how effectively the added material is degraded. The obtained values can be compared with a theoretical methane yield (*i.e.,* 0.350 liter per gram of COD degraded), which can be calculated assuming that the input substrate quality has not changed. Unfortunately, it is a rather time-consuming process, partly because the theoretical methane yield from complex substrate (*e.g.,* animal manure) can vary considerably. The relative gas production is therefore not practically useful for registering sudden changes in the process, although it can be quite useful for the subsequent evaluation of total collapses.

The concentration of hydrogen

Laboratory tests have determined that a low hydrogen pressure in a reactor is necessary to ensure an effective process. This is obtained when various bacteria convert hydrogen and carbon dioxide into methane and water. An increase of the hydrogen pressure may therefore be an indication of an imbalance between the different microorganisms, for instance when large amounts of easily convertible material have been added.

As hydrogen can be measured relatively easily in both the gas and the liquid phases, use of hydrogen as a control parameter has received quite a lot of attention in recent years (see for example the pioneer work of Archer *et al.,* 1986; Pauss *et al.,* 1990; 1993; Strong and Cord-Ruwisch, 1995 and later Bjornsson *et al.,* 2001). Unfortunately, the results have been very variable. The problem is that the concentration of hydrogen is often very sensitive to changes in the composition and amount of added material and it is to be expected that the concentration of hydrogen may give rise to many false alarms. The use of hydrogen as a single control parameter is therefore not realistic in many biogas plants and not at all as the only parameter.

Volatile fatty acids and pH

Volatile fatty acids (VFA) constitute most of the intermediates in the biogas process and among researchers, there is no doubt that the VFA level is very important for the maintenance of a stable digestion process.

There has been, however, much dispute about how to use a registration of VFA. Many studies have compared the concentration of the acid level with occurring interruptions of the process.

For instance, it has been mentioned that a concentration of 0.8 grams of acetate/liter indicates an interruption of the process, while up to 9 grams of VFA/liter, anaerobic digesters can still work efficiently without any sign of imbalance (Dupla *et al.,* 2004). However, it is not possible to generalize on the basis of such results, as the individual reactors have their own acid levels dependent on the operating conditions. Instead, the VFA level of each individual reactor should be used as an indicator. Several biogas plants measure the total acid level in the reactors from time to time. This may be done in a relatively simple way by means of titration and can give a hint of changes in the process, but it is advisable to use this with care as the only control parameter.

Despite some indication in the scientific literature (Clark and Speece, 1971), it can be debated whether pH can be used as a control parameter, because an increase in the concentration of acids must be expected to affect the pH. This is particularly true when treating highly buffered wastewater. An increase in the acid level will indeed not necessarily result in a substantial pH drop and often, a pH drop will not be registered until the VFA level has increased considerably and the process has become unstable. Therefore, pH is too slow to be useful as a single indicator, although it may be suitable in connection with other parameters.

On-line measurements of VFA

How often is it necessary to measure the VFA concentration? During a normal stable process operation, where no major disturbances are present, it might be sufficient to measure the level few times a week. However, in periods when operational changes are made, for instance by the addition of new types of waste in case of codigestion, measurements should be made as often as possible.

Unfortunately, measuring the VFA has been regarded as complicated for many years. One main reason is that, in practice, it is often difficult to undertake automatic sampling and the treatment of samples is itself time-consuming.

However, in recent years, there have been developments in this area (See for example Steyer *et al.,* 2002b, Feitkenhauer *et al.,* 2002, Pind *et al.,* 2003; Boe *et al.,* 2005; Ruiz *et al.,* 2005). Online measurement of the VFA level will make it much easier to avoid possible process interruptions and it will be safer to treat new and complex types of waste. At the same time, the use of the VFA measurements will provide various information that can be used to develop different "recipes" for regulating the operation of a biogas plant. Greater pressure can thus be put on the reactors than today without the risk of a collapse of the process (see for example Punal *et al.,* 2003, Steyer *et al.,* 2006).

Unfortunately, despite some reliable industrial sensors already available on the market (see for example the Anasense® VFA sensor from Applitek, a Belgium company), it is likely to take some years before these systems are commonly available, reasonable priced and sufficient reliable. Until then, much can be gained by following the below procedures:

- Generally ensure constant process temperature, agitation, load and composition of raw material,
- Make all necessary changes on the basis of a precautionary principle and gradually, especially with regards to changes in the composition of the material to be treated and the load,

- Always introduce new raw material with great care, especially if they contain a big amount of fat, proteins or similar. If possible, new types of raw materials should also be tested before being added by means of a simple test in a laboratory plant.
- Continuously evaluate the gas production in relation to expectations based on the addition over the last few days and hold back if inexplicable drops or major increases occur
- Undertake regular VFA measurements. The VFA level should be checked on a regular basis, even if there may be long periods without any "interesting" results.

CONCLUSION

Several advantages are recognized to anaerobic digestion processes: high capacity to treat slowly degradable substrates at high concentrations, very low sludge production, potentiality for valuable intermediate metabolites production, low energy requirements, reduction of odors and pathogens and possibility for energy recovery through methane combustion or even hydrogen production. However, AD processes also have drawbacks:

- The low sludge production is closely linked to the slow growth of micro-organisms. As a consequence, the start-up phase is often tedious and some time is required (*e.g.,* 2-4 months or longer) before steady state conditions are obtained.
- AD micro-organisms are highly sensitive to overloads of the process and disturbances of several causes.
- AD is a complex process involving many different micro-organisms which is still not completely understood.

These drawbacks explain probably that AD processes are not more widely used at the industrial scale. In the past, the lack of knowledge concerning AD processes led indeed to breakdowns, ranging from minor to catastrophic, mainly due to organic overloads of various origins. They created some kind of suspicion towards this process and delayed its development at the industrial scale. This is why actual research aims not only to extend the potentialities of anaerobic digestion, but also to optimize anaerobic processes and increase their robustness towards perturbations. Thus, the importance of implementing appropriate, carefully designed and efficient monitoring and control strategies (including use of on-line sensors) is of no doubt and will lead to a better economy of the biogas processes. It is indeed our strong belief that with appropriate information about the current state of the process, biogas plants are quite easy to operate and to optimize.

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ANNEXE III

MODIFIED ADM1
DISINTEGRATION/HYDROLYSIS STRUCTURES FOR MODELING BATCH THERMOPHILIC ANAEROBIC DIGESTION OF THERMALLY PRETREATED WASTE ACTIVATED SLUDGE

Modified ADM1 disintegration/hydrolysis structures for modeling batch thermophilic anaerobic digestion of thermally pretreated waste activated sludge[1]

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ABSTRACT

Anaerobic digestion disintegration and hydrolysis have been traditionally modeled according to first-order kinetics assuming that their rates do not depend on disintegration/hydrolytic biomass concentrations. However, the typical sigmoid-shape increase in time of the disintegration/hydrolysis rates cannot be described with first-order models. For complex substrates, first-order kinetics should thus be modified to account for slowly degradable material. In this study, a slightly modified IWA ADM1 model is presented to simulate thermophilic anaerobic digestion of thermally pretreated waste activated sludge. Contois model is first included for disintegration and hydrolysis steps instead of first-order kinetics and Hill function is then used to model ammonia inhibition of aceticlastic methanogens instead of a non-competitive function. One batch experimental data set of anaerobic degradation of a raw waste activated sludge is used to calibrate the proposed model and three additional data sets from similar sludge thermally pretreated at three different temperatures are used to apply it and to validate the parameters values.

KEYWORDS: ADM1, Contois model, Hill function, inhibition, thermophilic anaerobic digestion, waste activated sludge

1. Introduction

Anaerobic digestion is a multi-step biological process where the organic carbon is converted to its most oxidized (*i.e.,* carbon dioxide) and most reduced (*i.e.,* methane) states. The main product of the process is biogas, a mixture of CH_4 and CO_2 as well as trace gases such as hydrogen sulfide and hydrogen. With the increase of oil and natural gas prices and the increasing doubts on advantages of biofuels produced from energy crops, biogas more and more appears as a real valuable energy source to be developed (Tilche and Galatola, 2008).

Biogas production from sewage sludge depends on the composition and availability of organic compounds. Waste activated sludge (WAS) is composed of more or less stabilized and slowly biodegradable organic matter with a low biodegradability. Thermophilic anaerobic digestion allows one to enhance anaerobic degradation rates, hence to reduce the sludge retention time and increasing the methane production (Dohanyos *et al.*, 2004). However, several drawbacks are observed during the thermophilic anaerobic digestion. The main

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drawback is propionate accumulation (Speece *et al.*, 2006) that induces limitations in the conversion of organic mater into biogas. The accumulation of propionate is due to low microbial consortia proximity, the range of partial pressure of H_2 which needs to be maintained in a range from 10^{-4} to 10^{-6} atm (Fukuzaki *et al.*, 1990; Jackson, 1999) and the possible deficiency in inorganic nutrients which may not ensure enzymatic process efficiency (Espinosa *et al.*, 1995; Dohanyos *et al.*, 1997; Kim *et al.*, 2002).

WAS is mainly composed of proteins and thus, can release a large amount of ammonia during anaerobic degradation (Eskicioglu *et al.*, 2006; Jeong *et al.*, 2007). Although ammonia is an important buffer and an essential nutriment for anaerobic microbes, high ammonia concentrations can decrease microbial activities, particularly methanogens (Angelidaki and Ahring, 1994). Ammonia nitrogen is indeed one of the most common toxic substances encountered during anaerobic treatment of protein containing wastes. Unionized ammonia is toxic because, unlike ammonium ions, it can readily diffuse across the cell membrane (Kadam and Boone, 1996; Chen *et al.*, 2008). Within the two distinct methanogenic groups, acetate-consuming methanogens are usually found to be more sensitive than hydrogen-utilizing ones (Hansen, *et al.*, 1998). However, in some other studies, hydrogen-utilizing methanogens are defined as the most sensitive group (see for example Wiegant and Zeeman *et al.*, 1986). Another phenomenon due to a high ammonia concentration is the shift from the aceticlastic methanogens to the syntrophic acetate oxidation for methane production (Schnürer and Nordberg, 2008). Thus, in an anaerobic digester, methane production from acetate proceeds by either aceticlastic methanogenesis or syntrophic acetate oxidation. The shift of pathway resulted in a decrease in the specific gas and methane yield. This highlights the difficulty to have a clear understanding of all phenomena occurring in complex processes such as anaerobic digesters.

Pretreatment technologies are often a way to optimize WAS conversion into the methane. Over the years, several pretreatments were implemented and studied in the literature: physical (Nickel and Neis, 2007), chemical (Ardic and Taner, 2005), biological (Cirne *et al.*, 2006; Davidsson *et al.*, 2007) and thermal (Gavala *et al.,* 2003a; Jeong *et al.*, 2007) treatment in order to pre-hydrolyze the particulate organic matter and make it more available to the anaerobic biomass. In particular, thermal pretreatment can be combined with mesophilic anaerobic digestion and leads to an increase of biogas quantity and production rates (Li and Noike, 1992), energy costs being covered by the additional biogas production (Kepp *et al.*, 2000, Bougrier *et al.*, 2007). Climent *et al.*, (2007), Bougrier *et al.*, (2008) and Jeong *et al.*, (2007) also underlined the positive impact of solubilisation of particulate organic matter on the biogas production during anaerobic digestion. However, very few studies analyzed the combination of thermal pretreatment with thermophilic WAS anaerobic digestion, Gavala *et al.* (2003a, b) and Skiadas *et al.* (2005) being almost the only papers available in the literature on this topic.

In parallel, mathematical models are widely acknowledged to provide interesting and useful information about phenomena occurring in complex systems. In particular, the IWA Anaerobic Digestion Model No1 (ADM1 – see Batstone *et al.*, 2002) is a powerful tool for predicting the behavior of anaerobic digesters treating sewage sludge. This generalized model can indeed take into account chemical and biological interactions between solid, liquid and gas phases. However, ADM1 has also some drawbacks:

- A detailed characterization of the sludge is required a correct implementation of ADM1 (Huete *et al.*, 2006). In particular, a precise characterization of particulate and soluble

concentrations of carbohydrates, proteins, lipids and individual Volatile Fatty Acids (VFAs) is needed and from a practical point of view, this might be difficult to obtain. Fractionation of the particulate and soluble phases is another difficulty, sludge characterization being generally defined from particulate-soluble fractionation at 0.45 µm (Münch *et al.*, 1999; Kim *et al.*, 2002; Kampas *et al.*, 2007; Parravicini *et al.*, 2008) even though this might not be the limit for physical accessibility of biomass to the substrate.

- ADM1 uses some simplifications in reactions for particulate organic compounds. In particular, the first-order kinetic may be inaccurate to describe the disintegration and hydrolysis steps. Yasui *et al.* (2008) suggested to elaborate a more comprehensive degradation scheme to consider the model structure limiting factor and readily and slowly fractions of WAS. Fernandez *et al.* (2001) showed that the hydrolysis step might depend on the biomass concentration and activity. It is thus necessary to integrate an hydrolysis rate which takes into account the limitation by biomass concentration and by substrate concentration together with the impact of substrate accessibility and slowly biodegradable material content.

The objective of this study is to better characterize the disintegration and hydrolysis steps and to integrate them into ADM1 in order to obtain a model able to predict and interpret results from thermophilic anaerobic digestion of thermally pretreated WAS. In the following, an overview of the model structure, assumptions and main model parameters are presented. The dynamics of VFAs, pH and methane production obtained from four different batch thermophilic anaerobic digestion of untreated and thermally pretreated sludges are used to calibrate and to validate the proposed model.

2. Materials and methods

2.1. Experimental approach

2.1.1. Thermal hydrolysis

WAS samples from a highly loaded wastewater treatment plant in France were used during the experiments. Thermal hydrolysis was carried out in a 10 L agitated autoclave (Autoclave, class IV) allowing a temperature increase by electricity. Three temperatures of treatment were chosen: 110°C, 165°C and 220°C. Once the desired temperature was reached, treatments lasted for 30 min.

COD solubilisation was used to evaluate the impact of thermal pretreatment and was expressed as a percentage, according to the following equation:

$$S_X(\%) = \frac{S_S - S_{S_0}}{X_{p_0}} * 100$$

where S_S and S_{S_o} are the concentrations measured in the soluble fraction of treated and untreated sludge respectively and X_{p_o} is the concentration measured in the particulate fraction the untreated sludge. The COD solubilisation was calculated for COD, proteins and carbohydrates. The solubilisation of lipids was not determined because the difference between lipid concentrations in total and particulate fractions was very low and did not show a significant solubilisation.

2.1.2. Anaerobic biodegradability tests

Biochemical Methane Potential (BMP) tests are based on Buffiere *et al.* (2006) and adapted from Angelidaki and Sanders (2004). The assay method was based on the measurements of the end product (biogas) and the intermediate products such as VFAs, in a closed reactor wherein a measured quantity of substrate was introduced with a measured quantity of inoculum. The experiment was realized in favourable conditions for the anaerobic digestion of sludge. Five reactors, with a volume of 3.5 L each, were used in parallel. Anaerobic batch reactors were kept at 55°C (thermophilic conditions) by water circulation in a water jacket. The inoculum was taken from a full scale sludge anaerobic digester. One reactor was used with no feed to quantify the endogenous activity of the inoculum. Others reactors were fed with untreated sludge and with sludge treated at 110°C, 165°C and 220°C. Organic loading was 0.5 g_{COD} of WAS per g_{VS} of inoculum. For each condition, four successive 22 day batch experiments were carried out to minimize the effect of the inoculum. At the beginning of each BMP test, the reactors were purged with a N_2/CO_2 (75/25) gas mixture. Biogas production and pH were measured continuously. An electronic volumetric gas counter was used to monitor biogas production. During anaerobic digestion, total and soluble COD, VFAs and biogas composition were daily monitored in order to follow the formation of by-products involved in the biological reactions. In each case, only the fourth batch experiment was used to calibrate and to apply the model in order to minimize the influence of the initial inoculum composition.

2.1.3. Analytical methods

The soluble and particulate fractions were separated by centrifugation at 50 000 g, 15 min and 5°C, then by filtration through a cellulose acetate membrane with 0.45 µm pore size. Substrate characterization was realized on the sludge samples to determine initial values of the model variables. Some measurements were performed on total and soluble fractions: COD, proteins (measured according to the Lowry method – Lowry *et al.*, 1951) and total sugars (measured with the anthrone reduction method – Dreywood, 1946). Ammonia nitrogen, inorganic carbon and VFAs were measured only in the soluble fraction. Lipids were measured according to the Soxhlet method using petroleum ether as solvent, on both total and particulate fractions.

VFA concentrations were measured using a gas chromatograph (GC-8000 Fisons instrument) equipped with a flame ionization detector with an automatic sampler AS 800. The internal standard method allowed to measure acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate concentrations. A reference sample of known concentration was used to determine the standard deviation. The error related to this measurement was lower than 2% throughout all experimentals. The biogas composition was determined with a gas chromatograph (Shimadzu GC-8A) equipped with a CTRI Alltech column, a thermal conductivity detector and connected to an integrator, argon being the carrier gas.

2.2. Modeling Approach

2.2.1. Model Structure

Following the approach of O'Rourke (1968), Pavlostathis and Gossett (1988) and Vavilin *et al.* (1999), disintegration was included in ADM1 mainly to represent the pool of composite organic material and to facilitate the modeling of WAS digestion. The complex particulate pool is also used as a pre-lysis repository of dead biomass. The disintegration step was indeed originally intended to sum up an array of steps such as lysis, non-enzymatic decay, phase separation, and physical breakdown (*e.g.,* shearing). In accordance with Eastman and Ferguson (1981), this extracellular step was assumed to be of first order and was considered as an empirical function reflecting the cumulative effect of this multi-step process.

Although the hydrolysis of particulate organic material has been traditionally also modeled according to first-order kinetics and is usually considered as the rate-limiting step in anaerobic digestion (Pavlostathis and Giraldo-Gomez, 1991), some authors (see for example Bryers (1985) and Mata-Alvarez (1989)) have pointed out that the mechanisms, stoichiometry, kinetics and modeling of biological particulate hydrolysis have not yet been adequately studied. The complex multi-step process of carbohydrates, proteins and lipids hydrolysis may indeed include multiple enzyme production, diffusion, adsorption, reaction and enzyme deactivation steps (Vavilin *et al.*, 2008).

Consequently, the first order kinetics appears to be not applicable under all circumstances and an in-depth better understanding of the different processes involved is needed to accurately describe the disintegration and hydrolysis steps. Furthermore, it has been shown that models in which disintegration/hydrolysis is coupled to the growth of disintegration/hydrolytic bacteria and to substrate heterogeneity work well even at high or fluctuant organic loadings (Vavilin *et al.*, 2008). In particular, the Contois model has been demonstrated to be well adapted to represent different experimental data sets from a wide range of organic wastes (Sotemann *et al.*, 2006; Nopharatana *et al.*, 2007; Vavilin *et al.*, 2008). The Contois model that uses a single parameter to represent saturation of both substrate and biomass can be written as :

$$\rho_{process} = k_{m,process} X \frac{S}{K_{S,process} X + S} = k_{m,process} X \frac{S/X}{K_{S,process} + S/X}$$

where:

$\rho_{process}$	is the process rate ($kg_{COD}.m^{-3}.d^{-1}$)
$k_{m,process}$	is the maximum specific uptake rate of the process (d^{-1}),
$K_{S,process}$	is the half-saturation coefficient for the ratio S/X ($kg_{COD}.m^{-3}$),
X	is the hydrolytic (disintegration) biomass concentration ($kg_{COD}.m^{-3}$) and
S	is the particulate compound concentration ($kg_{COD}.m^{-3}$).

The Contois model has two particular cases with extreme values:

(i) first-order kinetic for biomass:

S/X ϕϕϕϕ $K_{S,process}$ $\quad \rho_{process} \approx k_{m,process} X$

(ii) first-order kinetics for substrate:

S/X ππππ $K_{S,process}$ $\quad \rho_{process} \approx k_{m,process} X \frac{S/X}{K_{S,process}} \approx k_{m,process} \frac{S}{K_{S,process}}$

In addition, the anaerobic biodegradation of WAS produces a large quantity of ammonia which is the main cause of inhibition since, as already pointed out, it is freely cell membrane permeable. Inhibition is usually indicated by a decrease of the methane production and an accumulation of VFAs (Kroeker, *et al.*, 1979; Chen, *et al.*, 2008). Free ammonia inhibition is included in ADM1 for aceticlastic methanogens using non-competitive functions, however, our experiments of pretreated WAS, acetate was not completely degraded and, in some cases, a second phase of acetate production was observed. This phenomenon cannot be explained with the non-competitive function included in ADM1 for modeling free ammonia inhibition of aceticlastic methanogens and the following Hill function (Hill and Barth, 1977) was used instead :

$$I_{NH_3} = b * \left[1 - \frac{S_{NH3}^{\alpha}}{K * S_{NH3}^{\alpha} + S_{NH3,\lim}^{\alpha}} \right]$$

where:

I_{NH3} is free ammonia inhibition factor of aceticlastic methanogens,

b is the maximum desired value for ammonia inhibition,

S_{NH3} is the free ammonia concentration ($kmole_N.m^{-3}$),

$S_{NH3,lim}$ is the mean free ammonia threshold concentration ($kmole_N.m^{-3}$),

K is a tuning parameter and

α is the Hill coefficient that defines the slope of the drop in the inhibition function.

These additional process rates and stoichiometry of the modified ADM1 can be found in appendices A and B, where only disintegration, hydrolysis and decay are described. The remaining reactions (*i.e.,* acidogenesis, acetogenesis and methanogenesis) are strictly equivalent to those present in the standard ADM1.

To summarize, this modified ADM1 involves few additional parameters: three disintegration biochemical parameters of composites X_c (*i.e.,* $k_{m,Xc}$, $K_{S,Xc}$ and $k_{dec,Xc}$), nine hydrolysis biochemical process parameters for carbohydrates, proteins and lipids (*i.e.,* $k_{m,ch}$, $K_{S,ch}$, $k_{dec,ch}$, $k_{m,pr}$, $K_{S,pr}$, $k_{dec,pr}$, $k_{m,li}$, $K_{S,li}$ and $k_{dec,li}$ respectively) and four stoichiometric parameters (Y_{Xc}, Y_{ch}, Y_{pr} and Y_{li}). Their tuning was performed by trial and error using the experimental data set obtained from the batch reactor fed with untreated WAS, then we apply the obtained values for simulated the other three experimental data sets (*i.e.,* those obtained from batch reactors fed with WAS thermally pretreated at 110, 165 and 220°C).

The liquid/gas transfers are described from Henry's law, which can describe the equilibrium between liquid and gas phases for CH_4, CO_2, H_2. The dynamic gas transfer rates (r_i) are expressed as:

$$r_i = k_L a_{,i} (S_{liq,i} - K_{H,i} p_{gas,i})$$

where:

$k_L a_{,i}$ is the overall transfer coefficient multiplied by specific transfer area of gas i (d^{-1}),

$S_{liq,i}$ is liquid concentration of gas *i* ($kg_{COD}.m^{-3}$),

$K_{H,i}$ is Henry's law coefficient of gas *i* ($M.bar^{-1}$) and

$p_{gas,i}$ is partial pressure of gas *i* (bar).

In ADM1, the liquid-gas transfer coefficient values are identical for all gas. However, k_La varies widely depending on reactor size, dimensions, turbulence, liquid flow, gas flow and diffusivity values. In order to take into account these differences, three kinetic coefficients were used, one for each gas : k_La_{CH4}, k_La_{CO2} and k_La_{H2} for methane, carbon-dioxide and hydrogen respectively. k_La_{CO2} was estimated for each batch reactor by fitting the CO_2 produced in the gas phase. Then, as recommended by Pauss *et al.* (1990), k_La_{CH4} and k_La_{H2} were estimated from the carbon-dioxide gas transfer coefficient and diffusivity coefficients, using the following equation:

$$k_L a_{,i} = k_L a_{CO_2} \left(\frac{D_i}{D_{CO_2}} \right)^{1/2}$$

where D_i is the diffusivity of gas i ($m^2.s^{-1}$).

2.2.2. *Model implementation and initial conditions*

The modified ADM1 was implemented using MatLab/Simulink. Values for initial conditions of most of the model variables were directly obtained from the experimental measurements on the WAS samples. The characterization of WAS samples was presented in the table 1. It was assumed that the substrate particulate COD was mainly present into X_c and little into X_{ch}, X_{pr} and X_{li} because, unlike other wastes such as food wastes, carbohydrates, proteins and lipids are embedded into the flocs of the WAS. The initial value of X_I was the sum of particulate COD concentrations from the reactor before feeding and the substrate. Initial values of S_{su} and S_{aa} were taken equal to the measured sugar and amino acids concentrations at time $t = 0$. The initial values for the different VFAs (*i.e.,* S_{ac}, S_{pro}, S_{bu} and S_{va}) were also obtained from the measurements performed just after feeding the reactor. pH was calculated from the ionized forms of VFAs, bicarbonate, ammonia and cation/anion concentrations. Ammonia (S_{IN}) and bicarbonate (S_{IC}) were measured by Kejdahl method and TOCmeter, respectively. Anion concentration (S_{an}) was taken equal to S_{IN} according to Rosen and Jeppsson (2002) and cation concentration (S_{cat}) was adjusted in each case according with experimental pH. Initial values of the different biomass concentrations were determined to fit to the VFAs curves obtained from each batch reactor.

2.2.3. *Model Calibration*

COD fractionation into particulate proteins, carbohydrates, lipids and inerts of the sludge composite particulate material is one of the most critical step when calibrating ADM1 for complex particulate substrates such as WAS. These stoichiometric coefficients are strongly correlated to the waste composition and intrinsic characteristics and, as a consequence, they have to be specified for each sludge. In the present study, particulate inerts from composites fraction, f_{Xi_Xc}, was determined from the final measured biodegradability (D) of each batch test. The remaining COD or inert fraction is $1-D$. Since it is difficult to clearly distinguish between particulate and soluble inerts and since our objective is to analyze the dynamics of non-inert materials, it was decided for simplicity to set all inert material to the particulate variable and f_{Si_Xc} was then taken equal to 0. The other stoichiometric coefficients (*i.e.,* f_{ch_Xc}, f_{pr_Xc} and f_{li_Xc}) were determined from the measured carbohydrate, proteins and lipids content of the WAS. These concentrations were expressed as COD and the stoichiometric coefficients were directly determined from the ratio of the COD of each component over the total COD particulate concentration. The fractionation results are presented in Table 1.

Another important critical step is well-define the values of initial conditions of the model variables. As already said, the values of initial dynamic state variables were directly obtained from the characterization of WAS and are presented in Table 1.

Yiel of product on substrate ($kg_{COD}.kg_{COD}^{-1}$)	ADM1 Default values[1]	WAS Untreated	WAS pretreated at 110°C	WAS pretreated at 165°C	WAS pretreated at 220°C
f_{Si_Xc}	0.10	0.0	0.0	0.0	0.0
f_{Xi_Xc}	0.25	0.606	0.506	0.475	0.528
f_{ch_Xc}	0.20	0.096	0.134	0.135	0.107
f_{pr_Xc}	0.20	0.160	0.164	0.149	0.113
f_{li_Xc}	0.25	0.138	0.196	0.242	0.252
Dynamic states variables	Units	Initial conditions values[2]			
X_c	$kg_{COD}.m^{-3}$	2.892	3.140	2.712	1.716
X_{ch}		0.100	0.100	0.100	0.100
X_{pr}		0.100	0.100	0.100	0.100
X_{li}		0.100	0.100	0.100	0.100
X_i		12.000	12.047	11.400	11.300
S_i		0.0	0.0	0.0	0.0
S_{su}		0.010	0.017	0.025	0.035
S_{aa}		0.010	0.003	0.401	0.347
S_{fa}		0.0	0.0	0.0	0.0
S_{va}		0.079	0.037	0.043	0.049
S_{bu}		0.144	0.092	0.034	0.002
S_{pro}		0.707	0.121	0.120	0.399
S_{ac}		0.321	0.257	0.240	0.317
S_{IC}	$kg\text{-}mole_C.m^{-3}$	0.0142	0.0159	0.0159	0.0151
S_{IN}	$kg\text{-}mole_N.m^{-3}$	0.0588	0.058	0.0568	0.0591

[1]Standard ADM1 values are those recommended in Batstone *et al.* (2002).
[2]Initial conditions values are determined from experimental data obtained in this study.

Table 1. COD flux for each sludge composite particulate material during the disintegration process and initial conditions values of the model variables

Total and individual VFAs, pH, cumulative methane and carbon dioxide production experimentally obtained using the untreated WAS were used to calibrate the model. Most parameters values were kept identical as those proposed originally by Batstone *et al.* (2002) except the stoichiometric coefficients of butyrate and valerate from amino acids (*i.e.,* $f_{bu,aa}$ and $f_{va,aa}$) that were respectively taken equal to 0.16 and 0.33 instead of 0.26 and 0.23. The decay rates (*i.e.,* k_{dec}) of the different microorganisms were also modified from 0.04 to 0.01. Finally, the ammonia inhibition coefficients of the Hill function were the following: $b = 1$, $\alpha = 100$, $K = 1.015$, $S_{NH3,lim} = 7.45*10^{-4}$. The biochemical and stoichiometric parameters of the new process rates related to disintegration and hydrolysis were obtained by trial and error and are presented in Table 2. As can be seen in Figure 1, modified ADM1 model simulations closely follow the dynamic evolutions of the different variables and they are improved when compared to standard ADM1 model simulations. The disintegration stoichiometric coefficients and initial conditions were the same for both models and are indicated in Table 1 which were determined from experimental data obtained in this study.

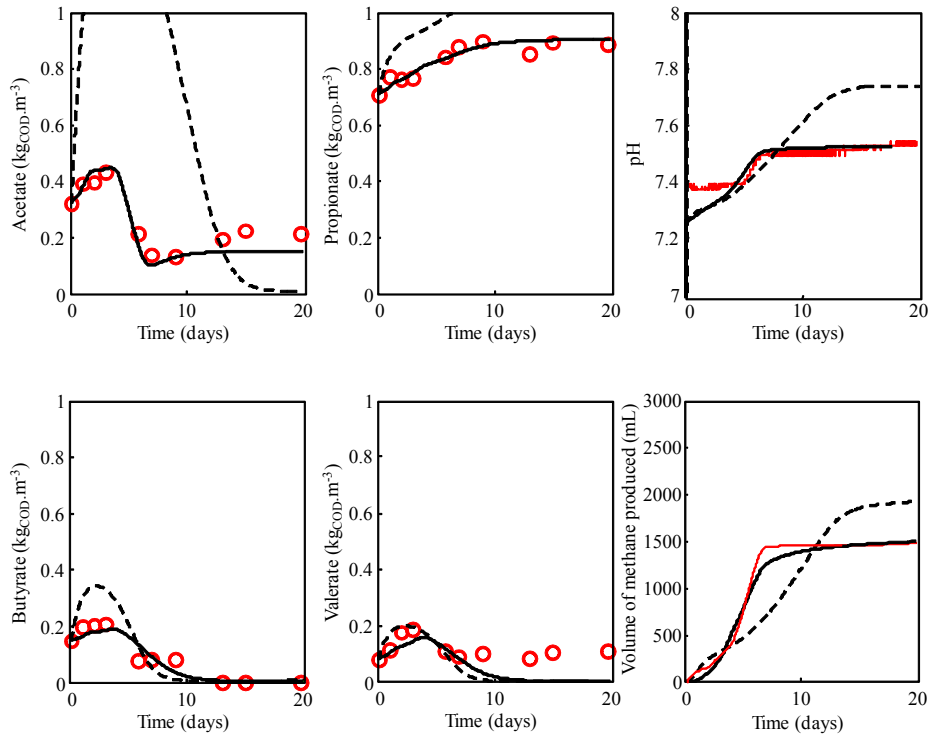

Figure 1. Simulated individual VFAs, pH and cumulative CH_4 production vs experimental data for untreated WAS. Comparison of the proposed disintegration/hydrolysis model with standard ADM1 (circles and thin plain line: experimental data points, dashed thin line: standard ADM1, thick plain line: modified ADM1).

Parameter	Composites	Carbohydrates	Proteins	Lipids
k_m	1.75	10	10	10
K_S	0.3	0.5	0.5	0.5
k_{dec}	0.01	0.01	0.01	0.01
Y	0.4	0.1	0.1	0.1

Table 2. Biochemical and stoichiometric parameters of the new process rates related to disintegration and hydrolysis.

3. Results and discussion

3.1. Impact of thermal pretreatment on sludge solubilisation and biodegradability

The parameter of COD solubilisation was used to evaluate the impact of thermal pretreatment on the transfer of organic matter from particulate phase to soluble phase obtained for the untreated and pretreated sludges. Results are summarized in Table 3. As can be seen the values of COD solubilisation increase with temperature from 3.8 (at 110°C) to 27% (at 220°C). Throughout applied thermal pretreatments, the total COD balance was maintained before and after treatment. The thermal pretreatment indeed leads to a transfer of particulate organic matter into the soluble phase (*i.e.,* particulates lower than 0.45 µm) and can be assimilated to a thermal hydrolysis. Thus, the application of thermal pretreatment to a largely particulate raw sludge (86 %VS in our case) makes organic components more available to the

anaerobic microorganisms and induces an increase of degradation rates and the volume of biogas produced.

Conditions	COD concentrations ($g_{COD}.L^{-1}$)		Solubilisation of COD (%)	BMP ($mL_{CH4}.g_{CODin}^{-1}$)	Biodegradability (%)
	Liquid phase	Particulate phase			
Raw sludge	5.6 ± 0.1	58.4 ± 0.8	-	165	47
110°C	7.8 ± 0.0	56.9 ± 3.0	3.8 ± 0.2	186	53
165°C	16.0 ± 0.6	45.5 ± 1.6	18.0 ±1.0	195	56
220°C	21.3 ± 0.5	38.7 ± 2.1	27.0 ± 1.0	142	41

Table 3. COD solubilisation, methane production and biodegradability of untreated and pretreated WAS at different conditions.

Morever, a maximum value can be noticed on methane production and WAS biodegradability when temperature reaches 165°C which can be considered as the optimal pretreatment temperature. This finding is in agreement with the literature and earlier studies showed that a pretreatment temperature of 170°C seems to be a limit for the improvement of methane production (Haug,et al, 1978; Stucky and McCarty, 1984; Li and Noike, 1992; Bougrier *et al.*, 2008). At 220°C, although a large solubilisation of particulate organic matter occurs, sludge biodegradability is indeed lower than the raw sludge biodegradability with only 142 $mL_{CH4}.g_{CODin}^{-1}$ being produced. This can be explained by the composition of solubilised organic matter (see Figure 2): at 220°C, protein solubilisation is similar to the one obtained at 165°C (*i.e.,* around 40 %) and carbohydrates solubilisation strongly decreases from 15% (at 165°C) down to 1.2 % (at 220°C). However, at the same time, COD solubilisation increases from 18 % to 27 %. Thus, at 220°C, carbohydrates in the soluble phase reacted with other components present to form slowly or hardly biodegradable products. These results are in agreement with those of Stuckey and McCarty (1984), Müller (2001) and Bougrier *et al.* (2007) and suggest the presence of "burnt sugar" reactions and Maillard reactions for high pretreatment temperatures. The brown color of the soluble phase noticed experimentally for the sludge treated at 220°C indeed confirmed the presence of new compounds, like Amadori compounds and melanoidins which are recalcitrant to anaerobic degradation.

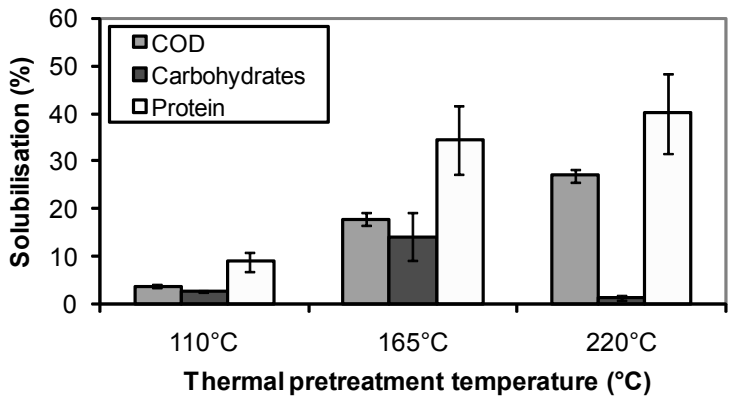

Figure 2. Impacts of temperature on solubilisation of WAS

3.2. Model Application

The behavior of the modified anaerobic digestion model has been compared to the one of the standard ADM1 (with the disintegration stoichiometric coefficients and initial conditions indicated in Table 1 which were determined from experimental data obtained in this study) and to experimental results in simulating the behavior of a batch thermophilic anaerobic digestion of thermally pretreated WAS. The calibration and the application of the model were only realized on the fourth successive batch experiment. Thus, an adaptation of the inoculum toward the substrate was performed. This adaptation was represented in the model by variations of the biomass concentrations between each WAS digestion.

In order to represent the difference in availability of organic matter between untreated and pretreated WAS, initial concentration values for disintegration biomass used in the Contois model were lower in the untreated WAS than in the pretreated ones. For the raw sludge, a low concentration of disintegration biomass (*i.e.,* X_{Xc} = 0.12) was applied because a large quantity was in a particulate form leading to a slow acetate kinetic and a low quantity of acetate being produced . For the pretreated sludge at 110°C and 165 °C, the limiting effect of organic matter availability was less important, thus the concentrations of disintegration biomass was arbitrarily increased to 0.5. On the other hand, in order to account for the negative effects of further increase of temperature, the initial value of the disintegration biomass was set equal to 0.1 for the WAS pretreated at 200 °C.

The ammonia inhibition function was also modified to account for the influence of temperature pretreatment: $S_{NH3,lim}$ was increased to $4.5*10^{-3}$ at 110 °C, $5*10^{-3}$ at 165 °C and $5.2*10^{-3}$ at 220 °C and, if *b* was kept equal to 1 for 110 and 165 °C pretreatment, it was decreased to 0.6 at 220 °C. Of course, this last value is not fully correct since it would mean that even at null ammonia concentration, inhibition is present. It thus account for the influence of other compounds (*e.g.,* Amadori compounds and melanoidins) and should be here only considered as a way to keep the model as simple as possible, not trying to represent components that were not measured.

Concerning acetate evolution Schnürer and Nordberg (2008) showed that a shift, from the aceticlastic mechanism to the syntrophic pathway, occurrs when the NH_4-N concentration increases above 3 $g.L^{-1}$. In our case, this dual acetate-degrading population should not be present since ammonium concentration were around 500 $mg.L^{-1}$. Acetate oxidation could also contribute to the total acetotrophic methanogenesis (Petersen and Ahring, 1991) but this pathway is usually considered as minor compared to aceticlastic methanogenesis and we decided to ignore it , as done in ADM1. Finally, the homoacetogenesis may be significant under psychrophilic conditions (Rebac *et al.*, 1995) and so it was not considered in our study. As a consequence, the incomplete degradation of acetate and in some cases the second phase of acetate production was represented taking into account the free ammonia inhibition of aceticlastic methanogens. The Hill function was chosen for this inhibition.

Besides these very few changes, all other parameters were strictly equivalent to those used to simulate the untreated WAS. Figures 3 to 5 display the simulated (with both models) and experimental results for the 110°C , 165°C and 220°C pretreated WAS. As can be seen, model simulations closely follow the dynamic evolutions of the main variables, in the liquid and but in the gas phases. The model predicts well the dynamics of the biogas production rate as a response of the pretreatment imposed. Small deviations in predicting the cumulative biogas production have been found. It appeared that the rate at which acetate is converted into

methane is somewhat underestimated. This may have resulted from either underestimation of the substrate consumption coefficients for aceticlastic methanogenesis or from an overestimation of the inhibition of this activity by ammonia.

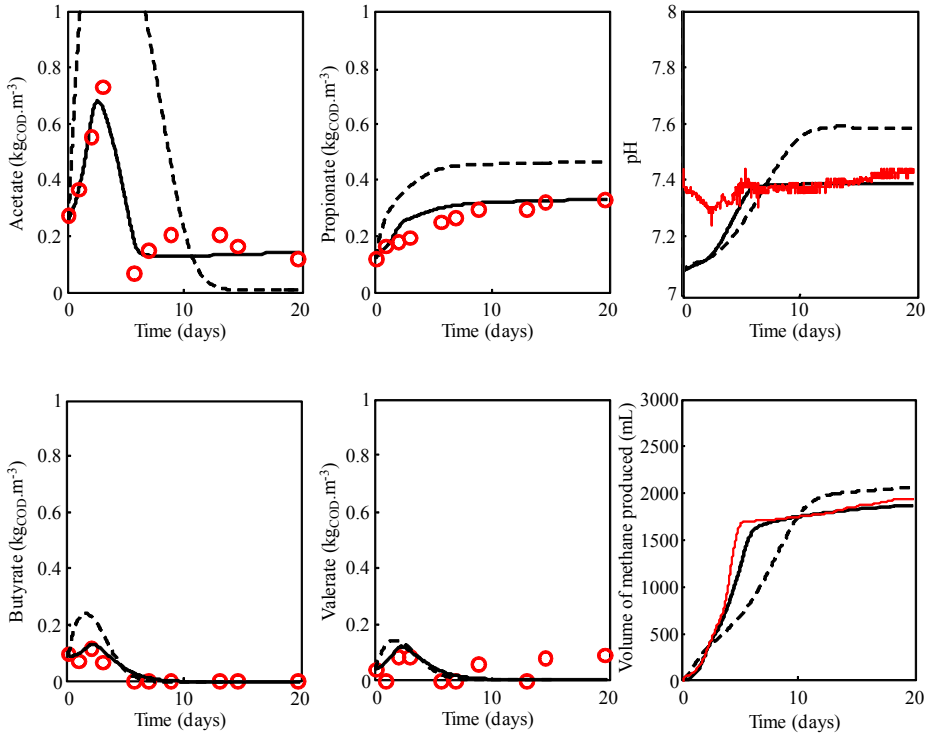

Figure 3. Simulated individual VFAs, pH and cumulative CH_4 production vs experimental data for 110°C pretreated WAS. Comparison of the proposed disintegration/hydrolysis model with standard ADM1 (circles and thin plain line: experimental data points, dashed thin line: standard ADM1, thick plain line: modified ADM1).

The pH model simulation was able to reflect the trends that were observed in experimental data. The pH prediction is closely related to the cation and anion concentrations in the reactor, and actually, the difference between the two concentrations. Since the input ion concentrations were not measured, it was calculated using the initial pH value and taking into account the initial concentrations of ammonia, alkalinity and ionized VFAs, in the reactor. The value of the input cation minus the input anion concentration from the reactor was arbitrarily increased in the model, so that the initial pH values were calibrated. For both untreated and pretreated WAS, pH was in general in a range of 7.18-7.59, with the low values corresponding to periods where VFAs accumulate in the thermophilic batch reactors. In all cases, pH varies within c.a 0.3 units, even when the process was inhibited and the VFA accumulated. The relatively large resistance against pH changes was probably due to the reactor buffering capacity.

The propionate accumulation reflected for both simulated and experimental data are in agreement with other investigations (Speece *et al.*, 2006). They showed that an accumulation of propionate is often present under thermophilic anaerobic digesters. From Figures 2 to 5, it is clear that until a temperature of 165°C, the propionate degradation is improved by the pretreatment, but as already said, in the 220°C pretreated WAS case, carbohydrates present in the soluble phase react with other components to form products slowly or hardly biodegradable. It is interesting to note that propionate accumulation limited the final accumulated methane volume.

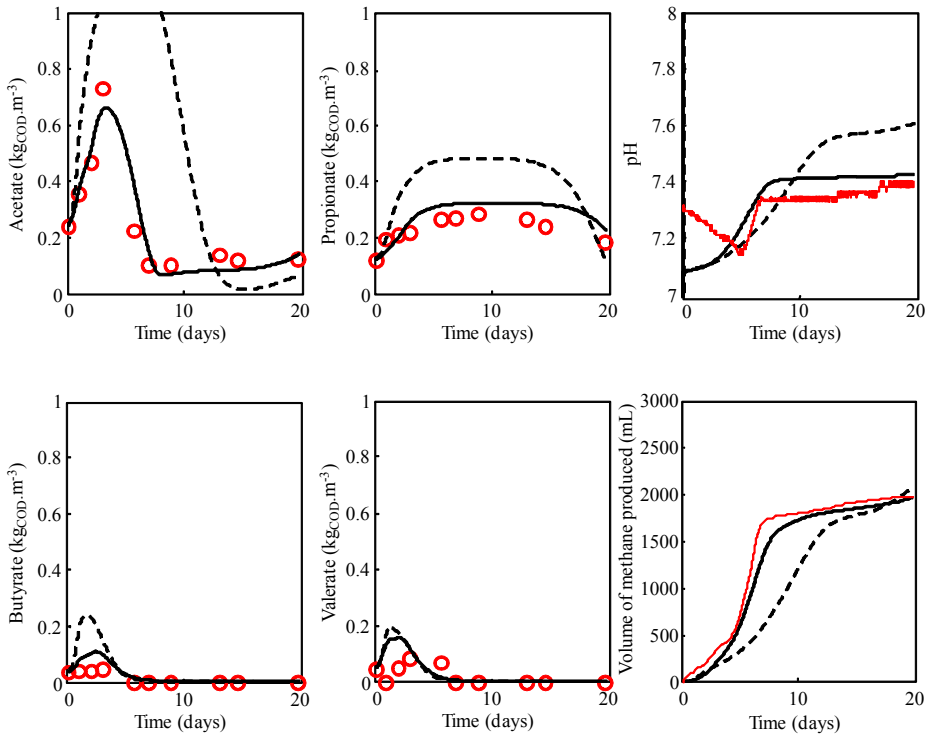

Figure 4. Simulated individual VFAs, pH and cumulative CH_4 production vs experimental data for 165°C pretreated WAS. Comparison of the proposed disintegration/hydrolysis model with standard ADM1 (circles and thin plain line: experimental data points, dashed thin line: standard ADM1, thick plain line: modified ADM1).

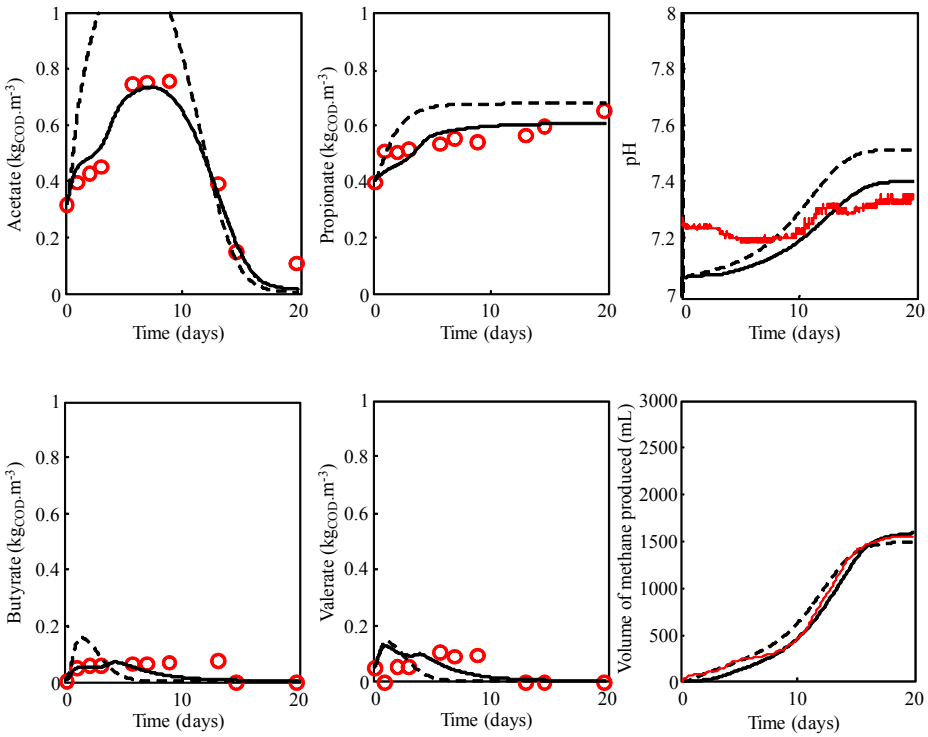

Figure 5. Simulated individual VFAs, pH and cumulative CH_4 production vs experimental data for 220°C pretreated WAS. Comparison of the proposed disintegration/hydrolysis model with standard ADM1 (circles and thin plain line: experimental data points, dashed thin line: standard ADM1, thick plain line: modified ADM1)

The acetate and propionate accumulations were due to an important limiting effect of the hydrolysis step. VFAs of higher molecular weights are degradation products of sugars and amino acids respectively and remain at low levels in all cases, meaning that they are rapidly consumed by acetogens bacteria. The individual VFAs and methane production experimentally observed were another sign showing the limitation of hydrolysis step because the maximal methane production and maximal acetate production were reached between day 4 and day 13. The modified model was also able to explain the dynamics of acetate accumulation obtained in the untreated and 110°C pretreated WAS, which was characterized by two peaks of acetate concentration and may be due to different hydrolysis rates for fats and proteins.

Total methane production increased with thermal pretreatment temperature. Nevertheless, no influence could be clearly observed on the kinetics of methane production for the different thermal pretreatment conditions. This can be explained by the conditions of BMP tests (notably low organic load) which are favorable to anaerobic digestion. For the sludge pretreated at 165°C, a low methane production was observed after day 10, arising from the slow degradation of propionate.

Figure 6 shows the agreement between VFAs concentration and methane produced predicted by the modified ADM1 model and the measured VFAs concentration and measured methane produced respectively, from the untreated and pretreated WAS. The agreement between the predicted and measured values was statistically significant in both cases ($\alpha \leq 0.005$), for each of the four WAS with correlation coefficients indicated as legends in Figure 6. If the overall data point are considered, r^2 of 0.9431 and 0.9915 (at the same significance level) are obtained for VFAs concentration and methane produced respectively. This agreement contributes to validate the modeling approach as well as the sixteen model parameters established in this study.

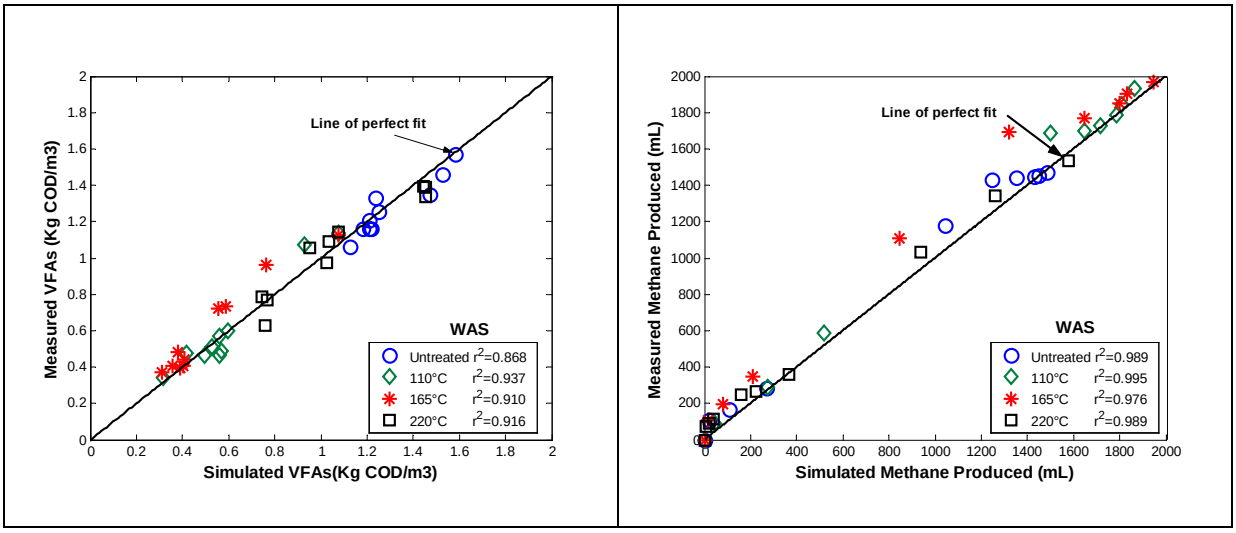

Figure 6. Comparison between (a) measured VFAs concentrations and simulated VFAs concentrations (b) measured methane produced and simulated methane produced, for untreated and pretreated WAS. The simulation data come from modified ADM1 model.

In order to achieve accurate model predictions, it is important to define the properties of the sludge stream entering the digester. For organic substances, the ADM1 model defines these

inputs in terms of soluble and particulate COD. For municipal sludge, the main part of the organic loading is associated with the particulate COD. The particulate COD entering the digester is defined in terms of biodegradable (*Xc*) and non-biodegradable components. Estimation of these parameters is often challenging for many data sets as in many cases the sludge COD is not reported and in almost all cases, the biodegradable fraction is not independently measured. Moreover, the value of *Xc* as such is not sufficient to precisely predict the amount of methane that will be produced. In our experiments, for example, *Xc* was equal to 3.19 $kg_{COD}.m^{-3}$ for the untreated sludge and to 3.44, 3.01 and 2.01 $kg_{COD}.m^{-3}$ for the thermally pretreated sludge at 110, 165 and 220 °C respectively whereas the maximum methane production was observed for the pretreatment at 165 °C. It is thus clear that the content of the composites in terms of carbohydrates, proteins and lipids – together with the availability of these substrates to the microorganisms – is at least as important as the concentration of composites in itself. As a consequence, if the model is to be used as an analysis and design tool, it would largely benefit from more careful characterization of these parameters. A standardized protocol for determining the anaerobically biodegradable fraction of the sludge COD would assist in this regard. The ammonia and TKN concentrations present in both the feed and the inoculum also need to be precisely characterized because of their impact on pH buffering and inhibition functions.

Overall the modified ADM1 model (Contois kinetic) fitted the measured data better than standard ADM1 model (first-order kinetic) as shown in Figures 1 and Figures 3 to 5. The trend and the goodness of fit of the modified ADM1 model was consistent throughout the full range of the tests. This finding supports the contention that the substrate-microorganism ratio (*Si/Xi*) may be a better limiting factor in the hydrolysis of particulate substrate, rather than the substrate concentration (*Si*) as modeled by the first-order reaction model. . Indeed, Miron et al. (2000) showed that none of the main components of primary sludge (carbohydrates, lipids and proteins) followed first order kinetics with respect to hydrolysis of anaerobic digestion under methanogenic conditions. The results are also in agreement with other studies that had used contois Contois model to describe anaerobic hydrolysis of particulate wastes. Notably, Vavilin et al (2008) underlined the accuracy of the Contois model that allows to take into account the hardly biodegrable material of certain complex substrates. As an other example example Myint and Nirmalakhandan (2006) evaluated three of the more common hydrolysis models—the first-order model; the second-order model; and the surface-limiting reaction model (also known as Contois kinetic model), for their suitability in describing hydrolysis-acidogenesis of cattle manure residues. They found that the two parameter, surface-limiting reaction model followed the trend of the measured data more closely and fitted the measured data slightly better than the other two models. In a later study they developed a mathematical model for the hydrolysis and acidogenesis reactions in anaerobic digestion of cattle manure (Myint *et al*., 2007). Their model is based on the premise that particulate hydrolysable fraction of cattle manure is composed of cellulose and hemicellulose that are hydrolyzed at different rates according to a surface-limiting reaction; and, that the respective soluble products of hydrolysis are utilized by acidogens at different rates, according to a two-substrate, single-biomass model.

Moreover in a study of digestion of primary sludge Yasui et al., (2008) found that anaerobic batch degradation of fresh primary sludge showed a complex MPR curve marked with two well-defined temporal peaks. The first immediate peak was associated with the degradation of relatively readily hydrolysable substrates, while the second delayed peak was associated with the degradation of large-sized particles. However they simplified ADM1model structure with

respect to soluble components and active biomass, so from their model we can not obtain neither individual VFAs nor total VFAs nor individual biomass for example.

Our model for disintegration/hydrolysis of batch thermophilic anaerobic digestion of thermally pretreated waste activated sludge incorporating two fractions of particulate hydrolysable substrat : readily hydrolysable substrates (Xcr) and slowly hydrolysable substrates (Xcs) is under further investigation.

4. Conclusion

A slightly modified IWA ADM1 model for thermophilic anaerobic digestion of pretreated WAS was calibrated and validated using batch experimental data sets. The model was based on the following hypothesis: (a) the disintegration and hydrolysis processes are described according to Contois model and (b) the ammonia inhibition for aceticlastic methanogens can be represented according to the general Hill function. The included model parameters involve three disintegration biochemical parameters, nine hydrolytic biochemical parameters and four stoichiometric parameter values. Predictions by the model using the parameters established in this study agreed well with the data measured under different pretreatment conditions. The resulting model was capable of explaining the dynamics of acetate accumulation obtained in some batch experiments and being possibly characterized by two peaks of acetate concentration, the result of different hydrolysis rates for fats and proteins. Since hydrolysis has been recognized as the rate-limiting step in the anaerobic digestion of complex particulate substrates, these findings can be of value in designing, monitoring, analyzing, and optimizing the anaerobic gasification process.

Acknowledgements

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Appendix A. Petersen matrix modified with biochemical rate coefficients and kinetic rate equations for soluble compounds (i=1-3,12,j=1-4,20-23)

Component	i	1	2	3	12	Rate (ρ_j, $kg_{COD}.m^{-3}.d^{-1}$)
j	Process	Ssu	Saa	Sfa	S_I	
1	Disintegration				$(1-Y_{Xc})*f_{sI,Xc}$	$k_{m,Xc}\frac{X_c}{K_{S,Xc}X_{Xc}+X_c}X_{Xc}$
2	Hydrolysis Carbohydrates	$1-Y_{ch}$				$k_{m,ch}\frac{X_{ch}}{K_{S,ch}X_{Xch}+X_{ch}}X_{Xch}$
3	Hydrolysis of Proteins		$1-Y_{pr}$			$k_{m,pr}\frac{X_{pr}}{K_{S,pr}X_{Xpr}+X_{pr}}X_{Xpr}$
4	Hydrolysis of Lipids	$(1-Y_{li})*(1-f_{fa,li})$		$(1-Y_{li})*f_{fa,li}$		$k_{m,li}\frac{X_{li}}{K_{S,li}X_{Xli}+X_{li}}X_{Xli}$
20	Decay of X_{Xc}					$k_{dec,Xc}X_{Xc}$
21	Decay of X_{Xch}					$k_{dec,ch}X_{Xch}$
22	Decay of X_{Xpr}					$k_{dec,pr}X_{Xpr}$
23	Decay of X_{xli}					$k_{dec,li}X_{Xli}$
		Monosaccharides ($kg_{COD}.m^{-3}$)	Amino acids ($kg_{COD}.m^{-3}$)	Long chain fatty acids ($kg_{COD}.m^{-3}$)	Soluble inerts ($kg_{COD}.m^{-3}$)	

Appendix B. Petersen matrix modified with biochemical rate coefficients and kinetic rate equations for particulate compounds (i=13-16,24-28,j=1-4,20-23).

Component	i	13	14	15	16	24	25	26	27	28	Rate (ρ_j, $kg_{COD}.m^{-3}.d^{-1}$)
j	**Process**	X_c	X_{ch}	X_{pr}	X_{li}	X_{Xc}	X_{Xch}	X_{Xpr}	X_{xli}	X_I	
1	Disintegration	-1	$(1-Y_{Xc})*f_{ch,Xc}$	$(1-Y_{Xc})*f_{pr,Xc}$	$(1-Y_{Xc})*f_{li,Xc}$	Y_{xc}				$(1-Y_{Xc})*f_{xI,Xc}$	$k_{m,Xc}\frac{X_c}{K_{S,Xc}X_{Xc}+X_c}X_{Xc}$
2	Hydrolysis Carbohydrates		-1				Y_{ch}				$k_{m,ch}\frac{X_{ch}}{K_{S,ch}X_{Xch}+X_{ch}}X_{Xch}$
3	Hydrolysis of Proteins			-1				Y_{pr}			$k_{m,pr}\frac{X_{pr}}{K_{S,pr}X_{Xpr}+X_{pr}}X_{Xpr}$
4	Hydrolysis of Lipids				-1				Y_{li}		$k_{m,li}\frac{X_{li}}{K_{S,li}X_{Xli}+X_{li}}X_{Xli}$
20	Decay of X_{Xc}	1				-1					$k_{dec,Xc}X_{Xc}$
21	Decay of X_{Xch}	1					-1				$k_{dec,ch}X_{Xch}$
22	Decay of X_{Xpr}	1						-1			$k_{dec,pr}X_{Xpr}$
23	Decay of X_{xli}	1							-1		$k_{dec,li}X_{Xli}$
		Composite ($kg_{COD}.m^{-3}$)	Carbohydrates ($kg_{COD}.m^{-3}$)	Proteins ($kg_{COD}.m^{-3}$)	Lipids ($kg_{COD}.m^{-3}$)	Composite degraders ($kg_{COD}.m^{-3}$)	Carbohydrate degraders ($kg_{COD}.m^{-3}$)	Protein degraders ($kg_{COD}.m^{-3}$)	Lipid degraders ($kg_{COD}.m^{-3}$)	Particulate inerts ($kg_{COD}.m^{-3}$)	

Appendix C

Nomenclature and description of main parameters and variables

Stoichiometric coefficients

Symbol	Description	Units
$\nu_{i,j}$	Stoichiometric coefficients for component i on process j	$kg_{COD}.m^{-3}$
$f_{product,substrate}$	Yield (catabolism only) of product on substrate	$kg_{COD}.kg_{COD}^{-1}$

Equilibrium coefficients and constants

Symbol	Description	Units
Hgas	Gas law constant (equal to K_H^{-1})	$Bar.M^{-1}$ ($bar.m^3.kmol^{-1}$)
Ka	Acid acid-base equilibria coefficient	M ($kmole.m^{-3}$)
K_H	Henry's law coefficient	M bar^{-1} ($kmole.m^{-3}.bar^{-1}$)
pKa	log10[Ka]	
R	Gas law constant (8.314×10^{-2})	$bar.M^{-1}.K^{-1}$

Kinetic parameters and rates

Symbol	Description	Units
$k_{A/Bi}$	Acid base kinetic parameter	$M^{-1}.d^{-1}$
$k_{dec,process}$	First order decay rate	d^{-1}
$I_{inhibitor,\ process}$	Inhibition function (see K_I)	
$k_{process}$	First order parameter (normally for hydrolysis)	d^{-1}
$k_{La,i}$	Gas-liquid transfer coefficient of gas i	d^{-1}
Di	Diffusivity of gas i	$M^2.s^{-1}$
$K_{I,\ inhibit,\ substrate}$	50% Inhibitory concentration	$kg_{COD}.m^{-3}$
$k_{m,process}$	Monod maximum specific uptake rate (μ_{max}/Y)	$kg_{COD_S}.kg_{COD_X}^{-1}.d^{-1}$
$K_{S,process}$	Half saturation value	$kg_{COD\ S}.m^{-3}$
ρ_j	kinetic rate of process j	$kg_{COD_S}.m^{-3}.d^{-1}$
$Y_{substrate}$	Yield of biomass on substrate	$kg_{COD\ X}.kg_{COD\ S}^{-1}$
μ_{max}	Monod maximum specific growth rate	d^{-1}

Algebraic variables

Symbol	Description	Units
pH	-log10[H^+]	
$p_{gas,i}$	Pressure of gas i	bar
p_{gas}	Total gas pressure	bar
S_i	Soluble component i	$kg_{COD}.m^{-3}$
$t_{res,X}$	Extended retention of solids	D
T	Temperature	K
V	Volume	m^3
X_i	Particulate component i	$kg_{COD}.m^{-3}$

Dynamic State Variables

Name	I[1]	Description	Units[2]
X_c	13	Composites	
X_{ch}	14	Carbohydrates	
X_{pr}	15	Proteins	
X_{li}	16	Lipids	
X_I	24	Particulate inerts	
S_I	12	Soluble inerts	
S_{su}	1	Monosaccharides	
S_{aa}	2	Amino acids	
S_{fa}	3	Total LCFA	
S_{va}	4	Total valerate	
S_{bu}	5	Total butyrate	
S_{pro}	6	Total propionate	
S_{ac}	7	Total acetate	
S_{h2}	8	Hydrogen	
S_{ch4}	9	Methane	
S_{IC}	10	Inorganic carbon	M
S_{IN}	11	Inorganic nitrogen	M
X_{su} .....X_{h2}	17-23	ADM1 Biomass	
X_{Xxc}, X_{Xch}, X_{Xpr}, X_{Xli}	24-27	Modified ADM1 Biomass	
S_{cat}		Cations	M
Sa_n		Anions	M

1. See process kinetics and stoichiometry matrix in Appendix A,B and (Batstone et al., 2002).
2. $kg_{COD}.m^{-3}$ unless otherwise stated.