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Par

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Titre :

Modélisation du devenir des micropolluants organiques au cours de la digestion anaérobie de boues contaminées

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RESUME : Beaucoup de micropolluants organiques sont présents dans les boues. Leur possible impact sur l'environnement contribue à accroître leur intérêt scientifique et social. La digestion anaérobie présente un potentiel certain pour dégrader ces composés. Dans ce travail, il a été développé un modèle dynamique pour décrire le devenir de micropolluants hydrophobes au cours de la digestion anaérobie de boues contaminées. Le modèle est basé sur une distribution des composés dans quatre-compartiments et il a démontré que la transformation des micropolluants est bien simulée si l'on considère une cinétique de co-métabolisme pour la dégradation et si la phase aqueuse constitue le compartiment biodisponible. Dans ce modèle, la sorption des micropolluants hydrophobes est envisagée sur deux phases différentes: la matière particulaire et la matière dissoute/colloïdale (DCM), car la sorption sur le compartiment DCM peut influencer la disponibilité des composés et donc leur biodégradation. Il a été conclu que le transfert de micropolluants hydrophobes ne limite pas leur biodégradation, et que leur devenir est régi par l'état d'équilibre de sorption-désorption. Afin d'identifier quelle(s) étape(s) de la digestion permet le co-métabolisme, de nouvelles expérimentations ont été menées en utilisant des inhibiteurs des Méthanogènes. Elles suggèrent que la dégradation anaérobie des micropolluants implique principalement des microorganismes non-méthanogènes. En effet, la transformation co-métabolique des micropolluants serait principalement liée à la population acidogène, comme le montre le modèle avancé proposé. Le modèle proposé est potentiellement utile pour mieux comprendre la distribution des micropolluants, prédire leur devenir dans des conditions anaérobies et aider à optimiser le processus de fonctionnement pour leur abattement.

MOTS-CLES : Biodégradation, co-métabolisme, hydrocarbures aromatiques polycycliques, méthanisation, nonylphenol, sorption

TITLE: Modeling the fate of micropollutant organics during anaerobic digestion of contaminated sewage sludge.

ABSTRACT: Many organic micropollutants are present in sludge. Their possible impact on the environment contributes to their increasing scientific and social interests. Anaerobic digestion has been shown as a potential biological process for removing these compounds. In this work, a dynamical fate model is developed for hydrophobic micropollutant under anaerobic digestion of contaminated sludge. The model is based on a four-compartment distribution and demonstrated that the micropollutant transformation is well simulated if considering a co-metabolic kinetic and the aqueous phase as the bioavailable compartment. In this model, the sorption of hydrophobic micropollutants is considered on two different phases: particulate matter and dissolved/colloidal matter (DCM). Indeed, the sorption onto DCM can influence the availability of compounds for biodegradation. It was concluded that hydrophobic micropollutant transfer does not limit their biodegradation, and that their fate is governed by sorption-desorption equilibrium state. In order to evaluate which step of the anaerobic pathway is implied in the co-metabolism of micropollutants, experimental set-ups were designed using different way to inhibit the Methanogens. The experimental inhibition of methanogenic activity suggests that the anaerobic degradation of micropollutants mainly involves non-methanogenic microorganisms. Indeed, the co-metabolic transformation of micropollutants would be mainly linked to the acidogenic population as it was shown through the proposed advanced model. This latter is potentially useful to better understand the micropollutant distribution, predict their fate under anaerobic condition and help to optimize the operation process for their depletion.

KEYWORDS: Biodegradation, co-metabolism, methanisation, nonylphenol, polycyclic aromatic hydrocarbons, sorption

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DISCIPLINE: Génie des Procédés

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List of abbreviations and symbols

Dimension

d	Days
g	Gram
Kg	Kilogram
L	Liter
mg	Milligram
mol	Mol
ng	Nanogram
µg	Microgram

Abbreviations

Name	***Description***	***Dimension***
AD	Anaerobic Digestion	
AP	Alkylphenols	
BES	Bromoethanesulfonic acid	
COD	Chemical Oxygen Demand	$g_{O2}.L^{-1}$
DCM	Dissolved and Colloidal Matter	
DM	Dry matter	$g.L^{-1}$
EDCs	Endocrine Disrupting Compounds	
EPA	U.S. Environmental Protection Agency	
EU	European Union	
NP	Nonylphenol	
NPE	Nonylphenol Polyethoxylates	
PAE	Phthalates	
PAHs	Polycyclic Aromatic Hydrocarbons	
PCBs	Polychlorinated Biphenyls	
PS	Primary Sludge	
JEA	Japan Environment Agency	
OMPs	Organic micropollutants	
OSPAR	Oslo and Paris Commission	
SS	Secondary Sludge	
USEPA	United States Environmental Agency	
UKEA	United Kingdom Environment agency	
VFAs	Volatile Fatty Acids	
WWF	World Wildlife Fund	
WWTPs	Wastewater Treatment Plants	

Roman and greek symbols

Name	***Description***	***Dimension***
b	First-order endogenous decay	d^{-1}
C_{aq}	Aqueous fraction of micropollutant	$\mu g.L^{-1}$
$C_{dissolved}$	Aqueous concentration of micropollutant	$\mu g.L^{-1}$
C_f	Free compartment concentration of micropollutant	$\mu g.L^{-1}$
C_g	Gas concentration of micropollutant	$\mu g.L^{-1}$
C_{sorbed}	Sorbed micropollutant concentration onto sludge	$\mu g.L^{-1}$
$C_{t,liq}$	Total liquid concentration of micropollutant	$\mu g.L^{-1}$
c_{DCM}	Micropollutant concentration sorbed to DCM	$mg.g_{COD\text{-}DCM}^{-1}$
c_p	Micropollutant concentration sorbed to particle	$mg.g_{COD\text{-}part}^{-1}$
K_{DCM}	Equilibrium constant of sorption to DCM	$L.g_{COD\text{-}DCM}^{-1}$
K_d	Solid-liquid partition coefficient	$L.Kg^{-1}$
K_H	Henry gas water partitioning coefficient	Dimensionless
K_{La}	Overall mass transfer rate	d^{-1}
K_{ow}	Octano-water partition coefficient	Dimensionless
K_p	Equilibrium constant of micropollutant sorption to particles	$L.g_{COD\text{-}part}^{-1}$
K_S	Half-saturation constant	$g_{COD}.L^{-1}$
K_{S1}	Half-saturation constant associated with S_S	$g_{COD}.L^{-1}$
K_{S2}	Half-saturation constant associated with S_a	$mmol.L^{-1}$
K_{SC}	half saturation constant of micropollutant	$\mu g_{OMP}.L^{-1}$
K_{I2}	Inhibition constant associated with S_a	$mmol.L^{-1}$
k_1	first-order kinetic constant of sorption to particle	d^{-1}
k_2	first-order kinetic constant of sorption to DCM	d^{-1}
k_c	the maximum specific rate of OMPs biodegradation in absence of primary substrate	$\mu g_{OMP}.g_{COD\text{-}X}^{-1}.d^{-1}$
k_{hyd}	First-order kinetic of hydrolysis	d^{-1}
q_M	Molar flow rate of methane	$mmol.d^{-1}.L^{-1}$
S_a	Concentration of volatile fatty acids	$mmol.L^{-1}$
S_p	particulate substrate concentration	$g_{COD\text{-}p}.L^{-1}$
S_S	soluble substrate concentration	$g_{COD\text{-}DCM}.L^{-1}$
T_c	transformation capacity of micropollutant	$\mu g_{OMP}.g_{COD\text{-}Ss}^{-1}$
X	Biomass concentration	$g_{COD}.L^{-1}$
X_1	Concentration of acidogenic bacteria	$g_{DCO}.L^{-1}$
X_2	Concentration of methanogenic microorganisms	$g_{DCO}.L^{-1}$
X_{SS}	Suspended solid concentration	$Kg.L^{-1}$
Y	growth yield	$g_{COD\text{-}X}.g_{COD\text{-}Ss}^{-1}$
Y_1	Yield for COD degradation	$g_{COD\text{-}Ss}.g_{COD\text{-}X1}^{-1}$
Y_2	Yield for VFA production	$mmol\ S_a.g_{COD\text{-}X1}^{-1}$
Y_3	Yield for VFA consumption	$mmol\ S_a.g_{COD\text{-}X2}^{-1}$
Y_4	Yield for CH_4 production	$mmol\ CH_4.g_{COD\text{-}X2}^{-1}$
μ	growth rate	d^{-1}

μ_{max}	the maximum bacterial growth rate	d^{-1}
μ_{max1}	Maximum acidogenic biomass growth rate	d^{-1}
μ_{max2}	Maximum methanogenic biomass growth rate	d^{-1}

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Résumé

La convention de Stockholm a défini le terme "*micropolluant*" comme "*des substances chimiques naturelles ou artificielles capables de présenter des effets toxiques à faible concentration dans un milieu défini*". Une grande variété de ces substances est présente à de très faibles concentrations ($ng.L^{-1}$ à $mg.L^{-1}$) dans notre environnement. La préoccupation majeure concerne les risques sur l'écosystème aquatique et la santé humaine du fait des caractères toxique, mutagène, cancérigène, tératogène ou des effets de perturbation endocrinienne observés avec ces composés. Les perturbateurs endocriniens sont des substances qui interfèrent avec les fonctions du système hormonal et qui risquent d'influer négativement sur les processus de synthèse, de sécrétion, de transport, d'action ou d'élimination des hormones.

Les nombreuses études scientifiques actuelles ont essentiellement mis l'accent sur les sources et le devenir de ces micropolluants: présence, concentration et persistance dans les différents compartiments de l'environnement (air, sol, eau), bioconcentration dans les organismes, processus et dynamique de dissipation (volatilisation, adsorption, dégradation chimique, biodégradation). Les systèmes de traitement des eaux résiduaires apparaissent comme des points majeurs du cycle de vie de ces composés, car ils constituent un point de convergence, d'action et de dissémination. Or, les technologies conventionnelles des stations d'épuration (STEP) n'ont pas été conçues pour l'abattement de ces micropolluants; en revanche, elles peuvent contribuer par divers mécanismes à la réduction de leur concentration et de leurs effets endocrines. Toutefois, leur taux global d'élimination varie fortement et cela montre clairement que leur élimination est souvent incomplète.

En effet, une grande quantité de divers micropolluants, particulièrement les composés hydrophobes, est transférée ou sorbée aux résidus solides, les boues, sous- produits du traitement des eaux usées. Or, leur élimination est souvent incomplète dans les processus de traitement des boues. Ces boues sont très largement utilisées en agriculture afin de pallier les pertes de matière organique par les sols et de valoriser des éléments fertilisants contenus dans ces boues. Cependant, cette pratique de recyclage ne peut se justifier que si l'innocuité des boues épandues est garantie. Or, elles peuvent être vecteurs de polluants, éléments traces metalliques et micropolluants organiques, ce qui, en fonction de leur devenir, peut conduire à la dégradation du patrimoine sol (accumulation de polluants, écotoxicité) puis à la contamination des eaux et de la chaîne alimentaire. Cependant, avant leur valorisation, ces

boues sont souvent traitées via divers procédés afin d'assurer leur stabilisation et leur assainissement: la digestion anaérobie est l'un de ces procédés.

La digestion anaérobie des boues présente plusieurs avantages, tels que (i) la réduction efficace des solides, (ii) la production d'énergie renouvelable sous forme de biogaz, (iii) la stabilisation et la réduction des germes microbiens et (iv) l'élimination partielle des micropolluants. Plusieurs mécanismes d'élimination des micropolluants ont été identifiés dans la littérature : la volatilisation (transfert à l'atmosphère), la sorption (transfert vers les solides) et la dégradation (par voie physico-chimique ou biologique). Seule la dégradation constitue un réel mécanisme d'élimination par la mineralization du micropolluant, les deux autres consistent en un déplacement de la pollution. Ces mécanismes dépendent des propriétés physico-chimiques des micropolluants, ainsi que des caractéristiques des boues et de l'état de fonctionnement du procédé biologique.

Si ces mécanismes commencent à être décortiqués au sein des procédés aérobies de traitement des eaux, très peu de données sont trouvées dans la littérature sur leur implication lors de la digestion anaérobie des boues contaminées en micropolluants. Les modèles mathématiques semblent être de bons outils pour mieux comprendre la dynamique des polluants au sein des procédés et *in fine* optimiser leur élimination, c'est aussi un moyen d'intégrer l'ensemble des connaissances acquises sur devenir, distribution et élimination des micropolluants.

L'objectif global de ce travail est de développer un modèle mathématique et dynamique qui décrit la distribution et le devenir des micropolluants lors de la digestion anaérobie des boues. Les objectifs secondaires ont été définis comme ci-dessous:

- Développer un modèle pour décrire et comprendre la répartition des micropolluants dans les différents compartiments de la boue et pour déterminer le compartiment disponible pour la dégradation biologique.
- Intégrer des mécanismes biotiques et abiotiques tels que la volatilisation, la sorption et la dégradation des micropolluants dans le modèle.
- Evaluer les hypothèses des cinétiques de co-métabolisme et déterminer les voies métaboliques impliquées dans la dégradation de ces composés.

Le manuscrit se divise en six chapitres.

Dans le **chapitre 1**, la motivation et les objectifs sont énoncés. Les publications et les présentations orales issues de ces travaux sont listées.

Le **chapitre 2** est consacré à la présentation de la revue bibliographique. Tout d'abord, il positionne le sujet dans son contexte global, à savoir la problématique des micropolluants organiques prioritaires et émergents au regard de la mise en œuvre de la Directive Cadre Eau (2000/60/CE) et de l'atteinte du bon état écologique et chimique de nos masses d'eau d'ici à 2015. Malgré leur très faible concentration dans les milieux, ces micropolluants peuvent provoquer un impact écotoxicologique important. Certains d'entre eux, classés perturbateurs endocriniens et présents dans les eaux de surface, peuvent induire la féminisation de poissons. Les systèmes de traitement des eaux sont ainsi pointés du doigt car ils apparaissent comme un maillon clé dans la chaîne de dissémination vers les écosystèmes récepteurs. Si la filière « eau » est abondamment regardée dans la littérature, la problématique de la gestion des boues ne doit pas être négligée. En effet, une bonne partie de ces composés, notamment les composés hydrophobes sont transférés dans les boues d'épuration. Or, ces dernières sont largement utilisées en épandage agricole, d'où une préoccupation croissante de la gestion de ces boues versus la présence de polluants.

Ensuite, les molécules sélectionnées pour notre étude sont présentées avec leur teneur respective dans les boues urbaines. Les nonylphénols et 13 composés représentatifs de la famille des hydrocarbures aromatiques polycycliques (HAP), identifiés comme perturbateurs endocriniens par différentes agences de l'environnement, sont des composés persistants, hydrophobes (log K_{ow} entre 3,4 et 6,6), s'adsorbant sur la fraction organique particulaire des boues.

Le procédé de digestion anaérobie est brièvement présenté pour se focaliser ensuite sur les connaissances acquises sur le devenir des molécules selectionnées présentes dans les boues urbaines au cours de leur méthanisation. Ce procédé, couramment utilisé pour le traitement des boues, a démontré tout son potentiel dans l'élimination de composés hydrophobes, mais les abattements restent souvent incomplets. Les facteurs et processus jouant un rôle déterminant dans le devenir de ces composés sont distingués. Entre autre: (i) la dégradation de

ces micropolluants est étroitement liée à la dégradation des matières organiques et (ii) la digestion anaérobie thermophile améliore la dégradation des micropolluants avec une augmentation concomitante de l'efficacité d'élimination des solides totaux. Aussi, la présence de substrats facilement dégradables provenant de boues peut accroître la dégradation anaérobie des micropolluants. Ces résultats combinent en fait deux aspects fondamentaux. Le premier est en lien avec la nature même de la matrice boue, système organo-mineral complexe, qui interagit avec les micropolluants via le processus de sorption, celui-ci détermine le ou les compartiments disponibles pour la dégradation. Le second, toujours en lien avec la nature de la matrice boue, concerne le métabolisme général développé par les populations microbiennes (diversité structurelle et fonctionnelle) et qui soustend le métabolisme des micropolluants.

Ce chapitre se termine par une revue des divers modèles mathématiques développés pour décrire la distribution et la dégradation des micropolluants. Les modèles développés emploient communément une approche bi-compartiment de la distribution du micropolluant, à savoir, le compartiment sorbé aux «particules», considéré comme non biodisponible, et le compartiment «phase aqueuse», considéré comme biodisponible. Du point de vue des cinétiques de biodégradation, des cinétiques avec biomasse spécifique de type Monod sont utilisées.

De façon générale, les modèles « micropolluants » ont été développés pour simuler leur devenir au sein des traitements aérobies, type boues activées. Pourtant la digestion anaérobie a montré un réel potentiel pour l'abattement de micropolluants et est un écosystème fréquemment rencontré dans notre environnement. Selon les récents travaux sur la digestion, la dégradation de composés récalcitrants, tels que les HAP et le NP, est souvent concomitante avec la transformation co-métabolique de la matière sèche et s'opère grâce à la présence d'une fraction biodisponible qui inclut le compartiment dissous/colloïdal : ces deux mécanismes pourraient être considérés et inclus dans la modélisation du devenir des micropolluants.

Le **chapitre 3** est dédié au développement d'un modèle dynamique du devenir des micropolluants au cours de la digestion anaérobie. Le modèle est basé sur une approximation de quatre compartiments, dans lesquels les micropolluants vont coexister sous quatre états: gazeux, libre, associé à la matière particulaire, associé à la matière colloïdale et dissoute. Ce

système est décrit par trois constantes d'équilibres: K_{part} équilibre entre les états libres et associé aux particules, K_{DCM} équilibre entre les états libres et associé à la matière colloïdale et dissoute et K_H la constante de Henry, équilibre entre les états libre et gazeux.

Le modèle à quatre compartiments intègre les processus abiotiques et biotiques de l'abattement des micropolluants: la volatilisation vers la phase gazeuse et la sorption sur les phases particulaire et dissoute/colloïdale. Cette approche améliore la prédiction de la biodisponibilité et de la biodégradation des micropolluants. En effet, cet article montre que le modèle simule bien le devenir des micropolluants dans le système anaérobie, considérant des cinétiques de co-métabolisme et le compartiment aqueux comme le compartiment biodisponible. Une explication écologique pour le co-métabolisme est que l'élimination des composés, présents seulement au niveau des traces ($ng.L^{-1}$ ou $mg.L^{-1}$), n'a pas entraîné de croissance importante de la biomasse. Il est à noter que la biodégradation des boues est représentée par un modèle simple à trois processus (hydrolyse, croissance et mort cellulaire) et une seule biomasse. Cette biomasse représente la communauté globale anaérobie qui produit des enzymes et des cofacteurs pour l'utilisation des substrats solubles et aussi pour la transformation des micropolluants par co-métabolisme. Finalement, le modèle a été calibré et validé à l'aide de données expérimentales obtenues précédemment à partir de deux réacteurs continus alimentés avec des boues primaires et secondaires exploités dans des conditions mésophiles. Une méthode non linéaire des moindres carrés a été utilisée pour optimiser les paramètres du modèle. Le modèle est en accord avec les données expérimentales.

Le **chapitre 4** est consacré à l'acquisition de données afin d'élucider quelles étapes de la digestion anaérobie sont impliquées dans l'élimination des micropolluants. Pour cela, deux stratégies d'inhibition de la phase de Méthanogénèse sont mises en œuvre : elles sont fondées sur la séparation apparente de la digestion anaérobie en deux étapes sur des digesteurs anaérobies continus. Un réacteur anaérobie avec de bonnes performances méthanogène a été divisé en deux réacteurs identiques. Sur un réacteur, l'ajout d'une concentration élevée d'acide acétique a produit un déséquilibre dans le système avec une accumulation des acides gras volatils (AGVs), une baisse de pH, une diminution de l'activité méthanogène (chute du rendement en méthane) et un maintien de l'activité de dégradation des micropolluants. Sur le second réacteur, du sélénite soluble (Na_2SeO_3) a été ajouté à une concentration élevée, celui-ci, théoriquement a un effet irréversible toxique sur les organismes méthanogènes. Cependant,

si les oxyanions de sélénium (SeO_3^{2-}, SeO_4^{2-}) peuvent influencer directement la méthanogénèse via l'inhibition des groupes microbiens impliqués, ils peuvent aussi agir indirectement en modifiant le flux des électrons dans la chaîne réactionnelle : ils peuvent en effet jouer le rôle d'accepteurs d'électrons. Dans ce cas ci, nous avons observé une réduction de l'activité de dégradation des micropolluants mais que l'on ne peut imputer à une baisse de l'activité méthanogène seule puisque le métabolisme général a lui aussi été perturbé. Les résultats montrent que la dégradation anaérobie des micropolluants implique principalement les microorganismes non méthanogènes, c'est-à-dire que la transformation co-métabolique du micropolluant serait principalement liée à la population acidogène. Cependant, les populations méthanogènes peuvent co-métaboliser les micropolluants mais avec des cinétiques plus lentes que les populations acidogène.

Dans le **chapitre 5,** un nouveau modèle a été développé en considérant la séparation du consortium total de microorganismes anaérobies en deux types de biomasse: une biomasse acidogène et une biomasse méthanogène. La biodégradation de la matière organique est décrite par trois étapes: la première étape est l'hydrolyse de la matière particulaire en substrat soluble. Puis, l'étape d'acidogénèse où les bactéries acidogènes consomment le substrat soluble et produisent du dioxyde de carbone et des acides gras volatils. Ensuite, la population de microorganismes méthanogènes utilise les acides gras volatils comme substrat de croissance et produit du dioxyde de carbone et du méthane. La transformation co-métabolique du micropollutant est liée à chaque type de biomasse: acidogéne et methanogéne. Le modèle a été calibré grâce aux données expérimentales obtenues à partir de deux réacteurs mésophiles continus alimentés avec des boues secondaires: un réacteur au fonctionnement optimal sous méthanisation (*Rnc=R2*-chapitre 3) et un autre réacteur avec inhibition de la phase méthanogène (*Raa,* chapitre 4). Les résultats montrent que la vitesse de co-métabolisme est plus élevée avec l'étape d'acidogénèse qu'avec celle de l'étape de méthanogénèse.

Le **chapitre 6** énumère les conclusions et les perspectives de ces travaux.

Les résultats obtenus sont présentés sous le format de leur valorisation par publication. Afin de rendre la lecture du document plus fluide, les chapitres sont brièvement introduits par un avant-propos et se terminent par une discussion générale.

1. General Introduction

1.1. Motivation and objectives

The Stockholm convention defined the term "micropollutant" as "natural or man-made chemical substances able to present toxic effect at low concentration in a defined medium". A huge variety of these substances is present at very low concentration ($ng.L^{-1}$ to $\mu g.L^{-1}$) in our environment. The major concern regards their risk on aquatic ecosystem and human health and involves acute toxicity, mutagenesis, carcinogenesis, teratogenesis or endocrine disrupting effects. Many scientific studies have focused on the sources and the fate of these micropollutants: presence, concentration and persistence in various environmental compartments (air, soil, water), organisms bioconcentration, processes and dynamic of dissipation (volatilization, adsorption, chemical degradation, biodegradation).

Numerous sources of micropollutants are reported in the water systems: chemical pollution in rainfall-runoff and wastewater resulting from atmospheric washout, erosion of building materials, traffic emissions, pesticides application, industrial production, use of household chemicals, personal care products and pharmaceuticals. Indeed, due to the persistence, the bioaccumulation potential and the toxicity of micropollutants, it is necessary to minimize their input into the sewage system and water bodies. There is thus a need for the micropollutants control from direct and indirect discharger point sources such as industrial enterprises, hospitals and wastewater treatment plants. The wastewater treatment plants can be considered as a point of convergence for various micropollutant sources but they were first designed for carbon and nitrogen removal, as well as microbial pollution, and consequently they are not specifically designed for micropollutant removal. In fact, a large amount of various micropollutants, especially the hydrophobic compounds, are transferred or sorbed to the sludge solid wastes produced during wastewater treatment, and their elimination is often incomplete in the sludge treatment processes. These sludge wastes could be of great advantage when recycled and used in farmland as fertilizer and as soil conditioner. However, the direct application of this waste containing micropollutants on agricultural fields may cause a potential soil and groundwater contamination. Before their disposal, sludge wastes can be treated by several processes in order to ensure stabilization and sanitation, anaerobic digestion being one of these processes.

In particular, anaerobic sludge digestion presents several advantages, such as (i) efficient reduction of solids, (ii) production of renewable fuel (reducing global warming emission), (iii) stabilization of sludge and microbial reduction for application in agriculture fields and (iv)

potential for micropollutant depletions. Indeed, several mechanisms of micropollutant elimination have been identified in the scientific literature. They depend on physicochemical properties of micropollutant, as well as sludge characteristics and operating condition of the biological process. Despite these facts, only few data are found in the literature dealing with the micropollutant fate during anaerobic sludge digestion. In addition, a mathematical model appears to be crucial to integrate and/or summarize the best available knowledge in order to predict the fate, the distribution and the removal of micropollutants. Models are also an important tool to better understand and optimize the micropollutant depletion in the process.

In this frame work, the overall aim of this work is to develop a dynamic mathematical model that describes the fate and the distribution of the micropollutant during the anaerobic sludge digestion. Secondary objectives were defined as listed below:

- Develop a model to describe and understand the distribution of micropollutants.
- Integrate biotic and abiotic mechanisms such as volatilization, sorption and degradation for micropollutant removals.
- Evaluate the hypothesis of co-metabolism kinetics and bioavailability in the degradation pathway of these compounds.

1.2. Outline of the thesis

This document is divided in 6 chapters. In chapter 1, the motivation and objectives are stated, and the publications and the oral presentations are listed. Chapter 2 presents the literature review on the micropollutant fate and removal in anaerobic systems as well as the existing models developed for micropollutant fate in wastewater and sludge treatment. Chapter 3 includes the four-compartment model for micropollutant fate developed with the data set obtained from lab-scale anaerobic reactors treated sewage sludge. Chapter 4 is dedicated to a new experimental set up and data acquisition in order to elucidate which anaerobic steps are involved in the micropollutant removal. Chapter 5 presents the advanced model formulated with the new data set. Finally, chapter 6 lists the conclusions and perspectives of this thesis. All the references are listed at the end of the document. Appendices include summaries of the articles published and/or submitted in which the author has contributed.

List of publications, "in preparation" articles, oral and poster presentations:

Publications

1. Pierre Buffiere, Liliana Delgadillo-Mirquez, Jean-Philippe Steyer Nicolas Bernet and Jean-Philippe Delgenes. 2008. *Anaerobic Digestion of Solid Wastes Needs Research to Face an Increasing Industrial Success*. International Journal of Chemical Reactor Engineering 6: A94.

2. Maialen Barret, Hélène Carrère, Liliana Delgadillo-Mirquez and Dominique Patureau. 2010. *PAH fate during the anaerobic digestion of contaminated sludge: Do bioavailability and/or cometabolism limit their biodegradation?* Water Research 44(13): 3797-3806.

3. Liliana Delgadillo-Mirquez, Laurent Lardon, Jean-Philippe Steyer and Dominique Patureau. 2011. *A new dynamic model for bioavailability and cometabolism of micropollutants during anaerobic digestion*. Water Research 45(15): 4511-21.

4. Maialen Barret, Liliana Delgadillo-Mirquez, Eric Trably, Florence Braun, Glenda Cea-Barcia, Jean-Philippe Steyer and Dominique Patureau. *Trace organic contaminant removal under anaerobic conditions: 15 years of experience*. Pedosphere, submitted.

5. Liliana Delgadillo-Mirquez, Laurent Lardon, Jean-Philippe Steyer and Dominique Patureau. *Effect of methanogenic inhibition on the hydrophobic micropollutant removal during anaerobic sludge digestion*. In preparation.

6. Liliana Delgadillo-Mirquez, Laurent Lardon, Jean-Philippe Steyer and Dominique Patureau. *Advanced model of micropollutant fate during anaerobic digestion*. In preparation.

Oral Presentations

1. Liliana Delgadillo-Mirquez, Laurent Lardon, Jean-Philippe Steyer and Dominique Patureau. *A four-compartment model of the PAHs fate during anaerobic sludge digestion*. International Water Association. 12th World Congress on Anaerobic Digestion. October 31st – November 4th. 2010. Guadalajara, Jalisco – Mexico.

2. Liliana Delgadillo-Mirquez, Laurent Lardon, Jean-Philippe Steyer and Dominique Patureau. *A mechanistic model describing the fate of Endocrine Disrupting Chemicals during anaerobic sludge treatment*. 20th Setac Europe Annual meeting. May 23 – 27th. 2010. Seville – Spain.

Posters

1. Florence Braun, Glenda Cea-Barcia and Liliana Delgadillo-Mirquez. *Approche intégrée et pluridisciplinaire de la dégradation des perturbateurs endocriniens dans un procédé modèle de digestion anaérobie de boues urbaines*. Juin 10, 2011. Journée de l'ED "Sciences des Procédés, Sciences des Aliments", Montpellier – France.

2. Liliana Delgadillo-Mirquez, Laurent Lardon, Jean-Philippe Steyer and Dominique Patureau. *Modélisation du devenir de micropolluants organiques au cours de la digestion anaérobie*. Juin 22, 2010. Journée de l'ED "Sciences des Procédés, Sciences des Aliments", Montpellier – France.

3. Liliana Delgadillo-Mirquez, Laurent Lardon, Jean-Philippe Steyer and Dominique Patureau. *Modélisation du flux de micropolluants organiques au cours du traitement des eaux*. Juin 8 -11, 2009. Ecole-Chercheurs. Université de Nancy – France.

2. Literature Review

Wastewater contains a complex mixture of organic micropollutants at low concentration ($ng.L^{-1}$ to $\mu g.L^{-1}$), originating from personal care products, pharmaceuticals, excreted hormones, vehicles, exhausts household and industrial chemical and rain that collect pollutants from air and surfaces before entering the sewer. Many of these chemicals are considered to be persistent pollutants and some of them can interfere with the endocrine system of a wide range of organisms. These substances are referred as endocrine disrupting compounds (EDCs) and they have attracted serious attention in environmental science research and policy due to their ubiquity and estrogenic activities (Sumpter and Johnson, 2005).

There have been many definitions for EDCs. However, the U.S. Environmental Protection Agency (EPA) has proposed a more detailed definition: *"an endocrine disrupter is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance or homeostasis, reproduction, development and or behavior*" (Kavlock *et al*., 1996). Table 2.1 includes a wide list of chemicals that have already been classified as endocrine disrupters by organizations worldwide (Barret, 2009).

The EDCs have been detected in different environmental media including wastewaters (Rowsell *et al*. 2009, Musolff *et al*. 2010), surface water and groundwaters (Latorre *et al.* 2003, Cailleaud *et al.* 2007), sediments (Fu *et al.* 2007), aquatic organisms (Belfroid *et al.* 2002, Pojana *et al.* 2007) and drinking waters (Maeng *et al.* 2010, Schriks *et al.* 2010, Morasch *et al.* 2010). Some studies aiming at assessing whether different groups of organisms show similar or dissimilar responses to selected EDCs have also been reported (Sumpter and Johnson, 2005). Besides, health impairment has been demonstrated in aquatic species exposed to EDCs in urbanized and industrialized areas (Couillard *et al.* 2008, Harris *et al.* 2011).

The wastewater treatment plants (WWTPs) play thus an important role in the life cycle of these compounds: they are a point of convergence of multiple sources but also a point of action where the diversity of bioprocesses can reduce the micropollutant load to the environment and finally, they are a point of dissemination because of the discharge either to the receiving waters or to the land through sludge spreading. Indeed, modern WWTPs can effectively accomplish carbon, nitrogen and phosphorus removal, as well as microbial pollution control. However, they are not designed to remove the large number of trace polluting compounds, such as PAHs (polycyclic aromatic hydrocarbons), PCBs

(polychlorinated biphenyls), hormones, etc. The reported overall removal rate of micropollutants in WWTPs varies strongly and they clearly show that their elimination is often incomplete (Morasch *et al.* 2010, Musolff *et al.* 2010, Rowsell *et al.* 2009).

Table 2.1. List of compounds classified as endocrine disrupters by various organizations: **UKEA** (United Kingdom Environment Agency), **USEPA** (United States Environmental Protection Agency), **OSPAR** (Oslo and Paris Commission), **JEA** (Japan Environment Agency) and **WWF** (World Wildlife Fund).

Compound	UKEA	USEPA	OSPAR	JEA	WWF
Steroids					
Ethinyl estradiol	X		X		
17β-estradiol	X		X		
Estrone	X		X		
Mestranol			X		
Diethylstilbestrol	X		X	X	X
Alkylphenols					
Nonylphenol	X	X	X	X	X
Nonylphenol ethoxylate	X				
Octylphenol	X	X	X	X	
Octylphenol ethoxylate	X				
Polyaromatic compounds					
Polychlorinated biphenyls (PCBs)	X	X	X	X	X
Brominated flame retardants				X	X
Polycyclic aromatic hydrocarbons (PAHs)		X	X		
Organic oxygen compounds					
Phthalates	X	X		X	X
Bisphenol A	X	X		X	X
Pesticides					
Atrazine	X	X		X	X
Simazine	X	X		X	X
Dichlorvos	X				
Endosulfan	X	X		X	X
Trifluralin	X	X			X
Demeton-S-methyl	X				
Dimethoate	X				X
Linuron					X
Permethrin	X	X		X	
Lindane	X	X	X		X
Chlordane	X			X	X
Dieldrin	X	X		X	X
Hexachlorobenzene	X			X	X
Pentachlorophenol	X	X		X	X
Others					
Dioxins and furans	X		X	X	X
Tributyltin	X	X	X	X	

Moreover, sewage sludge (or biosolids) produced as a results of wastewater treatment are known to contain a wide range of EDCs (Patureau *et al*. 2007, González *et al*. 2010, Poulsen and Bester 2010). The main options for sludge disposal are presently either recycling to agriculture land or landfilling either directly or as ash following incineration. However,

recycling of sludge to agriculture land is in most cases the least expensive option for disposal, and also results in a degree of recycling of carbon, nitrogen, phosphorus and other minerals. The use of sewage sludge in agriculture is based on the prerequisite that they are safe. It is thus important to see if one of the common processes used for sludge stabilization and sanitation anaerobic digestion may help to reduce the micropollutants fluxes on soils.

In this chapter, we will firstly focus on the occurrence of EDCs in sewage sludge and present the targeted compounds of this work. The anaerobic digestion process is presented afterwards. The third part is dedicated to the impact of anaerobic processes on the NP and PAHs removal. The last part summarizes the current modeling approaches developed to simulate the transport and the removal of micropollutants in wastewater and sludge treatments.

2.1. Endocrine disrupting chemicals in sewage sludge

More than 50 000 wastewater treatment plants are operating in the European Union (EU), producing more than 7.9 million tons of dry matter annually (Poulsen and Bester 2010, Schmidt *et al*. 2006). The agriculture disposal remains today one of the most popular options for disposal in the EU. The main obstacle for sludge utilization in agriculture is its ability to concentrate potential micropollutant such as pesticides, metals, pathogens, industrial solvents, dyes, plasticizers and other persistent organic chemical residues (González *et al*. 2010, Poulsen and Bester 2010, Patureau *et al*. 2007). Due to the presence of such refractory compounds, the disposal and the handling of sewage sludge is a growing problem in Europe.

The literature survey regarding observations of micropollutants in sludge showed that a relatively large number of compounds have been identified and quantified (Eriksson *et al*. 2008, Patureau *et al*. 2007). Eriksson *et al*. (2008) have found through literature survey 192 different micropollutants in sewage sludge that were grouped into 14 families according to their potential presence: aliphatic hydrocarbons, dioxins and furans, endocrine disrupters, flame-retardants, organotins, PAHs, PCBs, pesticides, pharmaceuticals, phthalates and plasticizers, as well as miscellaneous compounds. Sixty-three compounds could not be classified due to lack of available data and the remaining 99 compounds were identified as being hazardous and require further hazard assessment. Schmidt *et al*. (2006) have also shown a broad variation of the different compounds between EU countries. This broad variation in concentration may be explained by: various analytical methodologies, analysis of various type

of sludge (treated, non treated…), intrinsic variability between countries, etc. Table 2.2 shows some micropollutant concentrations in sludge reported in the literature; the micropollutant concentrations vary a lot and they depend of the sludge treatment used. But it seems that alkylphenols and organohalogen compounds are frequently found at high level (around 100-500 $mg.Kg_{DM}^{-1}$) whereas PAHs, PCBs, pesticides, dioxins, estrogens are present at low to very low levels from pg to $mg.Kg_{DM}^{-1}$. These levels have to be compared to the proposed limits of the third draft of the EU sewage sludge directive (EU, 2001): organohalogens, 500 $mg.Kg_{DM}^{-1}$; linear alkylbenzene sulfonate, 2600 $mg.Kg_{DM}^{-1}$; phthalate (DEHP), 100 $mg.Kg_{DM}^{-1}$; nonylphenol ethoxylates, 50 $mg.Kg_{DM}^{-1}$; PAH, 6 $mg.Kg_{DM}^{-1}$; PCB, 0.8 $mg.Kg_{DM}^{-1}$ and dioxins/furans, 100 $ng.Kg_{DM}^{-1}$. Patureau *et al*. (2007) reported the analysis of four sewage sludge samples for polycyclic aromatic hydrocarbons (PAHs), nonylphenol polyethoxylates (NPE) and phthalates (PAE) which reveals concentrations varying between $mg.Kg^{-1}$ to $g.Kg^{-1}$ of dry matter.

Table 2.2. Some EDCs concentration values in sludge reported in the literature.

Compounds	Unit	Sludge treatment	Concentration			Reference
			Min	***Max***	***media***	
Nonylphenol	$mg.Kg_{DM}^{-1}$	Digestion	44	199	72	Fernandez-Sanjuan *et al.* 2009
		No digestion	4.8	11.9		
Nonylphenol ethoxylate	$mg.Kg_{DM}^{-1}$	Digestion	11.4	47	24	
		No digestion	3.2	31.3		
Estrone	$\mu g.Kg_{DM}^{-1}$	Digestion	22.8	27.8		Andersen *et al.* 2003
		Liming	2.5	21.7		Bevacqua *et al.* 2011
17β-estradiol	$\mu g.Kg_{DM}^{-1}$	Digestion	4.9	5.4		Andersen *et al.* 2003
PAHs	$\mu g.Kg_{DM}^{-1}$	Unknown	2534.1	6926.6	3724.1	Zeng *et al.* 2010
PCBs	$\mu g.Kg_{DM}^{-1}$	Unknown	98.76	14.54	69.27	
Dioxins and furans*	$ng.Kg_{DM}^{-1}$	Liming	0.97	15	2.53	Li *et al.* 2011
		Dewatering	1.91	11	4.57	
Bisphenol A	$mg.Kg_{DM}^{-1}$	Unknown	0.10	1.75	0.53	Clarke and Smith 2011
Phthalate	$mg.Kg_{DM}^{-1}$	Unknown	0.26	3574	159	

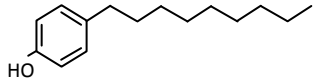
*TEQs total toxic equivalent values

This work is focused on the alkylphenols as nonylphenol and 13 representative compounds of the polycyclic aromatic hydrocarbons (PAHs) family. These compounds have been identified as EDCs by different environmental agencies (table 2.1). Polycyclic Aromatic Hydrocarbons (PAHs) and Nonylphenol (NP) are often persistent in the environment and they are listed in the Standards for sludge spreading.

2.1.1. Nonylphenol (NP)

Nonylphenol (NP) is by far the most commercially prevalent member of the Alkylphenols (AP) family, representing approximately 85% of the total AP market (Corvini *et al*, 2006). AP consists of a phenol ring, which is mono- or poly-substituted by alkyl chains of variable length. AP and their polyethoxylated derivatives are used directly as intermediates or as additives (emulsifiers, detergents, and flotation and dispersing agents) for a wide range of industrial products and processes. Due to nonyl chain, NP (table 2.3) is hydrophobic (log K_{ow} = 4.48) and tends to sorb onto various materials (Vinken *et al.* 2004). By this way, many studies report NP as a persistent pollutant in sewage sludge (González *et al*. 2010, Eriksson *et al*. 2008, Patureau *et al*. 2007). It has also been shown as a toxic and bioaccumulative in aquatic species (Nakamura *et al*. 2002).

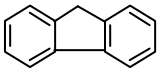
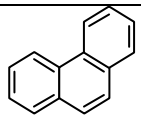
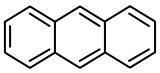
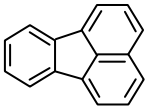
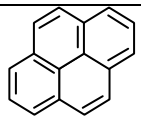
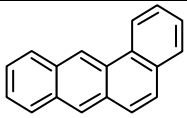
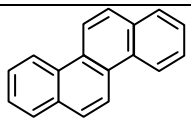
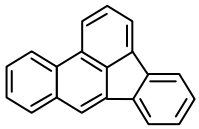
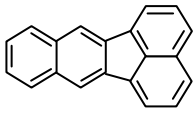
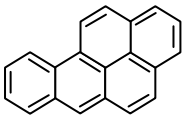
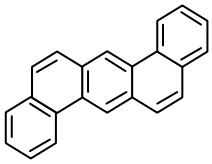
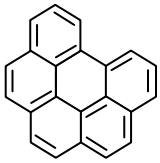
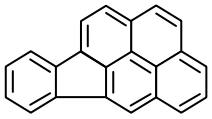
Table 2.3. Physicochemical properties of Nonylpehnol (NP).

Compound	**Structure**	**Molecular formula**	**Molecular weight ($g.mol^{-1}$)**	**Solubility in water ($mg.L^{-1}$)**	**log K_{ow} Octano-water partition coefficient**
Nonylphenol		$C_{15}H_{24}O$	220.35	6	4.48

2.1.2. Polycyclic Aromatic Hydrocarbons (PAHs)

These compounds generally consist of aromatic rings. PAHs have also the potential to bioaccumulate and have log K_{ow} values in a range of 4 to 6, indicating high degree of lipophilicity. Hence, partitioning to solid phase will predominate. PAHs are regarded as environmental pollutant by environmental and health agencies because they have toxic, mutagenic and carcinogenic effects on the living organisms (Samanta *et al*. 2002). PAHs are formed from both natural and anthropogenic sources, largely by the incomplete combustion of organic materials. Natural sources of PAHs include forest fires, volcanic activity, burning, and release of petroleum hydrocarbons. Anthropogenic sources of PAHs can be classified as stationary or mobile. The stationary category incorporates a wide range of activities, such as residential and commercial heating and industrial processes. Whiting the mobile category, petrol and diesel-engined vehicles are the predominant sources.

Table 2.4. Physicochemical properties of Polycyclic Aromatic Hydrocarbons (PAHs).

Compound	**Structure**	**Molecular formula**	**Molecular weight ($g.mol^{-1}$)**	**Solubility in water ($mg.L^{-1}$)**	**log K_{ow} Octano-water partition coefficient**
Fluorene		$C_{13}H_{10}$	166.22	1.68	4.18
Phenanthrene		$C_{14}H_{10}$	178.23	1.0	4.46
Anthracene		$C_{14}H_{10}$	178.23	0.045	4.5
Fluoranthene		$C_{16}H_{10}$	202.26	0.206	4.9
Pyrene		$C_{16}H_{10}$	202.26	0.132	4.88
Benzo(a)anthracene		$C_{18}H_{12}$	228.28	0.0094	5.63
Chrysene		$C_{18}H_{12}$	228.28	0.0018	5.63
Benzo(b)fluoranthene		$C_{20}H_{12}$	252.31	0.0015	6.04
Benzo(k)fluoranthene		$C_{20}H_{12}$	252.31	0.0080	6.21
Benzo(a)pyrene		$C_{20}H_{12}$	252.31	0.0016	6.06
Dibenz(a,h)anthracene		$C_{22}H_{14}$	278.35	0.0050	6.86
Benzo(g,h,i)perylene		$C_{22}H_{12}$	276.33	0.0007	6.78
Indeno(1,2,3,c,d)pyrene		$C_{22}H_{12}$	276.33	0.0002	6.58

The persistence of PAHs in the environment is dependent on a variety of factors, such as their chemical structure, their concentration and dispersion and their bioavailability (Bamforth and Singleton 2005). In general, high molecular weight PAHs are hydrophobic, toxic and persistent (Cerniglia 1992). This work is focused on 13 representative compounds of the polycyclic aromatic hydrocarbons family (table 2.4).

2.2. Anaerobic digestion of sludge

Anaerobic digestion is a process whereby organic matter is broken down in absence of oxygen into methane and carbon dioxide by naturally occurring microorganisms. Anaerobic digestion is a common process for treatment of sludge. Compared with other processes, its advantages are less requeriment of energy, a better stabilized product, and production of biogas being possibly used as bioenergy (heat, electricity). However, several limitations for anaerobic digestion include the slow reaction dynamics, the sensitivity to shock loads and toxic materials, and its complex operation.

2.2.1. The steps of anaerobic digestion

The main biochemical and microbial steps involved in anaerobic digestion process are summarized in figure 2.1 (Batstone *et al*. 2002).

Disintegration and Hydrolysis. These steps are extracellular biological and non-biological processes mediating the breakdown and solubilization of complex organic material into soluble substrates. Disintegration is largely a non-biological step, it converts the complex matter into inert substances and organic substances as carbohydrates, proteins and lipids. Complete enzymatic hydrolysis step transforms the degradation products into monosaccharides, amino acids and long chain fatty acids, respectively.

Acidogenesis. This step consists in the monomer compounds consumption by the fermentative bacteria without an additional electron acceptor or donor. This includes the degradation of soluble sugars and amino acids to a number of simpler products as volatile fatty acids (acetate, propionate, butyrate, etc.), alcohols, organic acids (lactate), but also hydrogen and carbon dioxide.

Acetogenesis. It is an oxidation step with no internal electron acceptor. Therefore the organisms oxidizing the organic acids are required to utilize an additional electron acceptor such as hydrogen ions or carbon dioxide to produce hydrogen gas or formate respectively. These electron carriers must be maintained at a low concentration for the oxidation reaction to be thermodynamically possible. Volatile fatty acids as propionate, butyrate and valerate are converted into acetate and hydrogen. Similarly, long chain fatty acids are oxidized anaerobically to produce acetate and hydrogen.

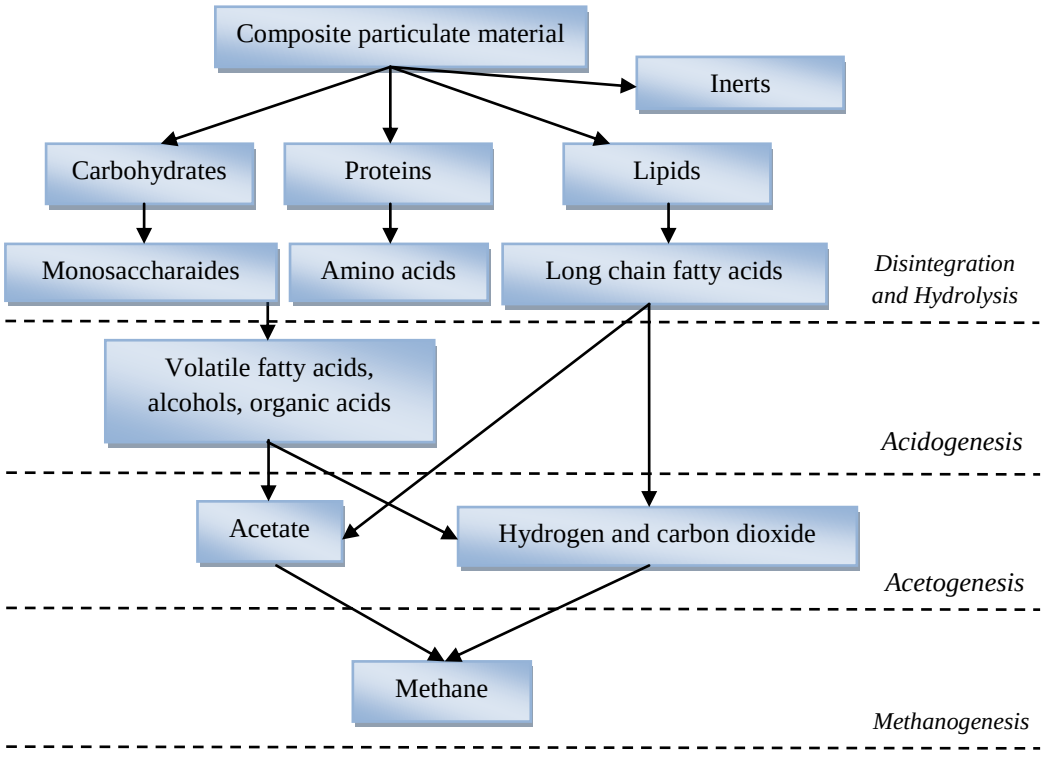


Figure 2.1. Anaerobic digestion process (Batstone *et al*. 2002).

Methanogenesis. Methane (CH_4) is produced by both cleavage of acetate to CH_4 (acetoclastic methanogenesis) and by H_2 to produce CH_4 (hydrogenotrophic methanogenesis). In the *hydrogenotrophic methanogenesis*, the hydrogen and formate are consumed by methanogenic organisms. The thermodynamics of syntrophic acetogenesis and hydrogen utilizing methanogenesis reactions are only possible in a narrow range of hydrogen or formate concentrations (and also influenced to a lesser degree by other product and substrate

concentrations). *Acetoclastic methanogenesis* is the main methanogenic step. Two genera utilize acetate to produce methane: *Methanosarcina* dominates above 10^{-3}M acetate while *Methanosaeta* dominates below this acetate level. Methanogenesis is regarded as the motive force of the anaerobic degradation as it is an energy producing process under standard conditions, as opposed to some of the other processes in the anaerobic degradation. Furthermore, it is the terminal step required for complete mineralization.

2.2.2. The physicochemical parameters

In essence, the anaerobic sludge behavior depends on the following physicochemical parameters.

- **Macro and micro nutrients.** Hydrogen, nitrogen, oxygen and carbon are the main ingredients in organic material. Sulphur is necessary for synthesis of the amino acids, cysteine and methionine. Phosphorus is found in nucleic acids, phospholipids, ATP, GTP, NAD and FAD. Potassium, calcium, magnesium and iron are required as cofactors for enzyme activity and as components in metal complexes. All these nutrients are thus required for the growth of anaerobic microorganisms.
- **Temperature.** Normally, the experiments with anaerobic digestion have been done in the mesophilic (30-40°C) and in the thermophilic (50-60°C) temperature range. Also, temperature influences the toxicity of ammonia: ammonia toxicity increases with increasing temperature. It is due to the fact that high temperature increases the solubility of various components as the proteins producing thus ammonia.
- **pH** influences the growth of the microorganisms, and can affect other factors such as dissociation of important compounds (ammonia, sulphide, organic acids) for the anaerobic digestion process. pH is mainly controlled by the bicarbonate buffer system, therefore depends on the partial pressure of CO_2 and the concentration of alkaline and acid components in the liquid phase. Methane formation is limited to a relatively narrow pH interval from approx. 5.5 to 8.5. Most Methanogens have an optimum pH between 7 and 8 while the acid forming bacteria often have a lower optimum (5.5).
- **Ammonia inhibition** of anaerobic fermentation is a well known phenomenon, especially inhibiting the *methanogenic* bacteria. Inhibition is higher under

thermophilic conditions than under mesophilic ones. Free ammonia, NH_3, is thought to be the fraction of ammonia which actually causes the inhibition.

- **Toxic compounds** can be tolerated in relatively high concentrations, due to absorption in inert material contained in the reactor. Experiments using semi-continuous, thermophilic digesters have shown that acclimatization to high heavy metal concentrations can occur.
- **Substrate inhibition**. Apart from specific toxic compounds in the waste, some relatively easily degradable compounds can also inhibit the digestion process. Especially lipids and proteins in the feed stream of biogas plants must, as well as other parameters, be carefully controlled.

2.3. Anaerobic degradation of NP and PAHs

2.3.1. Demonstration of an anaerobic degrading potential for NP and PAHs

Previous studies investigating the degradation of NP have focused principally on wastewater treatment based on activated sludge processes (Tanghe *et al*. 1998, Staples *et al*. 2001). This aerobic biotransformation of NP in sludge has been observed in both pure and mixed culture (Ahel, *et al*. 1994, Tanghe *et al*. 1998, Fujii *et al*. 2001, Corvini *et al*. 2006). Less work has been done on the degradation of NP in anaerobic sludge treatment units. Hence, working on methanogenic environments with municipal solid wastes and sludge, Ejlertsson *et al*. (1999) showed the production of NP from NP1EO and NP2PEO and an inhibition of methane production either by the ethoxylates or the NP at very high NPEO concentrations (above 46 $mg.g_{DM}^{-1}$). In contrast, in more recent studies, the NP degradation by anaerobic microbes in sediments (Chang *et al*. 2004) and sludge (Chang *et al*. 2005) has been demonstrated. These studies were performed with low concentration (2 $\mu g.g^{-1}$ to 5 $mg.L^{-1}$) of NP in batch reactors. As expected, probably at low concentration, the toxic effect is reduced and the micropollutant may then enter a particular metabolism pathway realized by anaerobic microbial populations.

Chang *et al*. (2004 and 2005) demonstrated that the optimal pH for NP degradation in sediments and sludge was 7 and that the degradation rate was enhanced when the temperature was increased. The NP biotransformation was also observed under sulfate-reducing conditions

but repressed under methanogenic and nitrate-reducing conditions (sulfate reduction conditions > methanogenic conditions > nitrate reduction conditions) in sediment and sludge rich in sulfate reducing bacteria. Furthermore, NP degradation was inhibited by the addition of molybdate or bromoethanesufonic acid, two microbial inhibitors of sulfate-reducing bacteria and methanogen microorganisms, respectively. These results indicate that sulfate-reducing bacteria play a main role in the NP anaerobic degradation, but methanogen microbial populations are also involved.

Additionally, Patureau *et al*. (2008) demonstrated that NP can be partially eliminated under methanogenic conditions in continuous reactors. It was observed that, (i) the NP degradation is linked to organic matter removal and (ii) the anaerobic digestion at thermophilic temperature improved NP degradation with a concomitant increase of the total solids removal efficiency. Similar results were obtained by Barret *et al*. (2010b): NP removals (33 to 62%) during continuous anaerobic digester with different sludge samples were linked to sludge matter biodegradation. Hence, the presence of easily degradable substrates (soluble substrate) from the sludge could enhance the micropollutant anaerobic degradation through either microbial diversity or metabolism versatility. In this sense, NP biodegradation could occur due to the co-metabolism coupled with the overall metabolism and the wide diversity of microbial population.

Concerning PAHs, most of the work is dedicated to their aerobic metabolism. Indeed, microorganisms capable of rapidly mineralizing the more recalcitrant and potentially carcinogenic PAHs have been isolated and characterized in aerobic condition (Cerniglia 1992, Bamforth and Singleton 2005). However, at the end of the eighties, Mihelcic and Luthy (1988) first reported on the degradation of naphthalene and acenaphtene under denitrifying conditions. Ten years later, naphthalene, phenanthrene and fluoranthene degradation was coupled to sulfate reduction (Coates *et al.* 1997, Hayes *et al.* 1999). Under such conditions, the performances remained always inferior to the aerobic one (Rockne and Strand, 1998). More recently, Trably *et al*. (2003), Dionisi *et al*. (2006) and Bernal-Martinez *et al*. (2009) observed the removal of PAHs under methanogenic condition. In these studies, it was demonstrated that PAH removal was mainly due to an effective biological activity and did not only result from an abiotic phenomenon of physical transfer, i.e. non specific PAH incorporation into the non-extractable fraction on the solid particles. However, the rates of biodegradation of individual hydrocarbons are found to be related to their chemical structure, their degree of aromaticity and their concentration and physicochemical properties (Cerniglia

1992). Generally, the rate of degradation of PAHs is inversely proportional to the number of rings in the PAH molecule. Thus, the lower molecular weight PAHs are biodegraded more rapidly than the higher molecular weight compounds (Chang *et al*. 2003). Several operational parameters were shown to influence the PAHs removal. In particular, biological PAHs removal was enhanced by thermophilic temperature for all the PAHs (Trably *et al.* 2003, Christensen *et al.* 2004, Benabdallah El-Hadj *et al.* 2006). In these experiments, it was always shown that the PAHs removal was closely related to the total solid removal. This means that micropollutants transfer and diffusion are involved in such process and that bioavailability remains an important limiting factor. It also means that microbial populations and their metabolic versatility could be considered as key points to enhance the micropollutant degradation. The evaluation of the diferent parameters of micropollutant degradation as bioavailability, co-metabolism and microbial population could be summarized in a mathematical model mathematical, in order to predict the micropollutant removal and fate during anaerobic digestion.

2.3.2. The conceptual approach

To this point, the anaerobic digestion processes were only studied empirically, following a "black box" approach. To go further and to improve our understanding of the driving mechanisms, a mechanistic approach had to be looked for micropollutant degradation. Thus, a conceptual approach was developed by Barret (2009) in which a complex network of interactions between the three actors of the system, i.e. micropollutant/ microorganisms/matrix, must be considered (figure 2.2).

On one hand, the pair matrix-microorganism determines a basal metabolic pathway, the final electron acceptor and fluxes of carbon and energy, i.e. the macroscopic performance of the process as organic material removal and biogas production in the anaerobic digestion, for example. The competition for the available substrate and environmental conditions such as pH, temperature, presence of inhibitors, etc., favors the presence of specific microorganisms and determine their relative abundance. Moreover, a direct link between the basal metabolism of the community and the biodegradation of micropollutant is the co-metabolism, i.e. the transformation of micropollutant (as not growth substrate) in the "obligate" presence of a growth substrate that generates carbon and energy fluxes in the cells.

Furthermore, the physicochemical interactions between micropollutants and the matrix, in terms of sorption, are likely to determine the bioavailability of micropollutants to the microorganisms, and consequently the biodegradation activity. Various definitions of bioavailability are used across many disciplines (Semple *et al.* 2004). In this work, a bioavailable micropollutant is defined as the compound fraction that can be freely transformed by a microorganism. From a general point view, a sorbed micropollutant is not available for microbial degradation; while its biodegradation occurs predominantly in the bulk aqueous phase (Byrns 2001, Artola-Garciano *et al.* 2003, Urase and Kikuta 2005, Dionisi *et al*. 2006, Plósz *et al.* 2010, Barret *et al.* 2010d).

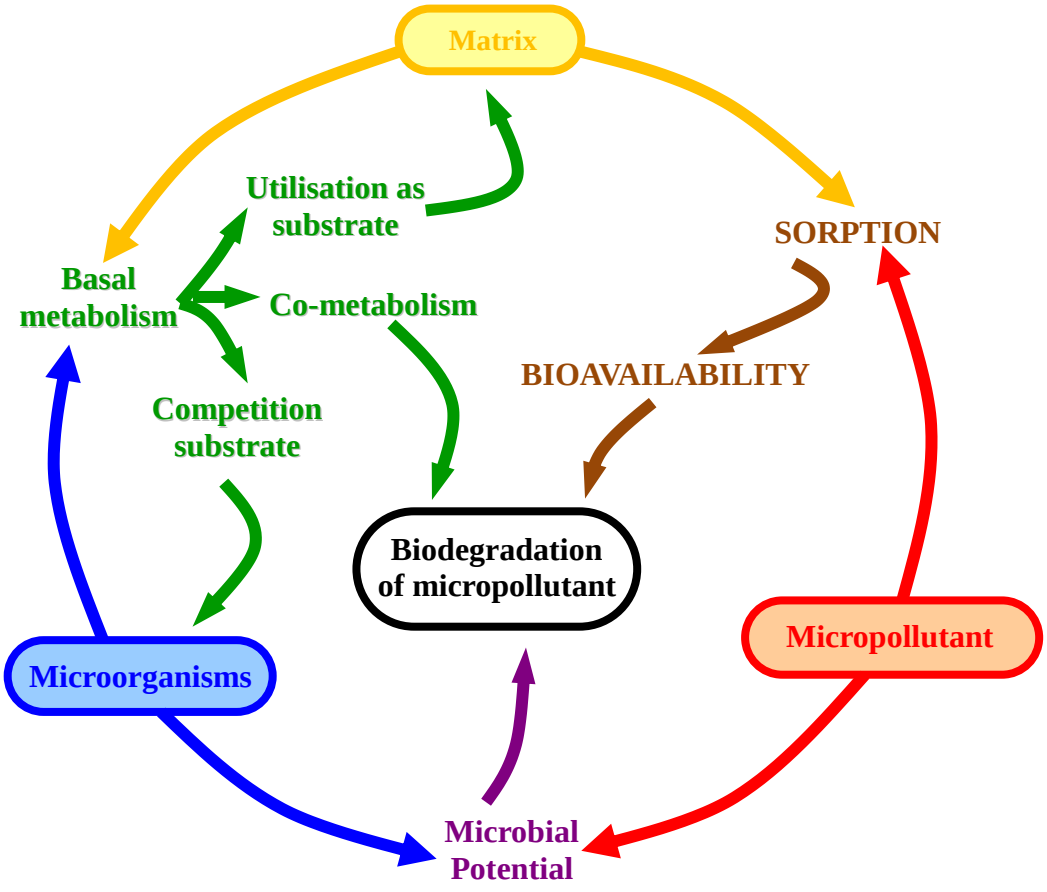


Figure 2.2. Network of interactions between the three actors micropollutant/matrix/microorganisms influencing the biodegradation of micropollutants during anaerobic digestion of contaminated sludge (Barret 2009).

Finally, the pair micropollutant-microorganism defines a microbiological potential for the presence of organisms directly involved in the pollutant anaerobic degradation. The state of knowledge on this subject involved the methanogenic populations in the anaerobic transformation of PAHs and NP (Trably *et al.* 2003, Chang *et al.* 2003 and 2005).

The important role of the anaerobic microbial populations is shown through sludge bioaugmentation with an adapted to PAHs consortium: the addition of this adapted consortium improved the PAH depletion (Trably *et al*. 2003, Larsen *et al.* 2009 and Hadibarata *et al.* 2009). This suggests that the abundance of micropollutants degrading microorganisms is a determining factor. However, the specific microbial population involved in hydrophobic micropollutant degradation is still a crucial research question. Thus, in order to identify the implied microorganisms, methanogenic inhibition procedures have been performed with bromoethanesulfonic acid (BES) as a selective inhibitor of methanogenesis. Chang *et al*. (2003) observed that the addition of this microbial inhibitor delayed PAH degradation, indicating that Methanogens populations are involved in these pollutants degradation in sludge. Similarly, Chang *et al.* (2006) shown that the addition of BES induced the partial inhibition of both naphthalene and phenanthrene degradation and suggested also that methanogenic organisms were involved in PAH degradation. Moreover, Trably *et al.* (2003) observed that PAH removal efficiencies are inversely correlated with methanogenic activity i.e. it was shown that biogas yield decreased proportionally with the increase of PAH removal. However, methanogenic activity was also essential for effective PAH removal, likely due to the thermodynamic limitation on anaerobic pathways (Trably, 2002).

2.3.3. The limiting factors: bioavailability and co-metabolism

Microbial potentiality for removing low amounts of micropollutants requires a long adaptation time and is often limited by the bioavailability of these compounds (Chang *et al*. 2003, Patureau and Trably 2006). Hence, the interactions between the matrix (organic and mineral matters) and the micropollutant, measured through sorption phenomenon, may help to better understand the limitation of micropollutant removal by bioavailability and/or bioaccessibility as shown on the figure 2.2. Generally, pollutants sorbed to particles are considered to be unavailable and pollutants found in aqueous phase are available to microbial activity (Byrns 2001, Artola-Garciano *et al.* 2003, Urase and Kikuta 2005, Dionisi *et al*. 2006, Plósz *et al.* 2010, Barret *et al.* 2010d). In recent studies, Barret *et al*. (2010c) have demonstrated that it is

essential in sludge system to consider the sorption phenomena occurring onto the aqueous phase containing dissolved and colloidal matter (DCM). In this study, the sludge has been thus considered as a three-compartment system with two equilibrium constants (figure 2.3). Furthermore, it was demonstrated that sorption onto the solid phase and DCM of sludge is a very fast mechanism in comparison with biological anaerobic kinetics (Dionisi *et al*. 2006, Barret *et al*. 2010c). The presence of this third compartment can indeed influence the pollutants distribution and their bioavailability. Thus, the bioavailable fraction of micropollutant can be considered as a limiting factor for anaerobic degradation.

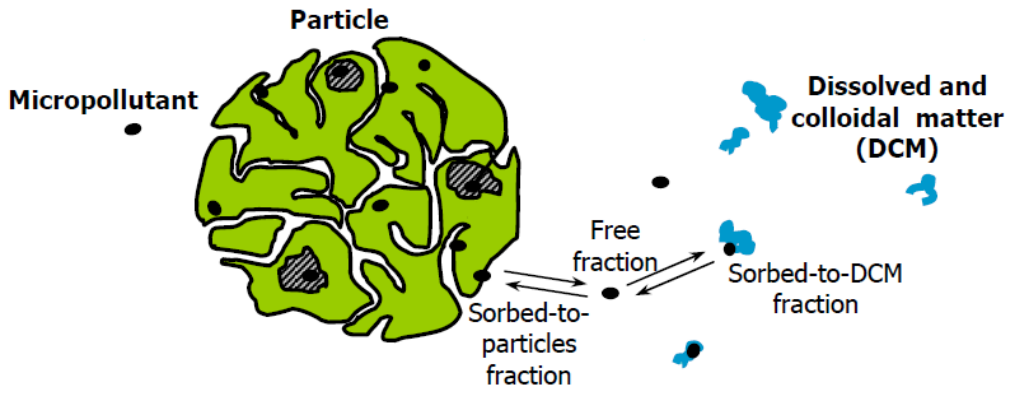


Figure 2.3. Representation of the three-compartment system (Barret 2009).

Previous publications have shown the co-metabolism as a relevant approach in the transformation of some recalcitrant contaminants (Chang *et al.* 1993; Criddle 1993; Tiehm and Fritzsche 1995; Yuan *et al.* 2001; Chang *et al*. 2003; Clara *et al.* 2005; Plósz *et al*. 2010; Barret *et al.* 2010c). Indeed, some micropollutants such as low-molecular weight PAH and NP can be used as sole carbon sources by methanogenic consortia (Chang *et al.* 2008 and Chang *et al.* 2006). However, this has not been shown for high molecular weight PAHs. In the case of highly recalcitrant compounds for which biodegradation is not thermodynamically favoured, the metabolic activity that leads to their removal is possible when combined to other metabolic routes. Furthermore, it was found that microbial co-metabolism is essential for micropollutant removal and dry matter biodegradation rate was identified as the most relevant co-metabolic flux (Barret *et al*. 2010d). This research demonstrated that co-metabolism was

one of the driving mechanisms in the micropollutant degradation and it had to be included in the modeling of these pollutants fate.

2.4. Modeling the micropollutant fate in wastewater and sludge treatments

A wastewater treatment plant (WWTP) usually consists of mechanical treatments (screening, grit removal and primary clarification), biological treatments (e.g. removal of organic matter, nitrogen and phosphorus) and sludge treatment. All these treatments determine the temporal and spatial distribution of a pollutant in the system. Consequently, the effective operation of WWTP plays an important role in minimizing the release of micropollutants into the environment.

Table 2.5. Micropollutant fate processes included in the various components in the integrated urban wastewater system model. Source: De Keyser *et al*. (2010).

Processes	Sewer	Stormwater unit (water)	Stormwater unit (sed.)	Primary settling	Aeration tank	Secondary setting	River water	River sediment
Adsorption-desorption	+	+	+	+	+	+	+	+
Aerobic biodegradation	+	+	+		+		+	+
Anoxic biodegradation	+	+	+		+		+	+
Hydrolysis	+	+	+		+		+	
Photolysis		+			+		+	
Sedimentation	+	+		+		+	+	
Resuspension	+		+					+
Sediment-water exchange							+	+
Volatilization	+	+		+	+	+	+	

Biotic and abiotic phenomena influence the distribution and the fate of these compounds throughout the treatment system. This distribution is governed by the physicochemical properties of compounds and sludge, together with the process design and operating conditions of the treatment plant. De Keyser *et al*. (2010) have identified and implemented the relevant removal processes for organic micropollutant in each unit of the Integrated Urban Wastewater System Model (table 2.5). Thus, the possible removal of micropollutants is determined by three main processes: volatilization, sorption to suspended solid and sludge, and degradation (biological and/or chemical). Only the degradation process is a real mechanism of elimination, while the two others consist of a displacement of pollution.

The models proposed in the literature are based on these processes and mainly addressed the simulation of micropollutant fate in WWTP as conventional aerobic systems (activated sludge).

2.4.1. Volatilization

Non-forced transport of a substance from the water phase to the gas phase is generally termed volatilization. At thermodynamic equilibrium, the distribution between liquid and gas phase has been successfully described by Henry's law:

$$K_H = \frac{C_g}{C_f} \qquad (2.1)$$

where K_H is the dimensionless Henry gas water partitioning coefficient, C_g and C_f are the concentration of a compound in the gaseous phase and the free compartment at equilibrium, respectively. The transformation can be modeled as:

$$\frac{dC_g}{dt} = K_{La}(C_g^{eq} - C_g) = K_{La}(K_H C_f - C_g) \qquad (2.2)$$

where K_{La} is the overall mass transfer rate and C_g is the bulk gas concentration of the micropollutant. For slowly volatilizing compounds ($K_H<4\cdot10^{-6}$), volatilization can be neglected. In the case of moderately volatile compounds ($4\cdot10^{-6}<K_H<4\cdot10^{-2}$), both the liquid film and gas film influence the mass transfer (Trapp and Harland, 1995). The mass transfer rate related to the gas phase must then be estimated. None of the model compounds (PAHs and NP) of this thesis are significantly affected by atmosphere transfers processes; however, for many other micropollutants transport from water to air could be an important fate process.

2.4.2. Sorption

Sorption of substances to suspended solids in the wastewater may be caused by adsorption (electrostatic interaction on the surface of particulate material) or by absorption (hydrophobic interaction of aliphatic and aromatic groups in the lipophilic cell membrane of biomass and the fat fraction of sludge) (Siegrist *et al*. 2003).

The importance of sorption depends of the physicochemical characteristics of the pair compounds-matrix; for example, PAHs and NP have high hydrophobicity (high log K_{ow} values) and will partition strongly onto the solid phase. A majority of previously published models for the environmental fate of hydrophobic micropollutants assume that the sorption phenomenon is reached instantaneously. The main reason for this assumption, in often dynamic models, is that the solid-liquid equilibrium is reached in a short time compared to the time scales of anaerobic degradation (Kordel *et al*. 1997, Dionisi *et al*. 2006, Barret *et al*. 2010c). The current models consider that sorbed micropollutant fraction is not available for the microbial degradation activity (Byrns 2001, Artola-Garicano *et al*. 2003, Joss *et al*. 2004 and 2006, Lindblon *et al*. 2009, Plósz *et al*. 2010); therefore the biodegradation rate can decrease as a result of sorption of compounds to a matrix. In these studies, the pollutant distribution is established with a solid-liquid partition coefficient (K_d in $L.Kg^{-1}$).

$$K_d = \frac{C_{sorbed}}{X_{SS} \cdot C_{dissolved}} \qquad (2.3)$$

where C_{sorbed} is the sorbed micropollutant concentration onto sludge ($\mu g.L^{-1}$), $C_{dissolved}$ the aqueous concentration of compounds ($\mu g.L^{-1}$) and X_{SS} the suspended solid concentration ($Kg.L^{-1}$).

However, sorption phenomenon also occurs inside aqueous phase considering sorption on dissolved and colloidal matter (DCM) (Barret *et al*. 2010c). This approach may also help to better assess the bioavailability concept. These authors have considered the sludge as a three-compartment system (figure 2.4), with three states of micropollutant: freely dissolved (C_f, $\mu g.L^{-1}$), sorbed to DCM (c_{DCM}, $\mu g.g_{COD\text{-}DCM}^{-1}$) and sorbed to particle (c_p, $\mu g.g_{COD\text{-}part}^{-1}$). At equilibrium, this system can be described by two equilibrium constants K_p and K_{DCM}.

$$K_p = \frac{c_p}{C_f} \qquad (2.4), \qquad K_{DCM} = \frac{c_{DCM}}{C_f} \qquad (2.5)$$

where K_p is the equilibrium constant of micropollutant sorption to particles ($L.g_{COD\text{-}part}^{-1}$) and K_{DCM} is the equilibrium constant of sorption to DCM ($L.g_{COD\text{-}DCM}^{-1}$). An experimental methodology was developed to measure these equilibrium constants in various sludge considering PAHs as modeled compounds. It was shown that the equilibrium constants were

correlated to the hydrophobic character of PAHs. This three-compartment approach can also better estimate the sorption equilibrium constants than the biphasic approach (Barret *et al*. 2010c).

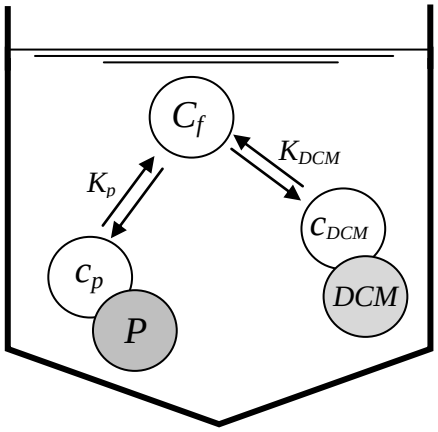


Figure 2.4. The three-compartment approach. P (particles). DCM (dissolved/colloidal matter).

The transfer of the compounds between aqueous phase (free and DCM compartments) and the particle at equilibrium states is assumed from equation 2.6 (Urase and Kikuta 2005, Dionisi *et al.* 2006):

$$r_i = -k_i(c_i^{eq} - c_i) = -k_i(K_{eq}C_f - c_i) \qquad (2.6)$$

where r_i ($\mu g.g_{COD}^{-1}.d^{-1}$) is the kinetics of sorption to particle or to DCM, k_i (d^{-1}) is the first-order kinetic constant of sorption to particle or DCM, K_{eq} is the equilibrium constant (K_p or K_{DCM}), c_i^{eq} is the micropollutant concentration at equilibrium, c_i is the micropollutant concentration sorbed onto particle or DCM compartment.

It is worth noting that when sorption is the primary removal mechanism, the desorption phenomenon is likely to occur, especially for hydrophobic micropollutants. This is referred as sorption-desorption hysteresis, as a result of partially irreversible sorption of a chemical onto a sorbent (Xu and Li 2010). Indeed, according to common hypothesis in sludge processes, microorganisms can probably only degrade dissolved micropollutant and slow desorption of these organic compounds from the particles to interstitial water is frequently cited as the cause of limited biodegradation (Artola-Garicano *et al*. 2003, Fountoulakis *et al*. 2006). Then, when hysteresis occurs, the removal capacity is reduced because some of the surface sites of sludge are unavailable. This reduction in removal capacity cannot be ignored, because neglecting it

may cause an overestimation of the sorption capacity and biological degradation of micropollutant. However, the irreversibility sorption can be due to sequestration phenomenon by physical or chemical interactions (Semple *et al*. 2003). These non-extractable residues might be mobilized back after a long time, but in the time scale of sorption experiment, they can be assumed to be irreversibly sequestrated. Nevertheless, excluding the sequestration fraction, the hysteresis phenomenon requires special attention in the micropollutant fate models. Thus, desorption kinetics is common modeled with a first order approach (Artola-Garicano *et al*. 2003, Joss *et al*. 2006, Lindblom *et al*. 2009, Plósz *et al*. 2010). Moreover, sorption-desorption phenomenon was investigated for hydrophobic compounds (Barret 2009). The results presented for 13 PAHs demonstrated that sorption and desorption kinetics are very quick in comparison with anaerobic kinetics and it was shown that the sorption to particles is strictly reversible and the hysteresis phenomenon is negligible (Barret *et al*. 2011).

2.4.3. Degradation

2.4.3.1. Conventional kinetics

The complete mineralization of organic micropollutants in treatment system is rare and the term degradation better accurately describes the potential changes of composition and molecular structure of the compounds. Some compounds are degraded into innocuous products that may then enter a particular metabolism pathway, whilst other compounds form products which may be less or more toxic than the parent. In general, degradation can occur biologically by the activity of viable biomass or chemically, such as photolysis and advanced oxidation. In the case of micropollutant anaerobic degradation, the chemical transformation is assumed not to be relevant, and is dismissed in this thesis.

Biological degradation of micropollutant is a complex process, and several models are used to describe it, by assuming zero order, first order, pseudo first order or based on saturation kinetics (Monod). Table 2.6 shows different approaches for modeling micropollutant biodegradation in WWTPs. The simplest zero-order kinetic assumes that the removed amount is independent of the influent concentration. Byrns (2001) considered that micropollutants are biologically degraded in the dissolved phase of the activated sludge stage. This process is modeled by a first order kinetics with respect to micropollutant available concentration (C_{mp}) and biotransformation in the dissolved phase is governed by the hydraulic retention time

(HRT). Similarly, Artola-Garicano *et al*. (2003) used this type of kinetics. This model distinguishes between an organic and an aqueous compartment with mass transfer of chemical between these two compartments and only the freely dissolved fraction is available for microbial degradation activity. Clearly, organisms responsible for micropollutant degradation are not explicitly modeled in both zero and first order equations. In fact, in both cases, the biomass concentration is assumed to be constant in the biological process. Nevertheless, the biomass growth can be affected by different environmental factors, such as, redox condition, electron acceptor, available substrate, inhibition, toxicity, etc.

Joss *et al*. (2004 and 2006) formulated a biological degradation model for estrogen, pharmaceutical and fragrances in municipal wastewater treatment plant. Here, degradation is described with a pseudo-first-order kinetics proportional to aqueous concentration of compounds and to the suspended solid concentration (X_{SS}). In this equation the suspended solid concentration ($g.L^{-1}$) is considered higher than the compounds concentration ($\mu g.L^{-1}$), meaning that on a short time scale it remains constant. Then, if the solid concentration can be assumed to be constant, the pseudo-first-order kinetics can be transformed to first-order-kinetics.

Table 2.6. Models for micropollutant biodegradation found in literature.

Kinetics	Removal rate	Parameters	References
Zero-order	$-k_o$	k_o = zero-order degradation rate coefficient ($\mu g.L^{-1}.d^{-1}$)	
First-order	$-k_1 C_{mp}$	k_1 = first-order degradation rate coefficient (d^{-1})	Byrns (2001), Artola-Garicano *et al*. (2003).
Pseudo-first order	$-k_2 C_{mp} X_{SS}$	k_2 = pseudo-first-order degradation rate coefficient ($L.g_X^{-1}.d^{-1}$)	Joss *et al*. (2004 and 2006), Fountoulakis *et al*. (2006), Plósz *et al*. (2010).
Saturation kinetic (Monod)	$-\mu_{\max,mp} \frac{C_{mp}}{K_{S,mp} + C_{mp}} X$	$\mu_{max,mp}$ = maximum specific microbial growth rate of compounds (d^{-1}) $K_{S,mp}$ = half saturation coefficient of compounds ($\mu g.L^{-1}$)	Lindblom *et al*. (2009).

Biodegradation models of some substrates are commonly based on saturation kinetic. The classical Monod models the dependence of the substrate degradation and the biomass growth as a function of a yield coefficient (Y, $g_{biomass}.g_{substrate}^{-1}$) and of the maximum specific microbial growth rate (μ_{max}). The rate of substrate utilization and biomass growth are

influenced by half saturation constant (K_S), which conceptually presents the affinity of the degrading enzyme for the substrate. When the microbial culture is in the log-growth phase, the biomass growth is reduced by a first order endogenous decay rate coefficient (b, d^{-1}). Lindblom *et al*. (2009) modeled the endocrine disrupting bisphenol-A (BPA) aerobic degradation in an activated sludge process with real wastewater using Activated Sludge Model No.1 (ASM1). Degradation of BPA is assumed to be performed by a specific microbial population ($X_{B,XOC}$, $\mu g_{COD}.L^{-1}$), which possesses the genetic capability to metabolize the compound under aerobic conditions and use it for growth. Previous batch observations (Lindblom *et al*. 2009) showed no BPA degradation under anoxic and anaerobic conditions. In the proposed model (equation 2.7), the BPA degrading bacteria grew with soluble concentration of compounds (S_{XOC}, $\mu g_{COD}.L^{-1}$) as a single substrate with growth rate determined by the maximum specific growth rate (μ_{XOC}, d^{-1}) and half saturation coefficients (K_{XOC}, $\mu g_{COD}.L^{-1}$ and $K_{O,XOC}$, $mg_{COD}.L^{-1}$). The decay followed the same first order kinetics as in ASM1 (b_{XOC}, d^{-1}) and the degradation process will finally depend on the yield coefficient (Y_{XOC}, $g_{COD\ biomass}.g_{COD\ oxidized}^{-1}$). Y_{XOC}, was assumed to be 0.67 $g_{COD\ biomass}.g_{COD\ oxidized}^{-1}$, which cooresponds to a typical yield for aerobic oxidation of organic matter.

$$r_{XOC} = -\mu_{XOC} \cdot \left(\frac{S_O}{K_{O,XOC} + S_O} \right) \cdot \left(\frac{S_{XOC}}{K_{XOC} + S_{XOC}} \right) \cdot X_{B,XOC} \qquad (2.7)$$

Plósz *et al*. (2010) modeled the antibiotic micropollutant (sulfamethoxazole, tetracycline and ciprofloxacin) removals in activated sludge treatment system and used the ASM1 model to simulate biomass growth under aerobic and anoxic conditions. Biodegradation of the selected micropollutants is described by a simplified version of the Monod-model, when the substrate concentration is significantly lower than the half saturation coefficient, and thus the biomass transformation capacity increases linearly with soluble micropollutant concentration and solid suspended concentration by a pseudo first-order kinetic expression ($-k_{bio}.X_{SS}.C_{mp,l}$). Furthermore, this model was optimized by accounting for competitive inhibition by readily biodegradable substrate on the co-metabolic micropollutant transformation process.

Fountoulakis *et al*. (2006) described the anaerobic degradation of Di-ethylhexyl phthalate (DEHP) with a pseudo-first-order kinetics and used the Anaerobic Digestion Model No.1 (ADM1) to fit the anaerobic digestion of secondary sludge. The DEHP biodegradation rate is proportional to the available micropollutant concentration and biomass concentration (sum of the concentration of all microorganisms groups in the ADM1 model). This study expanded the

term available concentration to the sum of micropollutant in the aqueous and the solid phase. Additionally, DEHP was assumed to be degraded by microorganisms that also consume other substrate as carbon source during anaerobic digestion of sludge i.e. co-metabolism. In fact, numerous papers have reported that for traces substances (as recalcitrant compounds) mostly occurring in the wastewater in the concentration range of 10^{-5} – 10^{-9} $g.L^{-1}$, biological degradation is only possible in the presence of another compound used as carbon and energy source (Chang *et al*. 1993; Criddle 1993; Tiehm and Fritzsche 1995; Yuan *et al*. 2001; Chang *et al*. 2003; Siegrist *et al*. 2003; Clara *et al*. 2005; Barret *et al*. 2010d). An ecological explanation of co-metabolism is that the removal of this traces compounds does not result in any significant biomass growth i.e. the biomass yield attributed to micropollutant degradation is insignificant.

2.4.3.2. Co-metabolism kinetics

Co-metabolic transformations are reactions that are catalyzed from enzymes and cofactors and that do not yield to carbon or energy benefits for the growing cells (Horvath 1972). Indeed, co-metabolism mechanism has been stated as the ability of the organism to attack the pollutant but it cannot assimilate the products of its oxidation. It is usually assumed that the co-metabolism may occur relatively slower than metabolism of growth substrate (Alvarez-Cohen and Speitel 2001). However, some models (table 2.7) have been developed in order to show the interrelationship between primary growth substrate (superscript *g*) and co-metabolite compounds (superscript *co*). The most commonly approach involves the Monod expression. Model A (table 2.7) accounts for interdependence of the growth substrate utilization rate (r_g, $g.L^{-1}.d^{-1}$), co-metabolic transformation rate (r_{co}, $\mu g.L^{-1}.d^{-1}$) and the biomass growth rate (r_x, $g.L^{-1}.d^{-1}$). With this approach, the rate of cell growth is typically expressed as a function of growth substrate consumption (function of yield coefficient, Y in $g_{cells}.g_{substrate}^{-1}$) and cell decay (decay rate, b in d^{-1}). This biomass is responsible of the enzymes production for both utilization of growth substrate and the compound co-metabolic transformation. The growth substrate rate is function of the substrate concentration (S_g, $g.L^{-1}$), the maximum specific rate of growth substrate (k_g, d^{-1}) and the half-saturation constant for growth substrate (K_{sg}, $g.L^{-1}$). Similarly, Monod expression is used for the rate of co-metabolic transformation; it is function of the co-metabolic compound concentration (S_{co}, $\mu g.L^{-1}$), the maximum specific rate of co-metabolic compounds degradation (k_{co}, d^{-1}) and the half-saturation constant for the co-

metabolic substrate (K_{sco}, µg.L^{-1}). However, the saturation kinetics can be simplified when the compounds concentration is significantly lower than half saturation constant ($S_{co} << K_{sco}$). Thus, r_{co} is a pseudo-first-order kinetics where k_1 is the pseudo-first-order co-metabolic degradation constant (L.g$_{cell}^{-1}$.d^{-1}) and is equivalent to k_{co}/K_{sco} in Monod equation. Nevertheless, this model does not take into account for the loss of transformation activity in the absence of growth substrate or for the toxicity product (Criddle 1993).

Table 2.7. Models for co-metabolism transformation found in literature.

Model	Rate equations	Refences
A	$r_{co} = -k_{co}\frac{S_{co}}{K_{sco}+S_{co}}X$ (If $S_{co} << K_{sco}$, $r_{co} = -k_1 S_{co} X$) $r_g = -k_g\frac{S_g}{K_{sg}+S_g}X$ $r_x = Y\cdot r_g - b\cdot X$	Alvarez-Cohen and Speitel 2001
B	$r_{co} = -k_{co}\frac{S_{co}}{K_{sco}(1+S_g/K_{isg})+S_{co}}X$ $r_g = -k_g\frac{S_g}{K_{sg}(1+S_{co}/K_{isco})+S_g}X$	Broholm *et al*. 1992, Plósz *et al*. 2010
C	$r_{co} = -k_{co}\frac{S_{co}}{K_{isco1}(1+S_g/K_{isg})+S_{co}(1+S_g K_{isco1}/K_{isg}K_{isco2})}X$ $r_g = -k_g\frac{S_g}{K_{sg}(1+S_{co}/K_{isco})+S_g(1+S_{co}/K_{isco2})}X$	Ely *et al*. 1995 and 1997, Pons *et al*. 2009
D	$q_g = k_g\left(\frac{S_g}{K_{sg}+S_g}\right)$ $q_{co} = (T_c^g q_g + k_c)\left(\frac{S_{co}}{K_{sco}+S_{co}}\right)$ $r_x = Yq_g X - bX - \frac{q_{co}}{T_c^b}X$	Criddle 1993, Chang and Criddle 1997

For many situations, the co-metabolic transformation may adversely affect the enzyme activity. An apparent decrease in enzyme affinity for the substrate may occur through the competition of active site when multiple substrates (primary and co-metabolic substrates) are

simultaneously available. This enzyme inhibition has generally been modeled by including competitive inhibition term in the saturation kinetic expression for the degradation of growth and co-metabolic substrates (model B, table 2.7). Two coefficients are added: K_{isg} ($\mu g.L^{-1}$) is the inhibition for growth substrate and K_{isco} ($\mu g.L^{-1}$) the inhibition coefficient for the co-metabolic substrate. Theoretically, the inhibition terms in the inhibition model are equivalent to the half saturation constants for such compounds. Plósz *et al*. (2010) have also used a modification of expression for competitive inhibition on antibiotics biotransformation caused for the growth substrate uptake.

On the other hand, Ely *et al*. (1995) developed a co-metabolism enzyme kinetics model (model C, table 2.7). This model takes into account changes in bacterial activity associated with enzyme inhibition caused by the presence of co-metabolic compound, enzyme inactivation resulting from toxicity product and recovery from bacterial synthesis of new enzyme in response to inactivation. It also incorporates both competitive and non-competitive inhibition for trichloroethylene degradation. In the model C (table 2.7): K_{isco1} is the competitive inhibition coefficient for the co-metabolic substrate (μmol or $mg.L^{-1}$) and K_{isco2} is the non-competitive inhibition coefficient for the co-metabolic substrate (μmol or $mg.L^{-1}$). However, Ely *et al*. (1997) simplified the model C for analysis and applied to situation with competitive inhibition alone. Similarly, Pons *et al*. (2009) have presented a conceptual model that incorporates both competitive and non-competitive inhibition in wastewater treatment plant BSM2 benchmark to evaluate the effect of biodegradable and none biodegradable micropollutant substances. Here, toxic micropollutant is supposed to affect only the activated sludge part and modeled with inhibition of enzyme-catalyzed reactions.

The co-metabolism kinetics (model D, table 2.7) proposed by Criddle (1993) introduce the presence of growth and energy substrate (energy substrate equation not shown) into a modification of the saturation kinetic model. The maximum co-metabolic transformation rate (q_{co}, $\mu g_{co}.g_{biomass}^{-1}.d^{-1}$) depends on: (1) the growth substrate utilization rate, q_g ($g_{substrate}.g_{biomass}.d$) and (2) the maximum specific degradation rate (k_c, $\mu g_{co}.g_{biomass}^{-1}.d^{-1}$) at which cells can transform co-metabolic compounds in the absence of growth substrate. Furthermore, this comprehensive model includes a growth substrate transformation capacity (T_c^g, $\mu g_{co}.g_{substrate}^{-1}$) defined as the stoichiometric mass of co-metabolic substrate transformed per mass of growth substrate consumed. Thus, the co-metabolic compound transformation is increased in the presence of growth substrate.

In addition, toxicity products associated with co-metabolism can cause a decrease to cell activity proportional to the amount of degraded compound (Alvarez-Cohen and Speitel 2001). Then, this toxicity product is different to classical inhibition model, in which cell activity decreases in proportion to substrate inhibitory concentration. In fact, for this approach, a new term is introduced in the growth rate, the transformation capacity of co-metabolizing cell, T_c^b ($\mu g_{co}.g_{biomass}^{-1}$). Thus, the relation q_{co}/T_c^b is the decay rate attributed to the co-metabolism alone. Small values of transformation capacity would be expected for toxic compounds, or compounds with toxic products; large values would be expected in the absence of toxicity. Chang and Criddle (1997) have successfully applied this model to describe co-metabolism of trichloroethylene in presence of methane.

It is worth noting that, the co-metabolism models, appropriate for describing the micropollutant degradation in wastewater or sludge treatment, depend of the goal of the modeling, the compounds concentration and available data. Indeed, a sensitive analysis can be very helpful to select appropriate kinetic expressions.

In the context of micropollutant degradation, the literature review of this thesis has demonstrated that the models developed are mainly focused on aerobic treatments, despite the fact that the anaerobic digestion is a potential bioprocess in the micropollutant depletion. Moreover, the degradation of recalcitrant compounds, such as PAHs and NP, is often concomitant with the co-metabolic transformation and the bioavailable fraction and both mechanisms could be considered and included in the modeling of micropollutant fate.

3. Micropollutant fate model

After discussing some concepts about micropollutant removals in wastewater and summarising the various existing models proposed to describe the fate and the distribution of these compounds, we propose a new dynamic model for hydrophobic micropollutant depletions in anaerobic process. In published studies, the micropollutant has usually been distributed as two compartments: the aqueous and the particle compartments. This distribution is usually calculated from a partitioning coefficient that is related to the octanol-water partition coefficient (K_{ow}) of the compound through the organic carbon content of the sorbent (matrix) (Byrns 2001). The matrix characteristics thus play a main role in the micropollutant distribution.

From a general point of view, micropollutant aqueous fraction is usually assumed to be available to biological activity and micropollutant sorbed-to-particle is considered as a bioaccessible compartment e.g. the micropollutant sorbed can be transferred to the aqueous phase during the process by the hydrolysis phenomenon, for example. However, the sorption phenomenon also occurs inside aqueous phase due to the presence of colloidal matter (Barret *et al.* 2010c). Thus, freely dissolved and sorbed-to-DCM (dissolved colloidal-matter) micropollutant states can be differentiated. Besides, the micropollutant distribution can be changed from a two-compartment to a three-compartment system. This new micropollutant distribution could be helpful to estimate the "real" bioavailable micropollutant fraction to be degraded in biological processes.

More precisely, according to Barret *et al.* (2010b), a micropollutant can be located in one among three physical compartments (figure 3.1): the freely dissolved one (concentration C_f, $\mu g.L^{-1}$), the sorbed-to-DCM one (concentration c_{DCM}, $\mu g.g_{DCM}^{-1}$) and the sorbed-to-particles one (concentration c_p, $\mu g.g_{PART}^{-1}$).

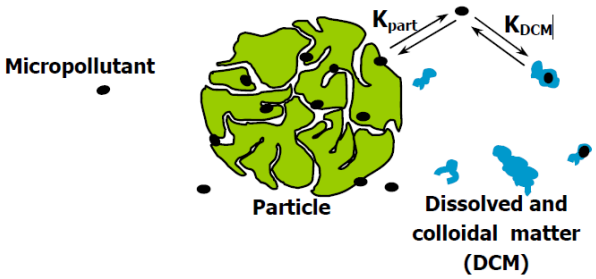


Figure 3.1. Representation of the three-compartment system of a micropollutant (Barret *et al.* 2010c).

In steady states, the compartment system can be described by two equilibrium constants: K_p is the equilibrium constant of micropollutant sorption to particles ($L.g_{PART}^{-1}$) and K_{DCM} is the equilibrium constant of sorption to DCM ($L.g_{DCM}^{-1}$).

$$K_p = \frac{c_p}{C_f} \qquad (3.1) \qquad\qquad K_{DCM} = \frac{c_{DCM}}{C_f} \qquad (3.2)$$

Barret *et al.* (2010b) have developed an experimental methodology for equilibrium constant determination. The freely dissolved concentration (C_f, $\mu g.L^{-1}$) is very difficult to obtain experimentally whereas the aqueous concentration (C_{aqu}, sum of the freely dissolved and sorbed-to-DCM states, $\mu g.L^{-1}$) and c_p are easy measurable. Moreover, C_{aqu} and c_p measurement can be related to K_{global} by the equation (3.3).

$$K_{global} = \frac{c_p}{C_{aqu}} = \frac{K_p C_f}{K_{DCM} C_f [DCM] + C_f} = \frac{K_p}{K_{DCM}[DCM] + 1} \qquad (3.3)$$

K_{global} is not a thermodynamical equilibrium constant but a partition coefficient, which is system-dependent. Thus, K_p and K_{DCM} can be extracted from the equation (3.3) by assessing K_{global} with various DCM concentrations ([DCM]) for each compound. A non linear regression algorithm of Levenberg- Marquardt type (Marquardt, 1963) was used to minimize the sum of square errors and to estimate the two parameters of the K_{global} model: K_p and K_{DCM}. Furthermore, this three-compartment methodology proposed to investigate micropollutants sorption into sludge was validated (Barret *et al.* 2010c) and the equilibrium constant values were showed to be dependent of both sludge and micropollutant characteristics (Barret *et al.* 2010a).

The degradation of micropollutant could be achieved either by metabolism, using the micropollutant as a source of primary carbon or nutrients for growth and/or energy; or by co-metabolism, in which the micropollutant could be transformed by the action of extracellular enzymes produced by the cells, but without any benefit for the microorganism (Ely *et al*. 1995). Moreover, no methanogenic consortium was shown until now to grow with PAHs over 4 rings as the sole carbon source and their removal is only possible when it is combined with other metabolic routes (Chang *et al*. 2003, Trably *et al.* 2003). These co-metabolic routes - and as a result, the micropollutants biodegradation – can also be stimulated by the addition of a readily biodegradable substrate (Chang *et al*. 2004, Chang *et al.* 2005, Dionisi *et al.* 2006, Chen *et al*. 2008).

In fact, the model developed in this chapter is directed at the solution of scientific questions (1) ***which fraction of micropollutant is bioavailable during anaerobic sludge digestion?***, (2) ***Is the sorption kinetics the rate-limiting step in the micropollutant removal?***, and (3) ***Is the co-metabolism the main mechanism of micropollutant degradation?***.

A new dynamic model for bioavailability and co-metabolism of micropollutants during anaerobic digestion

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Abstract

Organic micropollutants (OMPs) are present in wastewater and sludge. Their possible impact to the environment contributes to their increasing scientific and social interest. Anaerobic digestion has been shown as a potential biological process for removal of these compounds. An accurate description of OMP distribution in the environmental system can be used to better understand which compartment is used for degradation and to improve their depletion in conventional wastewater treatment technologies. In this work, we propose a dynamical model with a four-compartment distribution to describe the Polycyclic Aromatic Hydrocarbons (PAHs) fate during anaerobic digestion. The model is calibrated and validated using experimental data obtained from two continuous reactors fed with primary and secondary sludge operated under mesophilic conditions. A non-linear least square method was used to optimize the model parameters. The resulted model is in accordance with the experimental data. The PAH biodegradation rate is well modeled when considering the aqueous fraction (including free and sorbed to dissolved/colloidal matter PAHs) as the bioavailable compartment. It was also demonstrated in the simulations that the PAHs biodegradation is linked to a mechanism of co-metabolism. The model proposed is potentially useful to better understand the micropollutant distribution, predict the fate of PAHs under anaerobic condition and help to optimize the operation process for their depletion.

3.1. Introduction

Organic micropollutants (OMPs) have become an important environmental topic in recent years due to the risk they pose on aquatic environment and on human health e.g. endocrine disrupting effects (Press-Kristensen *et al.* 2007, Couillard *et al.* 2008) and to the development of highly accurate analytical methodologies with lower detection limits (Trably *et al.* 2004). OMPs are frequently detected in different environmental compartments (rivers, lakes, groundwaters, sediments, wastewaters, drinking waters) at low concentration (ng to $\mu g.L^{-1}$ and µg to $mg.Kg^{-1}$ dry matter). In wastewater treatment plants (WWTPs), OMPs are partially removed by abiotic and biotic processes, including volatilization, stripping, sorption to sludge and biological and/or chemical transformation (Alder *et al.* 1997, Byrns 2001, Lindblom *et al.* 2009). However, the conventional treatment technologies have not been specifically designed for removing OMPs but they can reduce OMPs concentrations as well as their potential environmental impact. Furthermore, these removals are dependant of the OMPs physico-chemical properties, the sludge characteristics and the WWTPs operational conditions (Clara *et al.* 2005, Dionisi *et al.* 2006, Joss *et al.* 2006).

Several mathematical models described the fate and the distribution of OMPs between the aqueous and the solid phase (Byrns 2001, Dionisi *et al*. 2006, Joss *et al.* 2004 and 2006, Lindblom *et al.* 2009, Plósz *et al.* 2010). In general, these models used the solid–water partition coefficient to describe equilibrium condition and assumed that the aqueous phase is available for microbial biodegradation activity and the solid phase is bioaccessible and can be transferred to aqueous phase during the process (Artola-Garicano *et al.* 2003). Nevertheless, Barret *et al.* (2010b) have demonstrated that the sorption phenomena also occur onto the aqueous phase containing dissolved and colloidal matter. In this study, the sludge has been considered as a three-compartment system with two equilibrium constants. The presence of this third compartment can thus influence the distribution and the pollutants bioavailability. In fact, the distribution in three compartments can help to find the real bioavailable fraction of OMPs. Furthermore, sorption onto the solid phase and dissolved-colloidal matter of sludge is a very fast mechanism in comparison with biological anaerobic kinetics (Chang *et al.* 2003, Dionisi *et al*. 2006, Barret *et al.* 2010b).

The bioavailability is influenced by a variety of factors including (i) sorption-desorption processes that could be rate-limiting for biodegradation, (ii) irreversibility or sequestration phenomena due to physical and/or chemical interactions and (iii) presence of other

compounds that might compete for sorption sites. Moreover, the biodegradation of multiple substrates can also take place during the co-metabolism process i.e. a compound of interest does not function as a growth substrate (Criddle, 1993). Previous publications have shown the co-metabolism as a mechanism approach in the transformation of some recalcitrant contaminants (Chang *et al.* 1993, Criddle 1993, Tiehm and Fritzsche 1995, Yuan *et al.* 2001; Chang *et al.* 2003, Clara *et al.* 2005; Plósz *et al.* 2010, Barret *et al.* 2010d).

This study aimed to propose, to analyze and to validate a dynamic model for the Polycyclic Aromatic Hydrocarbons (PAHs) fate under anaerobic condition considering sludge as a four-compartment system. To this end, two hypotheses were evaluated. The first hypothesis consists in modeling the PAHs biodegradation kinetics with a co-metabolism kinetics and to compare it with a Monod-type kinetics. The second hypothesis tests which one of the compartments is really available for degradation: the free dissolved one, the aqueous one or the sum of all compartments. This approach should improve the prediction of PAHs distribution, bioavailability and biodegradation.

3.2. Material and Methods

3.2.1. Sludge source

All experiments were performed using activated sludge from an urban wastewater treatment plant. The primary sludge sample (PS) was collected at the outlet of a primary settling tank of a domestic wastewater treatment plant treating 33 000 PE (Population Equivalent). The secondary sludge sample (SS) came from the biological aerobic unit of another domestic plant treating 250 000 PE with a very low hydraulic retention time (0.4 day). Prior to their direct use, PS and SS were stored at -20°C. All these samples were finally diluted with deionized water to reach 24 ± 5 $g_{COD}.L^{-1}$ and spiked at 5 $\mu g.g_{DM}^{-1}$ for each PAH except for indeno(1,2,3,c,d)pyrene (1 $\mu g.g_{DM}^{-1}$). Table 3.1 shows the main characteristics of these primary and secondary sludge.

3.2.2. Micropollutants

Polycyclic Aromatic Hydrocarbons (PAHs) were selected as model micropolluants (Table 3.2). All solvents were purchased from J.T.Baker. Mixtures are indicated in volume

percentage. PAH powders were obtained from Dr Ehrenstorfer GmbH. Each PAH was dissolved in dichloromethane at 1 $g.L^{-1}$. The spiking mix was prepared from these individual concentrated solutions, adding 5 mL of each, evaporating solvent under gentle nitrogen flow and dissolving in 50 mL of acetonitrile. Final concentrations were 100 $mg.L^{-1}$ for each PAH. The 10 $mg.L^{-1}$ standard solution of PAHs in acetonitrile was provided by Dr Ehrenstorfer GmbH. Dilution factors from 10 to 1 000 were applied to obtain 6 calibration levels. Standards were stored at – 20°C.

Table 3.1. Primary (PS) and secondary sludge (SS) characteristics (average value and standard deviation from 5 measurements performed at steady state).

Sludge	***COD***	***DM***	***DCM***	***Proteins***	***Carbohydrates***	***Lipids***	***VFA***
	$gO_2.L^{-1}$	$g_{DM}.L^{-1}$	*% of total DM*	$g_{eqBSA}.g_{DM}^{-1}$	$g_{eqGlu}.g_{DM}^{-1}$	$g_{PEEM}.g_{DM}^{-1}$	$g_{VFA}.g_{DM}^{-1}$
PS	28±4	22±2	5±1	0.27±0.03	0.29±0.09	0.13±0.05	0.03±0.02
SS	23±2	19±1	4±4	0.25±0.02	0.30±0.10	0.10±0.04	0.04±0.04

Source: Barret *et al.* 2010b. Chemical oxygen demand (COD), dry matter (DM), dissolved/colloidal matter (DCM) and volatile fatty acids (VFAs).

Table 3.2. Physicochemical characteristics of the PAHs. K_p ($mL.g_{COD\text{-}part}^{-1}$) and K_{DCM} ($mL.g_{COD\text{-}DCM}^{-1}$), equilibrium constants of sorption determined from a three-compartment methodology (Barret *et al*. 2010c) for PS and SS.

PAH	***M** (g.mol^{-1})*	**PS**		**SS**	
		log K_p	**log K_{DCM}**	**log K_p**	**log K_{DCM}**
Fluorene	166	0.098	0.481	0.283	0.902
Phenanthrene	178	0.398	0.681	0.683	1.102
Anthracene	178	0.398	0.681	0.583	1.102
Fluoranthene	202	0.398	0.781	0.603	1.202
Pyrene	202	0.698	0.981	0.883	1.302
Benzo(a)anthracene	228	0.798	1.281	0.983	1.302
Chrysene	228	0.898	1.281	1.083	1.502
Benzo(b)fluoranthene	252	0.898	1.381	1.083	1.602
Benzo(k)fluoranthene	252	0.998	1.281	1.183	1.802
Benzo(a)pyrene	252	0.898	1.281	1.083	1.702
Dibenzo(a,h)anthracene	278	1.198	1.481	1.383	1.902
Benzo(g,h,i)perylene	276	1.098	1.481	1.283	1.902
Indeno(1,2,3,c,d)pyrene	276	0.898	1.681	1.083	1.702

3.2.3. Experimental set-up

Two continuous reactors have been operated at a constant organic load of 1.2±0.2 $g_{COD}.L^{-1}.d^{-1}$ and a hydraulic retention time of 20 days. Temperature was regulated at 35°C using hot water circulation in the external jacket. The reactors were fed with primary (PS) and secondary sludge (SS). The feed was stored at 4°C. Six times a day, it was pumped into the reactor just after pumping out the digested sludge, collected in tanks at 4°C. For the start-up, reactors were filled with methanogenic sludge coming from an anaerobic mesophilic reactor adapted to PAHs-polluted sludge, and directly fed at the normal operating conditions. The pH and the biogas volumetric production were monitored on line. Seven days composite samples were taken once a week from the feed tank, the outlet tank and the gaseous phase.

3.2.4. Analytical methods

Inlet and outlet composite samples were analyzed for their chemical oxygen demand (COD) in both soluble and particulate fraction, dry matter (DM), organic matter (OM), proteins, carbohydrates, organic carbon in particles (POC) and in dissolved/colloidal matter (DCOC) and volatile fatty acids (VFAs), according to Barret *et al.* (2010a). The percentage of methane (CH_4) and carbon dioxide (CO_2) in the biogas were measured using a gas chromatograph (Shimadzu GC-8A), with argon as the carrier gas and equipped with a thermal conductivity detector. PAHs were extracted from the ORBO cartridge using a Soxhlet setup, operated during 16h at 60°C, with 200 mL of hexane/acetone (50:50 v:v). Extraction from inlet and outlet sludge samples were performed according to Trably *et al.* (2004).

Equilibrium constants (table 3.2) of sorption to particles (K_p) and to dissolved/colloidal matter (K_{DCM}) were determined according to the experimental methodology proposed by Barret *et al.* (2010b) for the thirteen PAHs and the two sludge (PS and SS).

3.2.5. Simulation software

Simulations presented in this work have been developed in MatLab®-Simulink. Optimization toolbox for solving non-linear least square problems has been used to estimate the model parameters.

3.3. Model description and assumptions

3.3.1. The four-compartment model

The model describes the physical exchanges of PAHs between compartments in the reactor. Hence, the physical distributions of these compounds influence their bioavailability and it may be the main limiting factor of OMP biodegradation in sludge. OMPs are assumed to be able to sorb onto eithers particles (P) or dissolved/colloidal matter (DCM) (Barret *et al.* 2010c). In the model, OMPs is thus assumed to be distributed between four physical compartments (figure 3.2): the free dissolved (C_f, µg.L^{-1}), the gas (C_g, µg.L^{-1}), the sorbed to DCM (c_{DCM}, µg.g$_{COD\text{-}DCM}^{-1}$) and the sorbed to particles (c_p, µg.g$_{COD\text{-}p}^{-1}$) compartments. At equilibrium, the four-compartment system can be described by the three following equations:

$$K_p = \frac{c_p}{C_f} \quad (3.4), \qquad K_{DCM} = \frac{c_{DCM}}{C_f} \quad (3.5) \qquad \text{and} \qquad K_H = \frac{C_g}{C_f} \quad (3.6)$$

where K_p is the equilibrium constant of OMPs sorption to particle (L.g$_{COD\text{-}p}^{-1}$), K_{DCM} is the equilibrium constant of OMPs sorption to DCM (L.g$_{COD\text{-}DCM}^{-1}$) and Henry constant (K_H, dimensionless) describes the equilibrium between gas phase and free dissolved concentration of OMPs.

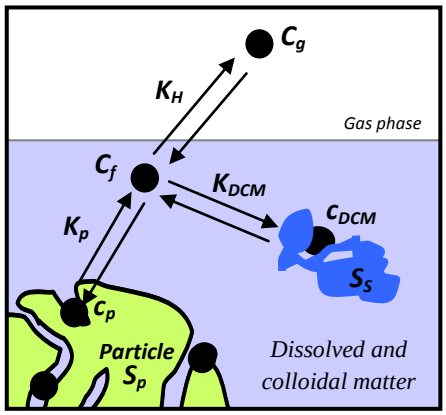

Figure 3.2. Representation of the four-compartment model of an OMP.

The total mass of OMP can be expressed as total liquid concentration ($C_{t,liq}$, µg.L^{-1}) and gas concentration (C_g, µg.L^{-1}) of OMP:

$$C_{t,liq} = C_f + c_P S_p + c_{DCM} S_S \qquad (3.7)$$

$$C_g = K_H C_f \qquad (3.8)$$

where S_p is the particulate substrate (particulate concentration, $g_{COD\text{-}p}.L^{-1}$) and S_S is the soluble substrate (dissolved and colloidal concentration, $g_{COD\text{-}DCM}.L^{-1}$).

Thus, based on the experimental measurement of total liquid concentration and on equations (3.4), (3.5) and (3.7), the concentrations in the different compartments and initial condition can be estimated from equations (3.9) to (3.11) for each pollutant. Indeed, the table 3.2 shows the equilibrium constants K_p and K_{DCM} calculated for the thirteen PAHs and the two sludge (PS and SS), according to previously developed methodology (Barret *et al.* 2010c).

$$C_f = \frac{C_{t,liq}}{1 + K_p S_p + K_{DCM} S_S} \qquad (3.9)$$

$$c_p = \frac{C_{t,liq} K_p}{1 + K_p S_p + K_{DCM} S_S} \qquad (3.10)$$

$$c_{DCM} = \frac{C_{t,liq} K_{DCM}}{1 + K_p S_p + K_{DCM} S_S} \qquad (3.11)$$

Kinetics of sorption and desorption of a pollutant between the free dissolved compartment and the particle one and between the free dissolved compartment and the DCM one are described by equations (3.12) and (3.13):

$$r_{sorp/desorp}^{part} = k_1 (c_p^* - c_p) = k_1 (K_p C_f - c_p) \qquad (3.12)$$

$$r_{sorp/desorp}^{DCM} = k_2 (c_{DCM}^* - c_{DCM}) = k_2 (K_{DCM} C_f - c_{DCM}) \qquad (3.13)$$

where c_p^* and c_{DCM}^* are the OMPs particle and DCM concentrations at equilibrium, respectively. k_1 and k_2 are the first-order kinetic constants of sorption to particle and DCM, respectively.

3.3.2. Biodegradation

A two-steps model has been used to describe the anaerobic digestion of sludge: first hydrolysis to particulate matter (S_p, $g_{COD}.L^{-1}$) and then soluble substrate (S_S, $g_{COD}.L^{-1}$) biodegradation to biogas.

$$S_p \xrightarrow{k_{hyd}} S_S$$

$$S_S \xrightarrow{\mu} X + CH_4 + CO_2$$

Table 3.3. Matrix representation the fate of OMP in the four-compartment model.

Components →			**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Rate**	
Process ↓			**X**	**S_S**	**S_p**	**C_g**	**C_p**	**C_{DCM}**	**C_f**		
1	Growth biomass		1	$-\frac{1}{Y}$						$\mu X = \frac{\mu_{\max} S_S}{K_S + S_S} X$	$g_{COD}.L^{-1}.d^{-1}$
2	Decay biomass		-1		1					$b \cdot X$	
3	Hydrolysis			1	-1					$k_{hyd} \cdot S_p$	
4	Abiotic	Volatilisation				1			-1	$K_{La}(K_H C_f - C_g)$	$\mu g.L^{-1}.d^{-1}$
5		Sorption to particles					1		-1	$k_1(K_{part} C_f S_p - C_p)$	
6		Sorption to DCM						1	-1	$k_2(K_{DCM} C_f S_S - C_{DCM})$	
7	Biotic (biodegradation by cometabolism)*	Degradation of free dissolved compartment							-1	$\left(T_c \frac{\mu}{Y} + k_c\right)\left(\frac{C_f}{K_{SC} + C_f}\right) X$	
8		Degradation of sorbed to DCM compartment						-1		$\left(T_c \frac{\mu}{Y} + k_c\right)\left(\frac{C_{DCM}}{K_{SC} + C_{DCM}}\right) X$	
9		Degradation of sorbed to particle compartment					-1			$\left(T_c \frac{\mu}{Y} + k_c\right)\left(\frac{C_p}{K_{SC} + C_p}\right) X$	
			Biomass (g_{COD}/L)	Soluble substrate (g_{COD}/L)	Particulate substrate (g_{COD}/L)	OMP gas concentration (µg/L)	OMP particle concentration (µg/L)	OMP dissolved/colloidal matter concentration (µg/L)	OMP free concentration (µg/L)	$C_p = c_p S_p$, $C_{DCM} = c_{DCM} S_S$ where, c_p and c_{DCM} (µg/g_{COD})	

*At the 4.2 section (bioavailability evaluation) the matrix model can be modified. Hypothesis 1: model with processes 1 to 7, hypothesis 2: model with processes 1 to 8 and hypothesis 3: model with processes 1 to 9.

Table 3.3 represents the mathematical model with seven components and nine processes. The metabolism of substrate growth is incorporated into the three first processes. Hydrolysis is described with a first order kinetics to represent the enzymatic degradation of particulate substrate in soluble substrate. Beside, decay is assumed in the transformation of activated biomass into particulate substrate. Biomass (X, $g_{COD}.L^{-1}$) growth is linked to soluble substrate uptake and modeled with Monod-type kinetics:

$$\mu = \mu_{\max} \frac{S_S}{K_S + S_S} \qquad (3.14)$$

where μ_{max} (1/d) is the maximum bacterial growth rate and K_S ($g_{COD}.L^{-1}$) is the half-saturation constant associated with the soluble substrate S_S.

OMP biodegradation may be considered as a classical metabolism with a specific OMP-degrader (figure 3.3b) and modeled with a Monod-type kinetics (equation 3.14: $\mu_{max,OMP}$, $K_{S,OMP}$). Nevertheless, the removal of pollutant present only in trace levels ($ng.L^{-1}$ or $\mu g.L^{-1}$) could not result in any significant biomass growth (Clara *et al*. 2005). Thus, we assumed that co-metabolism is the main OMP biodegradation mechanism (figure 3.3a).

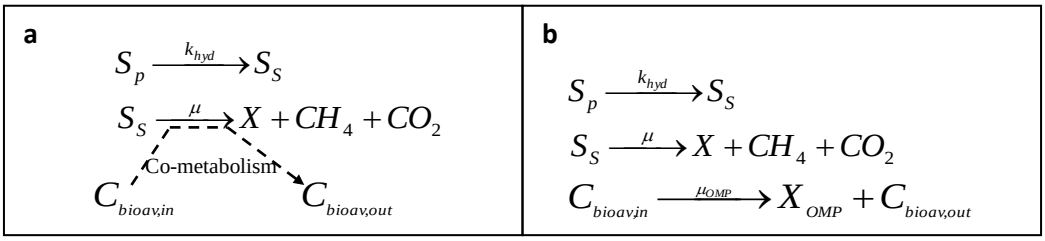

Figure 3.3. Scheme illustrating (a) co-metabolism and (b) classic metabolism of OMP.

Criddle (1993) proposed a co-metabolism model between a growing substrate and a non-growing substrate in simple ecosystem. This equation is based on the assumption that the co-metabolic degradation rate is enhanced by the generation of reductants caused by the degradation of growth substrate (S_S) and, in its absence, the co-metabolic transformation is linked to endogenous decay. Moreover, the co-metabolic model includes competitive inhibition between growth and non-growth substrate and negative effect of the toxic products. However, we did not include those two last terms in this work because of the low concentration of OMPs in the system and a large number of kinetic parameters can complicate the modeling effort under current conditions.

In our case, PAHs are the non-growing substrate and Criddle's equation has to be modified. In particular, the bioavailability limitation can be accounted for by replacing the total concentration by the bioavailable one (C_{bioav}):

$$r_{bio} = \left(T_c \frac{\mu}{Y} + k_c \right) \left(\frac{C_{bioav}}{K_{SC} + C_{bioav}} \right) X \qquad (3.15)$$

where T_c is the OMPs transformation capacity ($\mu g_{OMP}.g_{COD\text{-}Ss}^{-1}$) standing for cometabolic interaction between the soluble substrate metabolism and the OMPs metabolism, k_c is the

maximum specific rate of OMPs biodegradation in absence of primary substrate ($\mu g_{OMP}.g_{COD\text{-}X}^{-1}.d^{-1}$) and K_{SC} is the half saturation constant of OMPs in the Monod formalism ($\mu g_{OMP}.L^{-1}$). The k_c and K_{SC} parameters are representative of the response of the OMP metabolic route to the OMP bioavailable fraction including transporters and enzymes affinity for their substrate. μ is the growth rate (d^{-1}) and Y is the growth yield ($g_{COD\text{-}X}.g_{COD\text{-}Ss}^{-1}$).

Furthermore, the four-compartment distribution may help us to find which compartment is the real bioavailable fraction (C_{bioav}) to be biodegraded. Besides, the four-compartment model can be modified. By this way, the OMPs biodegraded fraction can be assumed to be the free dissolved fraction (C_f – only process 7) or the aqueous fraction (C_f and C_{DCM} – processes 7 and 8), or the sum of all fractions (C_f, C_{DCM} and C_p – processes 7, 8 and 9) as proposed by Fountoulakis *et al.* (2006).

3.3.3. Sensitivity analysis

The model has a cascade structure, which means that the variables X, S_S and S_p are not influenced by the other variables and, then, by the parameters associated with the other state variables. This cascade structure is an advantage to find the parameter set. First of all, we can estimate the parameters of biomass and substrates (μ_{max}, K_S, Y, b and k_{hyd}), and then, the parameters linked with the biodegradation of each OMP (T_c, k_c and K_{SC}).

A sensitivity analysis for OMP total concentration was conducted to identify the most sensitive parameters in the four-compartment model with free dissolved compartment (C_f) as the available fraction. In reference to a given set of parameter values, initial condition and characteristics of pollutants and reactors, eleven parameters were changed over ± 10%, ±20%, ±30% and ±50% of their based values. In steady state, nine simulations were run at each of these values to generate nine concentrations profiles of compartments (C_f, C_p, C_{DCM}, C_g) for each parameter. A sensitivity coefficient, σ_q, of the variable z to the parameter q, defined by equation 3.16 (Bernard *et al.* 2001, Myint *et al.* 2007), was calculated to quantify the average spread for each parameter.

$$\sigma_q = \frac{1}{t_f}\int_0^{t_f}\frac{(z_{q+\Delta q} - z_q)}{z_q}dt \qquad (3.16)$$

where t_f is the test duration, z_q is the variable z associated with base value of parameter q, and $z_{q+\Delta q}$ is the variable z when the parameter q is changed an amount Δq.

The sensitivity coefficient is presented in figure 3.4. Growth yield, Y, is the most sensitive parameter. A strong influence of parameters linked to soluble substrate uptake (Y, μ_{max}, K_S) can be noted. This is in agreement with co-metabolism concept, where the micropollutant fate is associated to growth substrate degradation. Half saturation constant of OMP K_{SC} and the specific biodegradation rate of OMP k_c show a relative sensibility. These parameters are representative of the OMP metabolic route and depend on the type of microbial consortium.

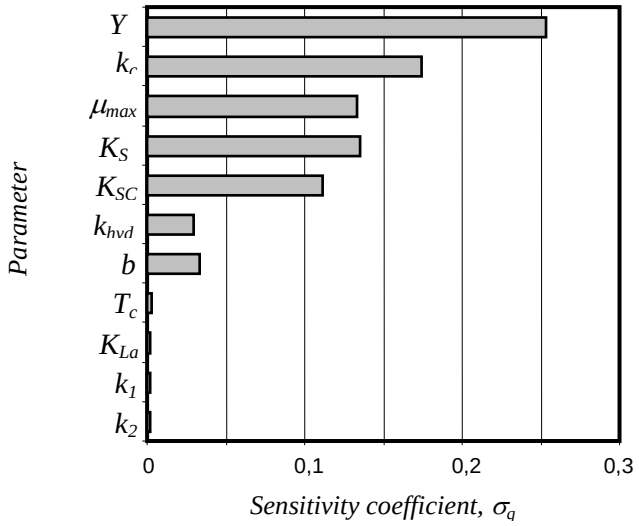

Figure 3.4. Sensitivity coefficient of OMP concentration for the model parameters.

Moreover, hydrolysis step (k_{hyd}) has little influence on the compartments concentration despite it is the rate-limiting step in the anaerobic digestion. As pointed out by the results, the least sensitive parameters are T_c, K_{La}, k_1 and k_2, and therefore they will be less precisely estimated. In fact, the volatilization of PAHs is negligible, and then the OMP gas concentrations are small, as a result K_{La} does not influence the OMP concentration. On the other hand, hydrophobic character of PAHs makes easy their sorption onto sludge. Therefore, the first order kinetic constants (k_1, k_2) have high values (Dionisi *et al.* 2006). Beside, Kordel *et al.* (1997), Dionisi *et al.* (2006) and Barret *et al.* (2010b) have demonstrated that the sorption equilibrium state for PAHs was achieved after 1 or 2h shaking. This sorption mechanism is faster compared to the biodegradation of these compounds under anaerobic condition (Chang

et al. 2003). As a consequence for hydrophobic pollutant, this little influence of the first-order kinetic constants of sorption to particle and DCM (k_1, k_2) on the model showed that the sorption kinetics are not the rate-limiting steps and that the equilibrium state can be sufficient to represent the sorption phenomenon in the OMPs removal. Indeed, the processes 5 and 6 can be replaced by their equilibrium in the model in the case of hydrophobic compounds.

3.4. Model calibration

The model simulations were compared with experimental data of anaerobic digestion of PAHs for two mesophilic continuous reactors fed with primary (PS) and secondary sludge (SS). The influent and effluent macroscopic performances of reactors and biogas production are represented in Table 3.4. Volatile fatty acids (VFAs) did not accumulate and the methane content in biogas was about 70% in the two mesophilic reactors. The overall removals in COD, dry matter and organic matter were higher than 60%. The variation of PAHs removal between reactors was slightly different while between PAHs in one reactor was high. The reported overall PAHs removal rates were from 32 to 74% and from 38 to 73% for PS and SS reactor, respectively.

Table 3.4. Anaerobic performances of PS and SS reactors. Average and standard deviation calculated from 5 measurements performed at steady state.

Parameter	***PS***	***SS***
COD input (g/L)	28.1 ± 3.6	23.0 ± 1.9
COD output (g/L)	13.4 ± 1.7	9.1 ± 2.6
% COD removal	52.4 ± 7.5	60.4 ± 6.5
DM input (g/L)	22.4 ± 1.7	19.5 ± 1.4
DM output (g/L)	12.1± 2.0	10.2 ± 1.8
% DM removal	46.0 ± 8.4	52.9 ± 6.9
VFAs input (gCOD/L)	0.70 ± 0.31	0.90 ± 0.47
VFAs output (gCOD/L)	0.11 ± 0.12	0.20 ± 0.24
% VFAs removal	86.2 ± 12.9	89.4 ± 9.6
% CH_4	0.67 ± 0.23	0.68 ± 0.25
L CH_4/d	0.80 ± 0.21	0.61 ± 0.10

In the model, the set of parameters were estimated using a non-linear least square method between simulated values and measurements. We take here advantage of the cascade structure of the model with the identification of a first set of parameters. Table 3.5 summarizes the values of the parameters linked to biomass and substrates for PS and SS. The K_S values suggest that the metabolism and the implied microbial population could be different in PS and SS biodegradation.

Table 3.5. Estimated values of the biomass and substrates parameters.

Parameter	Meaning	Unit	Value	
			PS	SS
μ_{max}	Maximum growth rate	d^{-1}	0.62	0.63
K_S	Half saturation of growth substrate	$g_{COD\text{-}Ss}.L^{-1}$	3.25	5.10
Y	Growth yield	$g_{COD\text{-}X}.g_{COD\text{-}Ss}^{-1}$	0.75	0.75
B	First-order endogenous decay	d^{-1}	0.05	0.05
k_{hyd}	First-order kinetic of hydrolysis	d^{-1}	0.07	0.13

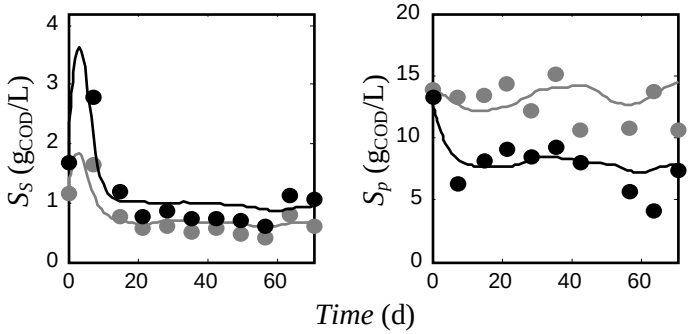

Figure 3.5. Behavior of soluble (S_S) and particulate (S_p) substrate for reactors PS (gray) and SS (black). Circles (•): experimental data and solid line: model.

Figure 3.5 shows the behavior of soluble and particulate substrate in PS and SS reactors. The simulations closely followed the dynamic evolution of the soluble substrate. Note the difference of the particulate substrate concentration between PS and SS, as well as, the fast decline of the particulate substrate in SS reactor. It could explain the dissimilarity of the hydrolysis coefficients found for both digesters. However, the particulate substrate in PS reactor is not well predicted by the model. This may be due to the first order kinetics used in the hydrolysis step which may be different between a non-stabilized sludge (PS) by comparison to a stabilized one (SS). Moreover, it is well known that the hydrolysis is the rate-limiting step in the anaerobic digestion for particulate substrate and the first order kinetics

may be inaccurate to describe the hydrolysis of certain complex substrates (Vavilin *et al.* 2008). The set of parameters estimated in this section were used in the model calibration for evaluation of co-metabolism and bioavailability hypotheses.

3.4.1. Co-metabolism evaluation

The physical and chemical characteristics of the OMP, as well as environmental factors, may influence their biodegradability. There are numerous references reporting that recalcitrant compounds may be transformed in the presence of another compound used as carbon and energy source, i.e. co-metabolism (Chang *et al*. 1993; Criddle 1993; Tiehm and Fritzsche 1995; Yuan *et al.* 2001; Chang *et al.* 2003; Clara *et al.* 2005; Plósz *et al.* 2010, Barret *et al.* 2010d). It is usually assumed that the co-metabolism may occur relatively slower than metabolism of growth substrate.

Consequently, the general scheme adopted for the co-metabolism of an OMP is shown on figure 3.3a. Total biomass consortium synthesizes enzymes for soluble substrate uptake and OMP degradation. Likewise, we propose the free dissolved compartment of OMP as bioavailable fraction. The behavior of the model with co-metabolism has been compared to a classic metabolism of OMP (figure 3.3b). In this case, a specific degrader uptake OMP (X_{OMP}) with Monod-type kinetics.

Besides, non-linear least square optimization method has been used to estimate PAH parameters in the cometabolism kinetics (T_c, k_c and K_{SC}) and classic kinetics (i.e. Monod kinetics for an OMP: $\mu_{max,OMP}$, $K_{S,OMP}$, Y_{OMP}) for each reactor (PS and SS) and thirteen PAHs. Figure 3.6 displays the simulated (with both models) and experimental results for the fluoranthene in PS and SS reactors. Similar behaviors have been obtained for the other PAHs (data not shown). The model simulations with co-metabolism closely follow the dynamic evolution of the total fluoranthene concentration and its compartments. In contrast, the model with a classic metabolism resulted in an overestimation of the experimental data. The residual values evaluated are 50.1 and 0.82 for metabolism and co-metabolism, respectively, in C_f compartment for fluoranthene and PS. Moreover, co-metabolic route of our results can be reinforced by following facts: (i) Barret *et al.* (2010c) reported a strong correlation between PAH and dry matter removal rates, it agrees with the results of Trably *et al.* (2003) and (ii)

under anaerobic condition, Chang *et al.* (2003) and Trably *et al.* (2003) shown no growth with PAH as source of carbon.

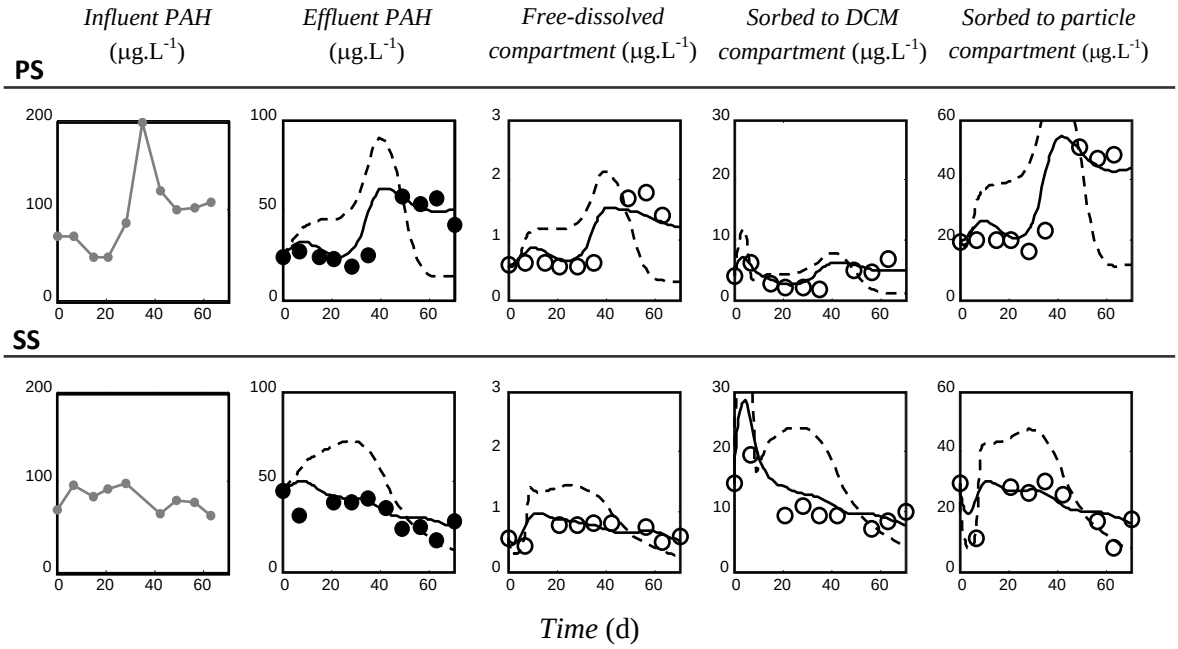

Figure 3.6. Fluoranthene behavior in PS and SS reactors: (gray line) influent concentration of PAH, (black circles) experimental data, (white circles) values estimated from equilibrium constants, (black line) model with co-metabolism and (dashed line) model with classic metabolism.

3.4.2. Bioavailability evaluation

Various definitions of bioavailability are used across many disciplines (Semple *et al.* 2004). In this paper, a bioavailable compound is the chemical fraction that can be freely transformed by a microorganism. From a general point of view, a sorbed micropollutant is not available for microbial degradation; while its biodegradation occurs predominantly in the bulk aqueous phase (Byrns 2001, Artola-Garicano *et al.* 2003, Urase and Kikuta 2005, Dionisi *et al.* 2006, Plósz *et al.* 2010, Barret *et al.* 2010d). However, few studies have concluded that at least some microorganisms are capable of degrading compounds directly from the sorbed phase (Haws *et al.* 2006; Fountoulakis *et al*. 2006).

In order to find the real OMP bioavailable fraction, the four-compartment model was modified into the biotic process matrix for C_f, C_{DCM} and C_p compartments (table 3.3 and processes 7, 8 and 9). To this end, we have tested three hypotheses in the model (table 3.3). Hypothesis 1 (processes 1 to 7): the bioavailable fraction was assumed to only be the free dissolved compartment, given that it is possible to separate the free dissolved fraction and sorbed to DCM of the aqueous phase in the model. Hypothesis 2 (processes 1 to 8): aqueous fraction is available for the microbial degradation activity. This is in concordance with the widespread assumption that the aqueous fraction of OMP corresponds to their bioavailable compartment (Chang *et al.* 2003, Artola-Garicano *et al.* 2003, Dionisi *et al.* 2006, Barret *et al.* 2010c). Indeed, two mechanisms were proposed for the degradation of micropollutant sorbed to DCM: (i) low molecular weight DCM might be able to cross the microbial membrane in form of micropollutant-DCM complex, because some molecules up to a few kDa were shown to cross bacterial membrane (Nikaido 2003) and (ii) recently it had been demonstrated that particles might transport the sorbed micropollutant directly or to vicinity of the cell surface (Smith *et al.* 2009), prior to the diffusion of free micropollutant throughout cell membrane. Finally, the hypothesis 3 (processes 1 to 9): All compartments are bioavailable as proposed by Fountoulakis *et al.* (2006). This is probably a mechanism of transport of sorbed micropollutant to vicinity of microorganism.

Non-linear least square optimization method has been used to estimate PAH parameters (T_c, k_c and K_{SC}) for each case, thirteen PAHs and two sludge. The residuals value taken up to quantify the best fitting do not present a high variation between cases, for example the residual values are 0.11, 0.10 and 0.12 for free dissolved, aqueous phase and all compartments as available fraction, respectively, for chrysene in C_f compartment and PS.

Figure 3.7 shows the comparison of model predictions (three hypotheses) for chrysene in PS and SS reactors. Similar behaviors have been obtained for the other PAHs (data not shown). Such results could suggest that the PAH degradation occurs at the same time into free, aqueous and solid fraction, i.e sorbed fractions into particle and dissolved colloidal matter could be bioavailable to degraders. However, the half saturation constants (K_{SC}) of PAHs estimated in the first case (the free dissolved fraction is the bioavailable one) are ten times and hundred times lower than for the two other hypotheses (aqueous fraction and the sum of all compartments, respectively) as shown on figure 3.8.

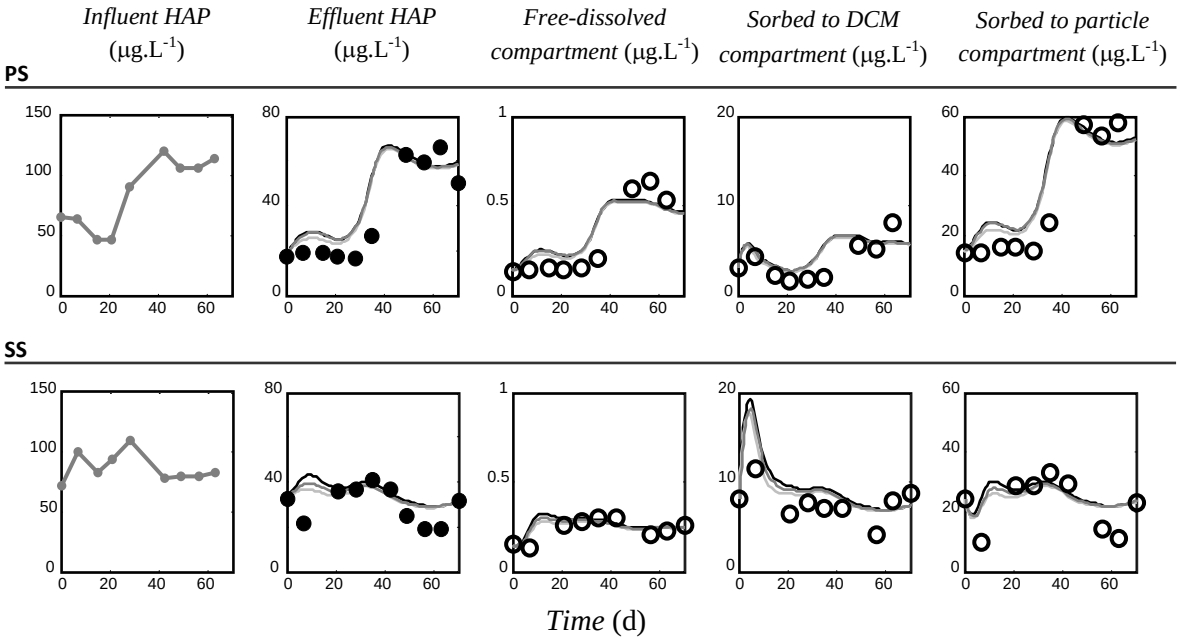

Figure 3.7. Chrysene behavior in PS and SS reactors: (gray line) influent concentration of PAH, (black circles) experimental data, (white circles) values estimated from equilibrium constants, (black line) hypothesis 1, (dark-gray line) hypothesis 2 and (light-gray line) hypothesis 3.

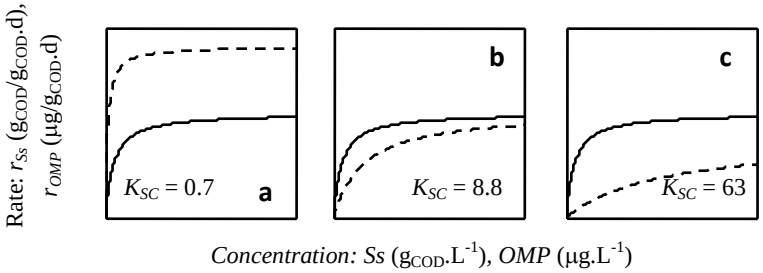

Figure 3.8. Comparison between substrate degradation rate (solid line) and OMP degradation rate (dashed line) for three hypotheses. (a) hypothesis 1: $C_{bioav} = C_f$, (b) hypothesis 2: $C_{bioav} = C_f$, C_{DCM} and (c) hypothesis 3: $C_{bioav} = C_f$, C_{DCM}, C_p.

Consequently, in the hypothesis 1, the degradation rate of OMP is faster than that of soluble substrate as shown on figure 3.8a. This disagrees with the co-metabolism studies demonstrating that the co-metabolism is relatively slow by comparison to the metabolism of growth substrate (Chang *et al.* 1993, Haws *et al.* 2006). In addition, half saturation value estimated in the hypothesis 3 is higher than the OMP concentration in the free compartment ($K_{SC} >> C_f$). This implies that the free compartment degradation is negligible compared to the

particle compartment one and suggests high affinity for the OMP sorbed to particle. This case is really atypical, since it is generally considered that the sorbed chemicals are unavailable for microorganisms unless desorption occurs first. Indeed, Feng *et al.* (2000) have demonstrated that some bacteria can degrade sorbed chemical but it is not more important than the OMP aqueous phase degradation. In contrast, hypothesis 2 presents a cometabolism slower than metabolism of soluble substrate (figure 3.8b) and the affinity for free compartment and DCM compartment are comparable. Finally, based on the three-compartment model, Barret *et al.* (2010c) reported a strong correlation between PAHs aqueous fraction degradation and the dry matter removal and shown that the PAH biodegradation depended on a combination of bioavailability and cometabolism. Thus, the widespread assumption that the aqueous fraction of PAHs corresponds to their bioavailable compartment (Chang *et al.* 2003, Artola-Garicano *et al.* 2003, Dionisi *et al.* 2006, Barret *et al.* 2010d) was reinforced by our results.

It is worth noting that PAH volumetric gas fraction does not exceed 0.05% of micropollutant total concentration for both low and higher molecular weight PAHs (data not shown). Moreover, hydrophobic character of PAH proves strong PAH affinity (higher to 98%) for both particle and DCM while PAH free concentration is hardly detectable (1.5%). However, PAH sorption to particle in PS reactor (85 ± 5%) was higher than SS (65 ± 5%) one and PAH affinity for DCM was 13 ± 5% and 33 ± 5% for PS and SS, respectively. As a consequence in this study, higher biological removal of individual PAH was observed in SS reactor in contrast with PS one.

3.4.3. Kinetic parameters

The results suggest that the three co-metabolism parameters (T_c, k_c and K_{SC}) could explain the different biodegradation rates between PAHs and between bioreactors. This is valid under the assumption that the aqueous fraction (sum of free and sorbed to DCM compartments) is the bioavailable compartment. Figure 3.9 shows the variation of T_c/K_{SC} and k_c/K_{SC} as function of molecular weight of PAHs.

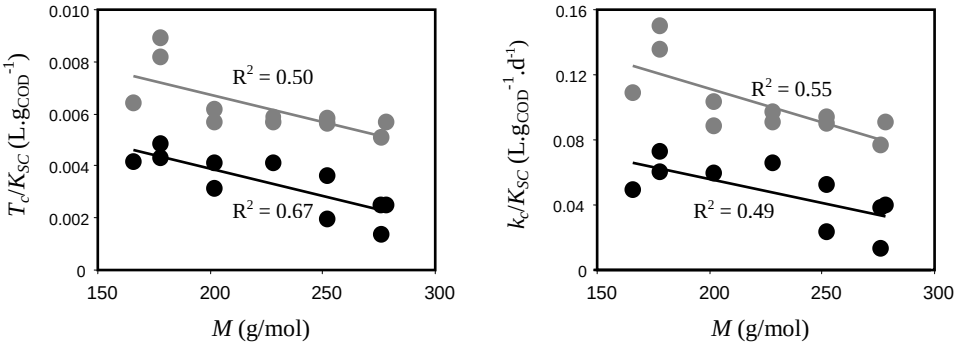

Figure 3.9. T_C/K_{SC} and k_C/K_{SC} as a function of molecular masses of PAHs. PS (gray) and SS (black).

The transformation capacity values T_c did not present differences between PAHs and reactors (value close to 0.05 $\mu g_{OMP}.g_{COD\text{-}Ss}^{-1}$). As a result, the T_c value could correlate with a molecular structure family. Indeed, this term links PAHs degradation to soluble substrate utilization, so it might play a role in the different fates of PAHs in the reactor fed with PS and SS. However, previous PS and SS characterizations presented similar composition and reported slight differences in proteins and lipids content (table 3.1). As a result, T_c can present trifling variation between substrates (PS and SS).

Half saturation constant K_{SC} is likely to vary as a function of PAH molecular weight. K_{SC} values indeed increase when PAHs molecular weight increases. This is in accordance with the idea that high molecular weight PAHs are less efficiently removed (Chang *et al.* 2003). Moreover, specific biodegradation rate, k_c was shown to vary between reactors in a similar range (PS: 0.70 – 0.85 and SS: 0.60 -0.90 $\mu g_{OMP}.g_{COD\text{-}X}^{-1}.d^{-1}$). Therefore, half saturation constant K_{SC} and k_c probably depend on microbial consortium. In this study, it was shown that different consortia exhibit different K_{SC} and k_c. This microbial effect could account for biodegradation differences reported when bioaugmentation strategy has been developed (Trably *et al.* 2003).

3.5. Conclusion

A four-compartment model of the fate of thirteen PAHs during anaerobic digestion of contaminated sludge was developed and confronted with experimental data. The model includes abiotic and biotic processes: volatilization, sorption, biodegradation as metabolism or co-metabolism. Furthermore, in the case of hydrophobic pollutants, the sorption process can

be represented by the equilibrium state. The model helps in elucidating which fraction of the PAHs distribution at equilibrium state is the real bioavailable compartment. Thus, the simulation carried out in this study validated the accepted assumption that the aqueous phase is bioavailable. Indeed, biodegradation affinity for OMP free dissolved and OMP sorbed to DCM are comparable. Furthermore, PAH biodegradation rate was coupled to co-metabolism. The PAH removal was linked to soluble substrate uptake in the anaerobic digestion of sludge. The modified co-metabolism model predicted well the relation between bioavailability and co-metabolism of OMP. As a result of the numerical simulation, the three co-metabolism parameters (T_c, k_c and K_{SC}) were shown to be molecule-dependant. These estimated parameter values could explain the different biodegradation rates between PAHs and between reactors. Nevertheless, the applied methodology for the parameters identification may converge toward several values but this study can be considered as a starting point, given that the parameter values comparison with previously published data was hardly feasible. A limitation in this model is that it does not include the OMPs inhibition and toxic effect, which could be considered in future work. However, the model proposed is potentially useful to better understand the micropollutant distribution, predict the fate of PAHs under anaerobic condition and help to optimize the operation process for their removal.

3.6. General Discussion

This article developed a fate model of hydrophobic micropollutant under anaerobic digestion of contaminated sludge. **The model is based on a four-compartment distribution and it reinforced the hypothesis that the micropollutant transformation is a function of co-metabolism and that the aqueous phase is the bioavailable compartment**.

Clearly, sorption phenomena play an important role in hydrophobic organic micropollutant fate during sludge treatment, because it can influence (i) the distribution of micropollutant fluxes between liquid phase and sludge throughout physical separation process, and (ii) the availability and degradation in biological process. In concordance with Barret *et al.* (2010b), two sorption-desorption equilibria are concomitant in sludge: sorption to particle and sorption to dissolved-colloidal matter (DCM) and both are assumed to fit linear isotherms at low micropollutant concentrations. Hence, an experimental methodology was designed to investigate the sorption-desorption phenomenon for hydrophobic compounds (Barret 2009). The results presented for 13 PAHs demonstrated that sorption and desorption kinetics are very

quick in comparison to biological treatments e.g. the equilibrium of sorption to particle and to DCM were achieved within one hour. In addition, it was shown that sludge aging did not affect the sorption equilibrium between free and sorbed to-particles micropollutants. In this sense, sorption to particles is strictly reversible and it suggests that the hysteresis phenomena could be only due to the transfer of micropollutants from sorbed fraction to sequestrated fraction, and not to a time-dependent increasing sorption affinity of micropollutants for the matrix (Barret *et al*. 2011). It was concluded that PAHs transfer does not limit their biodegradation, and that their fate is governed by sorption-desorption equilibrium state. Similar conclusions were obtained from sensitivity analysis of the four-compartment model i.e. the sorption processes to particle and to DCM were shown to be not dependent of their time constants (k_1 and k_2).

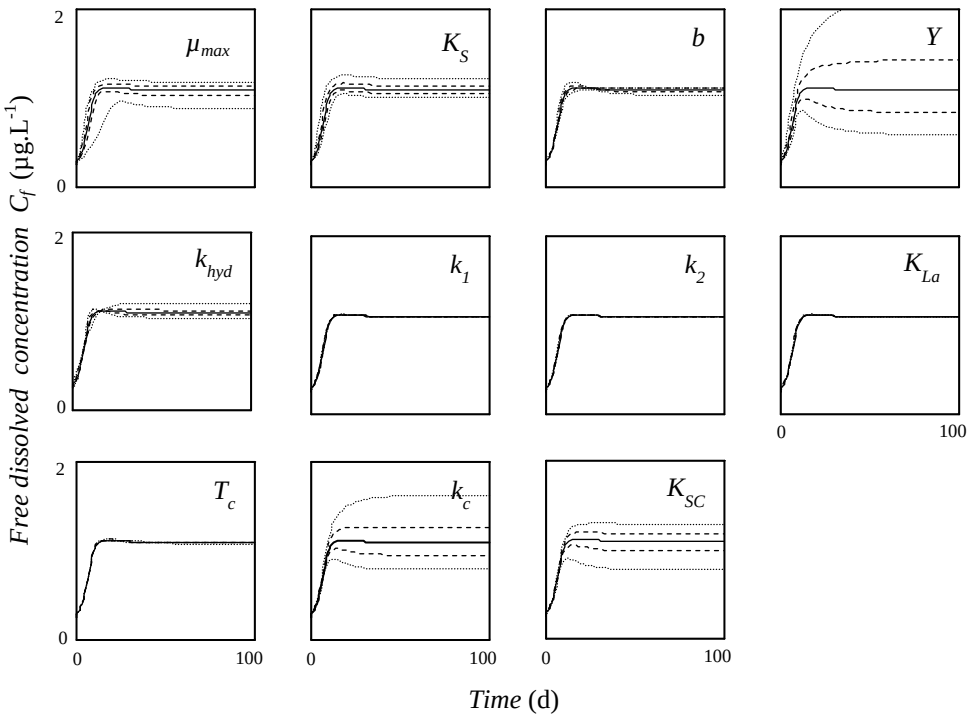

Figure 3.10. Behavior of free dissolved concentration of micropollutant in steady state for different parameter changes. (Solid line) based parameters, (dashed line) parameter changed ±20% and (dotted line) parameter changed ±50%.

Figure 3.10 shows the behavior of free dissolved concentration of micropollutant in steady state for different parameter changes (±20% and ±50%) over a base value of parameter. The sensitivity analysis results of the parameters model were already discussed in section 3.3.3. Thus, the equilibrium state is sufficient to represent the sorption phenomenon and the sorption-desorption kinetics are not the rate-limiting step in the hydrophobic micropollutant removal.

Besides, at equilibrium, the hydrophobic micropollutant distribution is well predicted by the two equilibrium constants (K_p and K_{DCM}). They can be determined by experimental methodology (Barret *et al.* 2010c) or by a statistical model as a function of both micropollutant and sludge characteristics (Barret *et al.* 2010a). Hence, this detailed distribution can help to drive the micropollutant degradation onto sludge digestion. However, during the biological treatment, the variation of micropollutant degradation is influenced by the dynamical and operational condition due to different levels of bioavailability, co-metabolism and microbial potential.

Indeed, this article showed that a co-metabolism kinetic model is quite well adapted to simuilate the fate of micropollutants in anaerobic systems considering concomitantly the aqueous compartment as the bioavailable one. An ecological explanation of co-metabolism is that the removal of compounds present only at traces levels (ng/L or µg/L) does not result in any significant biomass growth (Clara *et al.* 2005). Indeed, co-metabolism mechanism has been stated (Horvath 1972) as the ability of the organism to attack the pollutant but it cannot assimilate the products of its oxidation. In our model, the co-metabolic transformation was modeled with an adaptation of the co-metabolism kinetics proposed by Criddle (1993). This kinetic was developed for a specific co-metabolism i.e. it was applied for a specific growth substrate, non-growth substrate and pure cultures. Nevertheless, in our work, the Criddle co-metabolism was successfully applied to complex substrate (as sludge) and the presence of several co-substrates (as micropollutants) that are catalyzed by enzymes and cofactors during anaerobic digestion. Moreover, this adapted formula allows the combination of the micropollutant bioavailability concept to the co-metabolism concept i.e. the soluble substrate consumption (as DCM, a readily bioaccessible substrate) is linked to the transformation of micropollutant in the aqueous fraction (equation 3.17 and 3.18).

$$r_{bio} = \left(T_c \frac{\mu_{\max}}{Y} \left(\frac{S_S}{K_S + S_S} \right) + k_c \right) \left(\frac{C_{aq}}{K_{SC} + C_{aq}} \right) \quad (3.17)$$

$$C_{aq} = C_f + c_{DCM} S_S \qquad (3.18)$$

Equation (3.17) indicates that the lack of growth substrate (S_S) can limit the potential of the micropollutant degradation capacity by bacteria community. Thus, transformation capacity coefficient (T_c) links the soluble substrate utilization and the micropollutant transformation; so that co-metabolic transformation rate of micropollutant is increased in the presence of soluble substrate (figure 3.11). However, due to the poorly available carbon source and low level of nutrients, the micropollutant transformation is also linked to the biomass decay. In this sense, the half saturation constant of micropollutant (K_{SC}) and the specific maximum degradation rate (k_c) are probably dependent of microbial consortium. This fact should explain the different micropollutant removals found during anaerobic digestion of different sludge sources (Barret *et al*. 2010d).

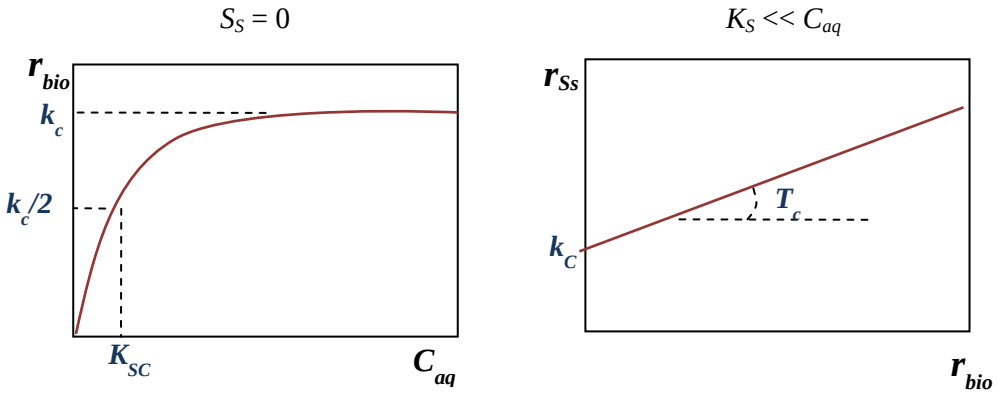

Figure 3.11. Kinetics parameter of co-metabolism.

This co-metabolims model of micropollutant was included to anaerobic digestion model. It is worth noting that the sludge biodegradation is represented by a simple model with three processes and one biomass (figure 3.12). This biomass represents the overall anaerobic community that produced the enzymes and cofactors for soluble substrate utilization and also for micropollutant transformation by co-metabolism. However, co-metabolism of recalcitrant compounds, such as PAHs is often concomitant with competitive inhibition on readily bioaccessible substrate (Criddle 1993, Chang *et al.* 1993, Alvarez-Cohen and Speitel 2001, Haws *et al.* 2006, Plósz *et al.* 2010), which possibly resulted from competition on energy or sharing a common enzymatic system. The toxicity effect is also related to recalcitrant compound degradation (Criddle 1993, Alvarez-Cohen and Speitel 2001, Haws *et al.* 2006):

toxicity of micropollutant, toxicity of products or the depletion of biomass energy reserves could deactivate or destroy biomass. Despite this fact, both mechanisms (competitive inhibition and toxicity) were not considered in our co-metabolism model because of the low micropollutant concentrations. Furthermore, a large number of kinetic parameters can complicate the modeling effort under current conditions. However, the comprehensive co-metabolism model proposed by Criddle (1993) considers the inclusion of these mechanisms that could be pondered over future works.

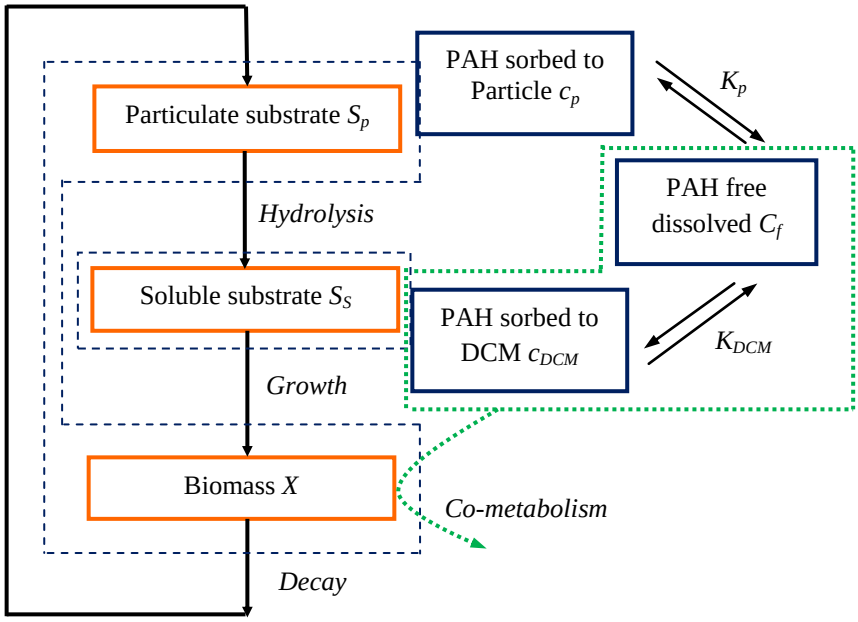

Figure 3.12. Presentation of PAH fate model.

Besides, the bioavailable concentration of micropollutant could be the limiting factor for the biological transformation. This study demonstrated that micropollutants located in the free dissolved and sorbed-to-DCM compartments are completely available for biological activity. In the meanwhile the micropollutants sorbed-to-particle can be considered as bioaccessible to bacterial species, because they can be transferred to aqueous phase during the hydrolysis steps of the process. Indeed, Barret *et al*. (2010d) have observed that the DCM concentration was a positive parameter for micropollutant removal, probably because (i) it determines the aqueous and bioavailable fraction of micropollutants, and (ii) it is a readily bioaccessible substrate. In this sense, the micropollutant removal could be improved by increasing the colloidal

concentration or improving the sludge degradability. However, understanding the anaerobic steps involved in the micropollutant hydrophobic degradation, and also identifying the bacteria contributing directly or indirectly to the transformation of the micropollutant will likely play a critical role in elucidating the mechanisms and interpreting the factor influencing the bioavailability and the co-metabolism.

The next chapter is focused on determining which anaerobic steps are involved in the micropollutant removal. We propose experimental strategies to evaluate the removal rate of PAHs and NP of methanogenic consortia when exposed to different inhibition source.

New kinetic parmaters

A key limitation of the anaerobic digestion modelling field is a lack of reliable, verified parameters, and parameter acquisition methods. Problems with estimation in anaerobic digestion systems include correlation between key parameters, poor identifiability, and difficulty in applying suitable dynamic tests to the process. In particular, it is very difficult to properly identify the initial conditions in continuous systems, it needs long periods to achieve model convergence, and stable operation. Parameter estimation, with realistic uncertainty estimates, is becoming more reliable, repeatable, and practically useful, largely due to application of iterative parameter estimation methods, and statistical methods for estimating parameter uncertainty. A new set of kinetic parameters were estimated in order to found the more realistic values according to literature (K_S and Y). Table 3.6 summarizes the new values of the parameters linked to biomass and substrates for PS and SS.

Table 3.6. New set of kinetics parameters.

Parameter	Meaning	Unit	Value	
			PS	SS
μ_{max}	Maximum growth rate	d^{-1}	0.31	0.27
K_S	Half saturation of growth substrate	$g_{COD\text{-}Ss.}L^{-1}$	0.95	1.12
Y	Growth yield	$g_{COD\text{-}X}.g_{COD\text{-}Ss}^{-1}$	0.10	0.10
b	First-order endogenous decay	d^{-1}	0.05	0.05
k_{hyd}	First-order kinetic of hydrolysis	d^{-1}	0.050	0.087

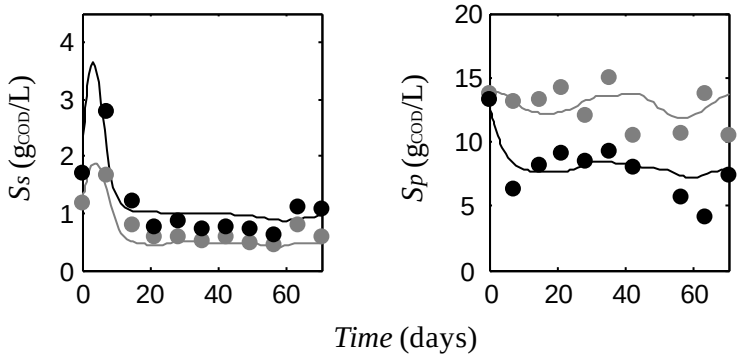

Figure 3.13. Behavior of soluble (S_S) and particulate (S_p) substrate for reactors PS (gray) and SS (black) with a new set of kinetics parameters estimed. Circles (•): experimental data and solid line: model.

The new kinetic parameters can modify the co-metabolic parameters of PAHs (table 3.7). However their behavior is similar to the previous simulations (comparison figures 3.6, 3.7 and 3.14). The applied methodology for the parameters identification may converge toward several values but this study can be considered as a starting point. In this sence, the design of micropollutant removal experiment could be conducted in order to inprove the co-metabolism parameters identifiability and the microbial pathway of micropollutant anaerobic degradation.

Table 3.7. Co-metabolic parameters of the PAHs for PS and SS reactors. Bioavailable fraction is the aqueous compartment. T_c (µg.g_{COD}^{-1}), k_c (µg.g_{COD}^{-1}.d^{-1}) and K_{SC} (µg.L^{-1}).

PAH	M ($g.mol^{-1}$)	PS			SS		
		T_c	k_c	K_{SC}	T_c	k_c	K_{SC}
Fluorene	166	1.87	2.53	2.59	1.37	1.54	3.18
Phenanthrene	178	2.13	2.67	2.24	2.03	2.26	3.02
Anthracene	178	2.28	2.75	2.24	2.03	2.26	3.02
Fluoranthene	202	2.08	2.45	2.52	1.96	2.12	3.44
Pyrene	202	2.12	2.50	2.44	2.09	2.33	3.24
Benzo(a)anthracene	228	2.12	2.58	2.44	2.98	3.17	3.24
Chrysene	228	2.12	2.58	2.44	2.64	2.81	3.42
Benzo(b)fluoranthene	252	2.12	2.58	2.44	2.91	3.02	3.42
Benzo(k)fluoranthene	252	2.12	2.58	2.44	2.61	2.80	3.42
Benzo(a)pyrene	252	1.77	1.92	3.61	1.62	1.87	5.29
Dibenzo(a,h)anthracene	278	2.12	2.58	2.44	2.32	2.60	3.72
Benzo(g,h,i)perylene	276	2.12	2.61	2.52	2.32	2.60	3.72
Indeno(1,2,3,c,d)pyrene	276	1.11	1.33	5.05	0.95	1.10	6.11

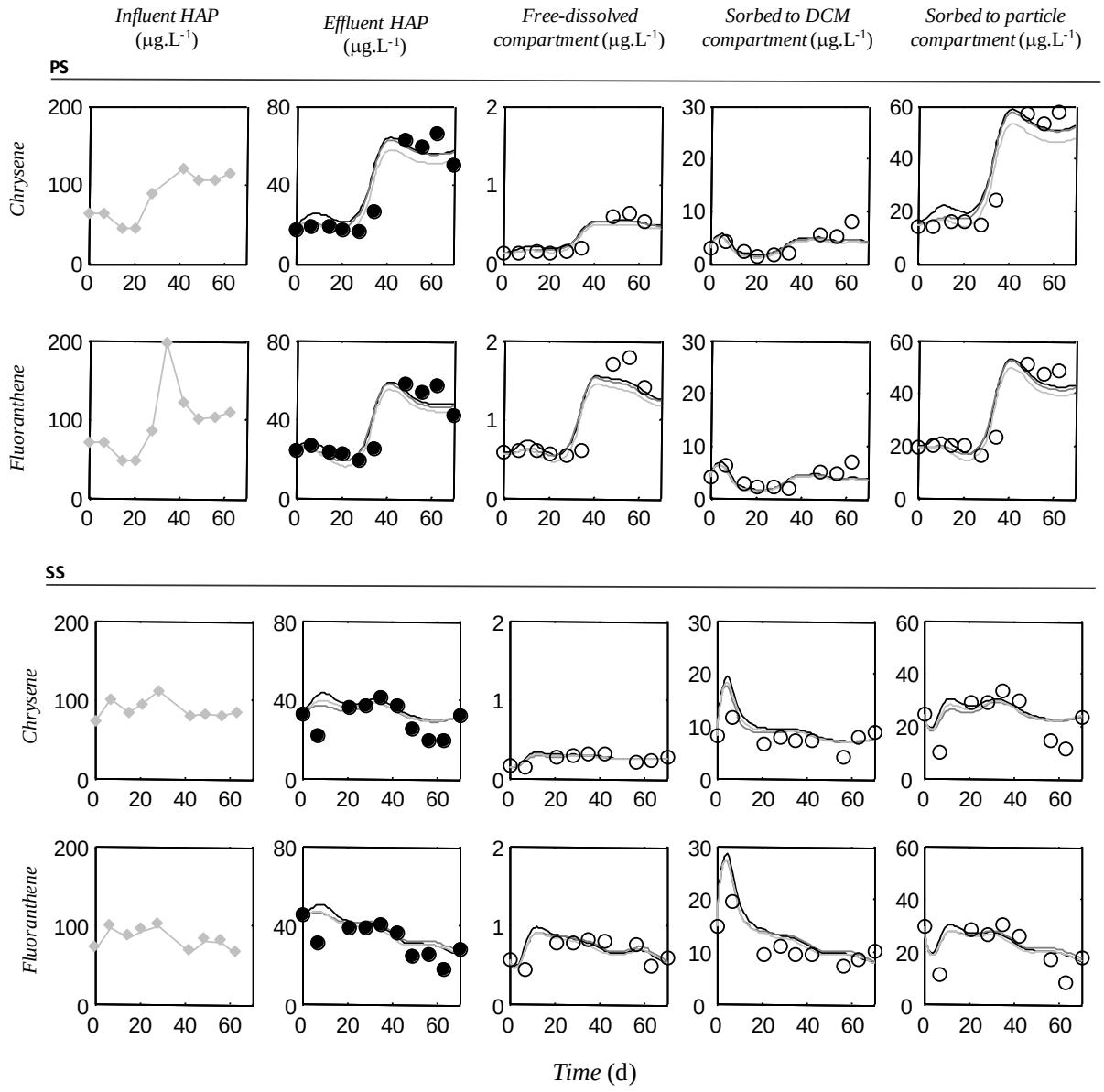

Figure 3.14. Chrysene and Fluoranthene behaviors in PS and SS reactors: (gray line) influent concentration of PAH, (black circles) experimental data, (white circles) values estimated from equilibrium constants, (black line). Bioavailability hypotheses: hypothesis 1, (dark-gray line) hypothesis 2 and (light-gray line) hypothesis 3.

4. Anaerobic digestion steps involved in micropollutant removal: the experimental part

The fate model of hydrophobic compounds, developed in the previous chapter, has demonstrated that the sorption phenomenon is independent of time constant i.e. the sorption can be successfully represented by the equilibrium states. The two hypotheses, co-metabolism transformation and aqueous phase as available compartment were also validated. Besides, the bioavailable concentration in conjunction with the co-metabolism could be the limiting factors for the biological transformation of micropollutant. Our first model approach involves one biomass that represents the overall anaerobic community responsible for both utilization of soluble substrate and micropollutant transformation by co-metabolism. However, anaerobic digestion is a complex process that involves several microorganisms and several substrates all along the organic matter degrading pathway and this pair microorganism/substrate or one part of it could be responsible of the hydrophobic micropollutant depletion. Then, the organisms directly involved in the micropollutant anaerobic removal represent a microbial potential throughout the presence and abundance of these active microorganisms. Some authors (Chang *et al.* 2003, 2005 and 2006) have investigated the role of Methanogens using specific methanogenic inhibitor, as bromoethanesulfonic acid (Freedman and Gossett 1989, Pulliam Holoman *et al*. 1998, Le Van *et al*. 1998 and Trably 2002). Their results suggested that methanogenic populations are fully involved in the hydrophobic micropollutant removal. The main objective of this chapter is to test this hypothesis: ***is only the methanogenic step involved in the micropollutant degradation?*** We propose two strategies in order to modify the anaerobic metabolic pathway and to see the effect on micropollutant removals.

The two strategies are based on the apparent separation of the anaerobic digestion in two phases in continuous anaerobic digesters. One anaerobic reactor with good methanogenic performances was divided into two reactors. In one reactor, the addition of high acid acetic concentration produced an imbalance in the system with (i) volatile fatty acids (VFAs) accumulation and consequently (ii) a pH drop, (iii) a decrease in the methanogenic activity and in the methane yield. One of the critical steps in the anaerobic digestion process is the oxidation of organic acids: the accumulation of acetate from acetogenic bacteria inhibits the propionate and butyrate degradation (Ryan *et al*. 2010). Indeed, Schnürer *et al.* (1999) have investigated on pure culture that as much as 6 $g.L^{-1}$ of acetate, propionate or butyrate in combination with low pH (dissociated form of acids) may be responsible for 50% inhibition of both acetoclastic and hydrogenotrophic Methanogens. However, it has been previously reported that the hydrogenotrophic Methanogens are less susceptible to VFAs induced inhibition than the acetoclastic Methanogens or Acetogens (Ryan *et al*. 2010).

In the second reactor, soluble selenite (Na_2SeO_3) was added at high concentration, producing an irreversible toxic effect on methanogenic organisms. Selenium oxyanions can influence fermentation and methanogenesis either directly via inhibition of the microbial groups involved, or indirectly by altering the electron flow in the organic degrading web, as selenium reducers use hydrogen (Chung *et al*. 2006) and acetate (Macy and Lawson 1993) as electron donors. Lenz *et al*. (2008) have evaluated the inhibitory effects of selenite and selenate towards hydrogenotrophic and acetoclastic methanogenesis in anaerobic toxicity assays: inhibition occurs at low concentrations ($<10^{-4}$ M SeO_3^{2-}; $<10^{-5}$ M SeO_4^{2-}) and within a short time span (SeO_3^{2-}: 1 week; SeO_4^{2-}: 2 weeks).

Due to the imbalance produced or the electron flow modification, it is worth noting that different levels of co-metabolism and various available fraction of micropollutant could be observed. Indeed, these inhibition procedures can favor the growth of certain organisms and the production/accumulation of certain metabolites potentially involved in the co-metabolic micropollutant transformation.

Effect of methanogenic inhibition on the hydrophobic micropollutant removal during anaerobic sludge digestion

Abstract

Anaerobic process has been shown as a potential treatment for removal of recalcitrant compounds. Long term adapted to micropollutant anaerobic ecosystems is required to observe such removal, implying the presence of adapted archaeal and bacterial species. In order to identify the microorganisms involved in these compound degradations, removal of thirteen Polycyclic Aromatic Hydrocarbons (PAHs) and Nonylphenol (NP) was measured during methanogenic inhibition in continuous anaerobic reactors. The reactors were fed with secondary sludge spiked with micropollutant and their methanogenic population was inhibited by two different strategies: high acetate concentration and selenium oxyanion. It was shown that micropollutant degradation is not correlated only with methanogenic activity and the results suggested that the bacterial populations responsible for acetate and/or H_2 production could be also involved in the micropollutant degradation pathway. Moreover, micropollutant

fate is fully affected by organic matter hydrolysis. Indeed the hydrolysis can modify either the micropollutant sorption equilibrium and thus the bioavailable concentration of micropollutant or the metabolic fluxes in the overall anaerobic pathway and thus the co-metabolism of micropollutants.

4.1. Introduction

Organic micropollutants, such as Polycyclic Aromatic Hydrocarbons (PAHs) and Nonylphenol (NP) are often persistent in the environment. However, anaerobic digestion has been shown as a potential biological process for removing these compounds. Trably *et al.* (2003) observed the removal of thirteen PAHs under mesophilic and thermophilic methanogenic condition. In particular, biological PAHs removal was enhanced by thermophilic temperature, especially for the heaviest PAHs. Moreover, degrading activity of these pollutants was enhanced by addition of an anaerobic micropollutant-adapted consortium (Chang *et al.* 2003, Trably *et al.* 2003). Bioaugmentation of sludge improved the PAH removal (Trably *et al*. 2003, Larsen *et al.* 2009 and Hadibarata *et al*. 2009). Trably *et al*. (2003) observed also that PAH removal efficiencies seemed to be closely associated to the methanogenic activity, e.g. biogas yield decreased proportionally with the increased of PAH removal at a constant but low PAH load. The bioaugmentation experiments combined with the observation of Trably *et al*. (2003) underlined the main role played by the Methanogens in PAHs degradation. Chang *et al.* (2005) investigated the effect of various factors on the anaerobic sludge digestion of NP. They have shown that the NP biotransformation was also observed under sulfate-reducing conditions but repressed under methanogenic and nitrate-reducing conditions (sulfate reduction conditions > methanogenic conditions > nitrate reduction conditions) in sludge rich in sulfate reducing bacteria. Beside, Patureau *et al.* (2008) and Barret *et al.* (2010a) have shown that anaerobic consortia are involved in the partial removal of NP. However, the specific microbial population involved in hydrophobic micropollutant degradation is still a crucial research question that this paper aims to address.

Based on a three compartment distribution of PAHs in the sludge matrix, Barret *et al*. (2010b) have shown that hydrophobic micropollutant degradation is driven by either the existence of a bioavailable compartment or the presence of a co-metabolic pathway. Indeed, these authors identified the dry matter biodegradation as the most relevant co-metabolism flux and the aqueous phase as the bioavailable fraction. Similar conclusions were obtained using a four-

compartment model of PAH fate developed by Delgadillo-Mirquez *et al*. (2011). In this dynamic model, the micropollutant removal was linked to the soluble substrate uptake by a co-metabolism kinetic proposed by Criddle (1993). The simulation demonstrated that the co-metabolism was the driving mechanism.

However, the collected data did not allow the authors to further investigate which part(s) of the anaerobic degradation pathway was/were responsible of the micropollutant degradation. Former studies have tried to elucidate the involvement of different anaerobic microbial groups into micropollutant removal; most of them were based on the introduction of bromoethanesulfonic acid (BES). BES is a specific inhibitor of the methanogenesis, it inhibits the methyl-coenzyme M reductase of Methanogens (Martin and Macy 1985). Chang *et al*. (2003) showed that the addition of BES reduced removal rate of PAHs but did not stop it. Similarly Chang *et al*. (2006) showed that the introduction of BES reduced drastically the removal rate of phenanthrene and naphthalene and hence concluded: "*methanogenic metabolism is coupled to anaerobic PAH degradation. The inhibited degradation, the ceased methane production, and the vanished methanogenic populations provided evidence for this conclusion"*.

However it is hard to guaranty the specificity of a given inhibitor and to ensure that the inhibition of an actor of a trophic chain does not affect other parts, even in the upstream. Indeed, inhibition of the Methanogens could induce accumulation of acetate and hydrogen, this latter inducing inhibition of the upper part of the metabolic pathway (Xu *et al*. 2010, Wang *et al*. 2009, Ahring *et al*. 1995). For example, Chiu and Lee (2001) have shown that BES addition on culture's ability to reductively dechlorinate compounds can also inhibit non-methanogenic microorganisms. Moreover, it was found that BES can inhibit non-Methanogens that reductively dechlorinate polychlorinated biphenyls (PCBs) (Ye *et al*. 1999) and chlorinated ethens (Löffler *et al*. 1997). Ye *et al.* (1999) reported that BES may compete with PCBs for electrons, since the sulfonate moiety of BES can serve as an alternative electron acceptor for sulfate-reducing bacteria, which were presumably responsible for PCB dechlorination.

As co-metabolism is clearly a question of electron flows, one should question carefully the effect of substance able to affect electron flows by representing a new electron donor or acceptor. However, such perturbation is likely unavoidable. Based on this consideration, we

propose to evaluate the removal rate of PAHs and NP of methanogenic consortia when exposed to different inhibition sources. VFAs are known to inhibit methanogenesis without perturbing the upstream processes of hydrolysis and acidification, at least in the first stage of VFA accumulation (Ryan *et al*. 2010). Consequently we chose to substitute a part of the incoming COD by a high enough amount of acetic acid to stop the methanogenesis. Selenium oxyanion is another way to inhibit methanogenesis, it is reported that it directly inhibits hydrogenotrophic and acetoclastic Methanogens (Lenz *et al.* 2008). Selenium oxyanions can influence fermentation and methanogenesis either directly via inhibition of the microbial groups involved, or indirectly by altering the electron flow in the food web, as selenium reducers use hydrogen (Chung *et al*. 2006) and acetate (Macy and Lawson 1993) as electron donors for selenium reduction.

4.2. Materials and Methods

4.2.1. Chemicals

All solvents were purchased from J.T.Baker. 13 Polycyclic Aromatic Hydrocarbons (PAHs) and the Nonylphenol (NP) were measured during this experiment. Fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3,c,d)pyrene powders were obtained from Dr Ehrenstorfer GmbH. Each PAH was dissolved in dichloromethane at 1 $g.L^{-1}$. The pure 4-nonylphenol (NP) isomer mixture was purchased from Interchim. It was diluted in hexane to obtain 40 $g.L^{-1}$. The spiking mix was prepared from these individual concentrated solutions, adding 5 mL of each, then evaporating solvent under gentle nitrogen flow and finally dissolving in 50 mL of acetonitrile. Final concentrations were 100 $mg.L^{-1}$ for each PAH, except for indeno(1,2,3,c,d)pyrene (20 $mg.L^{-1}$) and nonylphenol (2$g.L^{-1}$).

Standard solutions were used to calibrate the HPLC analytical chain. The 10 $mg.L^{-1}$ standard solution of PAHs in acetonitrile and 100 $mg.L^{-1}$ of NP in hexane were provided by Dr Ehrenstorfer GmbH. Standards were stored at –20 °C. Standard solutions were diluted to obtain 6 calibration levels from 10 to 1000 $\mu g.L^{-1}$ of PAHs in acetonitrile and from 500 to 5000 $\mu g.L^{-1}$ of NP in hexane.

4.2.2. Sludge source

The experiments were carried out using secondary sludge sampled in the biological aerobic unit of a domestic plant treating 250 000 PE. Retention time in the aerated tank was 0.4 day. Secondary sludge was frozen at -20°C for storage. After defrosting, the sludge contained 52.8 ± 1.1 $g_{COD}.L^{-1}$ and 41.8 ± 1.5 $g.L^{-1}$ of dry matter (DM). The samples were diluted with deionized water to reach 24 ± 5 $g_{COD}.L^{-1}$ and spiked at 5 $\mu g.g_{DM}^{-1}$ for each PAH except for indeno(1,2,3,c,d)pyrene (1 $\mu g.g_{DM}^{-1}$) and for NP (100 $\mu g.g_{DM}^{-1}$).

4.2.3. Experimental setup

Experiments were performed using three CSTR lab-scale reactors. Similar and constant feed conditions were imposed to all of them, a hydraulic retention time of 20 days and a constant organic load of 1.2 ± 0.2 $g_{COD}.L^{-1}.d^{-1}$. Temperature was regulated at 35°C using hot water circulation in the external jacket, pH was monitored but not regulated. The reactors were fed with the spiked secondary sludge. First, a control reactor (*Rco*) with a working volume of 5L was inoculated with an adapted-to-micropollutant anaerobic sludge. After 90 days at steady-state, it was stopped and the digested sludge was used to inoculate two reactors (*Raa* and *Rss*) with a working volume of 2L each. The feed of the *Raa* reactor was composed of the same secondary sludge (20.2 ± 0.4 $g_{COD}.L^{-1}$) and acetic acid at a concentration of 3.0 ± 0.3 $g.L^{-1}$ (i.e. 3.21 ± 0.32 $g_{COD}.L^{-1}$). The feed of the *Rss* reactor was composed of the secondary sludge complemented by sodium selenite (1.7 ± 0.1 $g.L^{-1}$). The pH and the biogas volumetric production were monitored on line. Seven days composite samples were taken once a week from the feed tank and the outlet tank that were stored at 4°C. The gaseous phase was sampled in the reactor twice a week.

4.2.4. Analytical methods

The composite samples were analyzed. The chemical oxygen demand in total and soluble fraction (COD, $g_{O2}.L^{-1}$) were determined thanks to Merck Spectroquant kits, in accordance with the ISO 15 705. The samples were diluted to be in the range 150 – 1 500 $mg_{COD}.L^{-1}$. The dry matter (DM, $g_{DM}.L^{-1}$) in total and soluble fraction were measured by weighing the sample after heating at 105°C during 24 h. The volatile fatty acids (VFAs): acetate, propionate, iso-

butyrate, butyrate, iso-valerate and valerate concentrations were determined in soluble phase by gas chromatography (GC800, Fisons Instruments). The percentage of methane (CH_4) and carbon dioxide (CO_2) in the biogas were measured 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 CR-8A). A fraction of each sample was freeze-dried and ground in order to quantify the micropollutants. Extraction from inlet and outlet sludge samples and quantification in all extracts were performed according to Trably *et al*. (2004) and Patureau *et al*. (2008).

4.2.5. Calculation of the apparent first-order degradation rate

The dynamic of the degradation rate of the micropollutant is difficult to capture as feed conditions vary always. Hence instead of following the concentrations or an instantaneous removal degradation ratio, we propose to determine the dynamic of an apparent degradation rate. Let us assume the dynamic of the micropollutant follows a first-order degradation kinetic (equation 4.1).

$$\frac{dC_t}{dt} = D(C_t^{in} - C_t) - k_{obs}C_t \qquad (4.1)$$

where D is the dilution rate (d^{-1}), C_t is the inlet and outlet total micropollutant concentration ($\mu g.L^{-1}$) and k_{obs} is the apparent first-order coefficient kinetic (d^{-1}).

If we integrate the former relation between t-T and t, we have the equation (4.2).

$$C_t(t) - C_t(t-T) = \int_{t-T}^{t} D(C_t^{in} - C_t)dt - k_{obs}\int_{t-T}^{t} C_t dt \qquad (4.2)$$

If we define the terms $u(t)$ and $v(t)$ as

$$\begin{cases} u(t) = C_t(t) - C_t(t-T) - \int_{t-T}^{t} D(C_t^{in} - C_t)dt \\ v(t) = \int_{t-T}^{t} C_t dt \end{cases} \qquad (4.3)$$

Hence we can follow the evolution of the apparent k_{obs} over the experiment by calculating the ratio *u/v*. This ratio is independent of fluctuations in the feed or in the dilution rate.

4.3. Result and discussion

4.3.1. Control reactor (Rco)

The control reactor (*Rco*) was run for 5 retention times at steady state. *Rco* maintained favorable methanogenic condition without VFAs accumulation (figure 4.1*a-e*). Removal rates of COD and dry matter were higher than 70% (Table 4.1). The methane content in biogas was about 70%.

Figure 4.2(*a-d*) shows the behavior of some micropollutants in *Rco* reactor. In general, the overall removal of PAHs and NP was about 69 ± 9% (table 4.1). Beside, low molecular weight PAHs (fluorene to pyrene) are more efficiently removed (81 ± 7%) in contrast of high molecular weight PAHs (62 ± 5%) as shown in figure 4.3. This is in accordance with results of Chang *et al*. (2003) and Barret *et al*. (2010a) that have shown that high molecular weight PAHs are less efficiently removed. Moreover, as already shown by Trably *et al*. (2003), the PAH average removal are closely related to the dry matter removal (table 4.1).

Table 4.1. Anaerobic performances of *Rco, Raa* and *Rss* reactors. Average and standard deviation calculated from 5 measurements.

Reactor		*Removal COD (%)*	*Removal DM (%)*	*Removal VFAs (%)*	*pH*	*Methane content (%)*	*$mLCH_4.d^{-1}$*	*% average removal micropollutants (13 PAHs + NP)*
Rco		70.9 ±10.4	73.3 ±6.3	99.8 ±0.2	7.18 ±0.15	73.2 ±1.7	800.7 ±23.7	69.2 ±8.5
Raa	*Period 1*	56.2 ±6.5	62.3 ±4.9	7.2 ±16.5	5.28 ±0.23	21.5 ±4.3	8.3 ±7.8	65.4 ±10.1
	Period 2	36.2 ±0.8	28.8 ±8.1	-3.9 ±11.5	5.26 ±0.20	26.3 ±16.5	8.2 ±9.3	41.4 ±15.1
	Period 3	64.9 ±3.2	57.4 ±7.2	73.1 ±11.8	7.01 ±0.17	68.3 ±6.9	423.7 ±173.2	61.6 ±14.1
Rss		33.6 ±8.1	17.6 ±21.0	-17.1 ±43.1	7.84 ±0.18	7.3 ±5.1	6.3 ±5.7	41.0 ±12.2

In the case of NP, the removal was 68 ± 6% which is also related to the dry matter removal as shown by Patureau *et al*. (2008). Indeed, Barret *et al*. (2010a) have shown NP removal between 33 to 62% during continuous anaerobic digestion with five different feed sludge samples. In contrast, other studies have shown that NP removal varies from 0% in continuous mode (Hernandez-Raquet *et al*. 2007) to 40% in batch mode (Chang *et al*. 2005).

4.3.2. Inhibition with high acetate concentration (Raa)

The *Rco* content was divided into two reactors of 2L. Acetic acid was added to the reactor to reach immediately a concentration of 3g.L^{-1} and then the same concentration was maintained in the feed. The addition of acetic acid implied a rapid decrease of the pH (5.7 ± 0.1) which was expected to stop the methanogenic activity. Indeed, the methane production dropped quickly and VFAs started to accumulate, the activity of methanogenic microorganisms became repressed. However, total VFAs accumulation (4.62 $g_{COD}.L^{-1}$) was evidenced on day 14 of acid period (figure 4.1*i*). Indeed, it is well known that, before being degraded to methane gas, all VFAs are first degraded to acetic acid. As a consequence, in *Raa* reactor, the acetic acid addition implied the accumulation of propionate (0.59 $g_{COD}.L^{-1}$), butyrate (0.66 $g_{COD}.L^{-1}$) and valerate (0.43 $g_{COD}.L^{-1}$) in the first weeks of the process. Likewise, soluble COD accumulation appeared on day 7 (figure 4.1*g*); this represents mostly the VFA concentrations.

Additionally, in the first 28 days of process (period 1, figure 4.1*f-j*), *Raa* is in complete methanogenic inhibition without methane gas production and low pH (5.3 ± 0.2); the micropollutants removal (PAHs and NP) seemed to be not affected. In this period 1, micropollutant removals were 79 ± 7%, 57 ± 4% and 60 ± 4% for low molecular weight PAHs (fluorene to pyrene), high molecular weight PAH and NP, respectively. These removal values present slight statistical differences with the control reactor (*Rco*) one, despite the decrease of the micropollutant load on *Raa* reactor, due to the dilution of the feed by the acid addition (figure 4.3). A possible reason for such observation is that the micropollutant biotransformation is supported by the upper anaerobic pathway (hydrolysis, acetogenesis and acidogenesis).

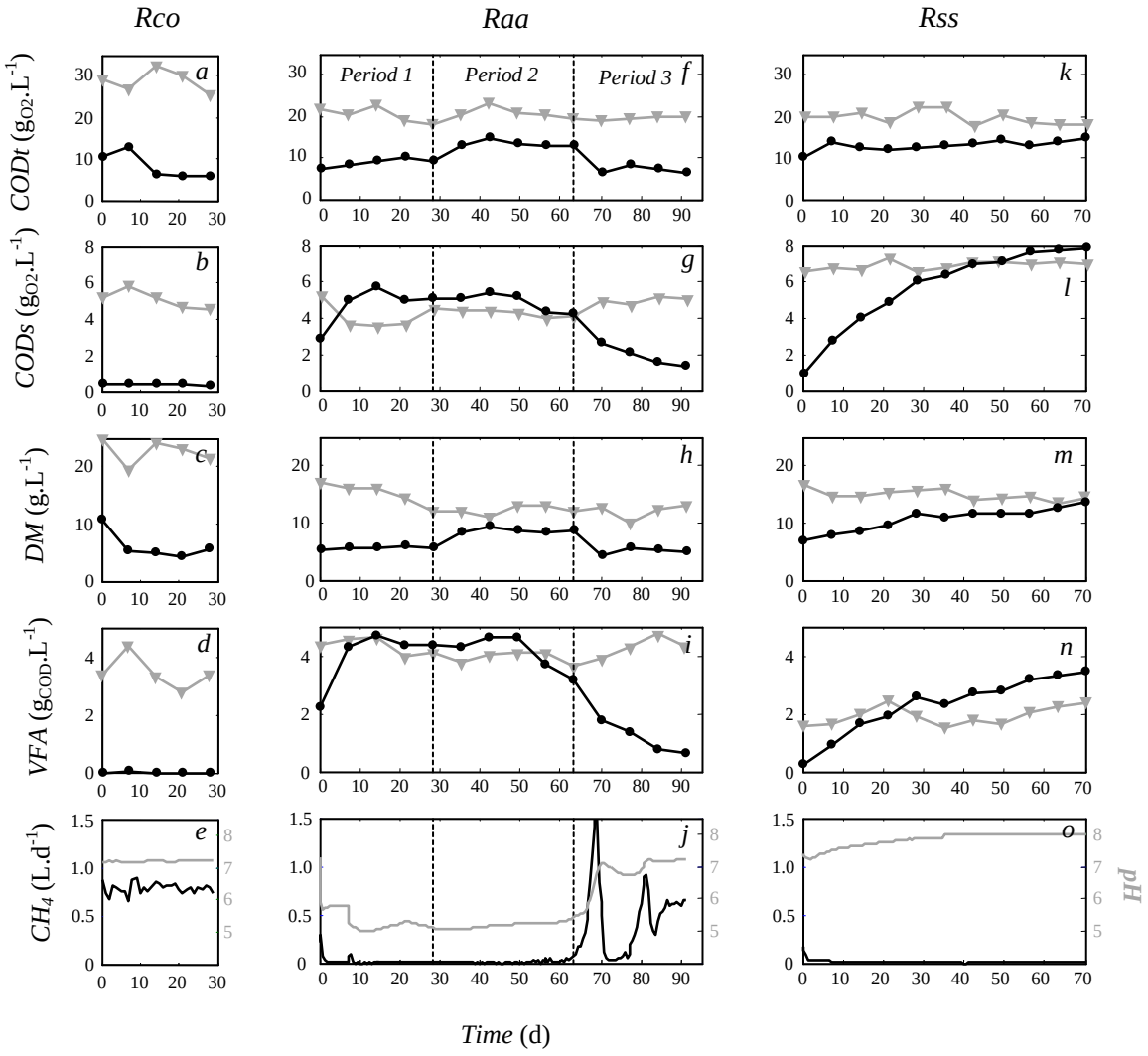

Figure 4.1. Anaerobic performance of *Rco*, *Raa* and *Rss* reactors. Inlet (gray triangle-down), oulet (back circle), methane production (black solid line) and pH (gray solid line).

However, the low pH value in *Raa* can also influence the PAHs and the NP degradations by two others actions: (i) a direct action with respect to molecular dissociation, especially for NP and (ii) an indirect action relative to the modification of the organic matter combined to the modification of the sorption and thus the bioavailable fraction of micropollutant. Indeed, NP has a covalent bond between hydrophobic alkyl chain and hydrophilic phenol share and high acid dissociation constant (pKa = 10.7). However, the observed pH variation from 7 to 5 by comparison to the pKa, may not influence the decomposition of NP, implying no influence on

its removal. PAHs are neither influenced by variation of pH due to their stable molecular structure.

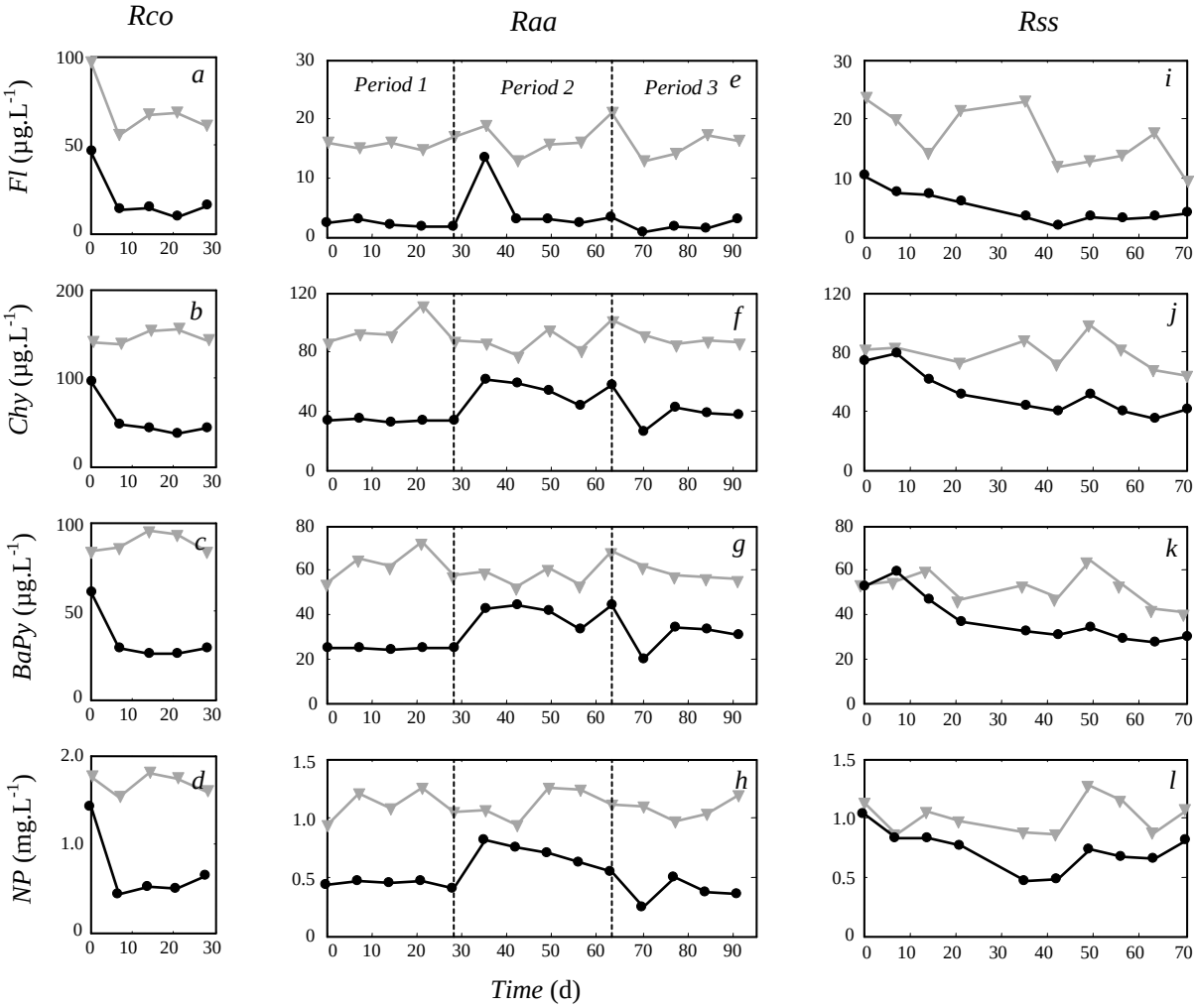

Figure 4.2. Micropollutant performance in *Rco*, *Raa* and *Rss* reactors. Inlet (gray triangle-down), oulet (back circle), Fluorene (Fl), Chrysene (Chy), Benzo(a)pyrene (BaPy) and Nonylphenol (NP).

The pH variation could also affect the physicochemical sludge structure. However, Chen *et al*. (2007) have investigated the effect of pH on the hydrolysis and acidification of waste activated sludge and their observation showed that acid pH (from 5 to 7) and neutral pH slightly differed in soluble COD (including VFAs) and presented smaller differences in

soluble proteins and carbohydrates. Thus, it can be concluded that in our experiments the pH did not influence significantly the sludge characteristics and thus the micropollutant removal.

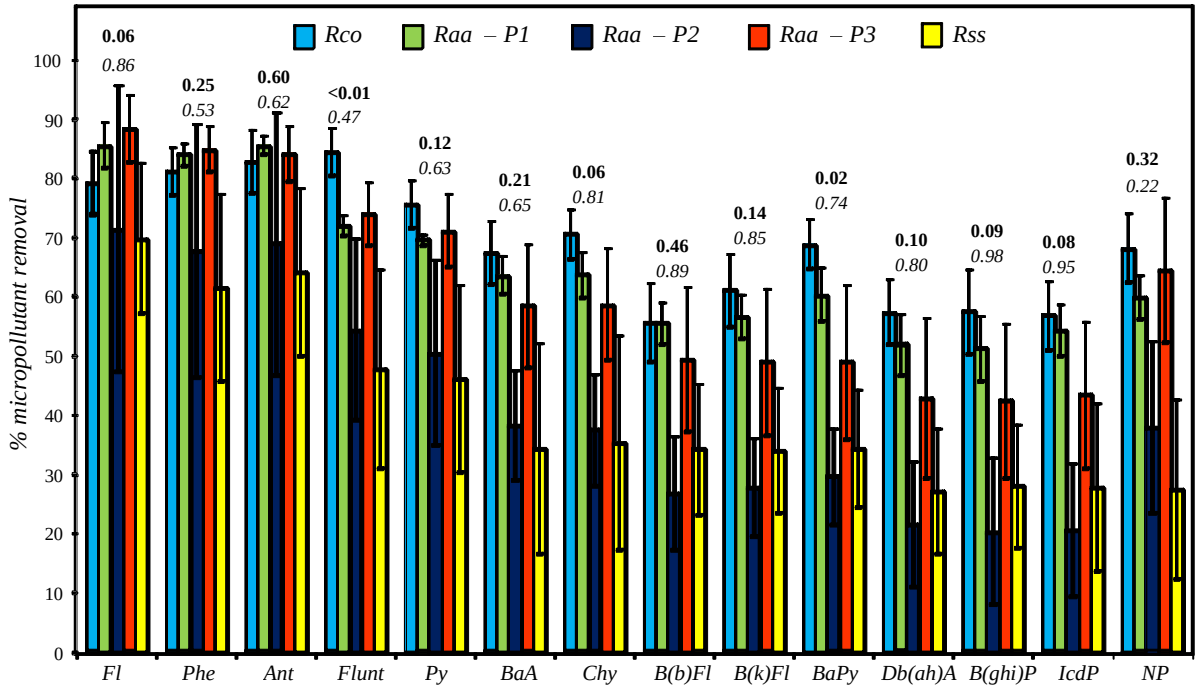

Figure 4.3. Comparison of the micropollutant removals in *Rco*, *Raa* (periods 1, 2 and 3) and *Rss* reactors. For each compound, the *p*-value between 5 sets of measurement is given. Removal can be considered as statistically different for *p*-values below 0.05. Two groups were distinguished. ***p*-values**: group of good methanogenic performances (*Rco*, period 3 of *Raa*) with complete methanogenic inhibition (period 1 of *Raa*) and *p-values*: group of complete methanogenic inhibition and hydrolysis changes (period 2 of *Raa* and *Rss*).

On day 28 (period 2, figure 4.1*f-j*) the *Raa* feed was made with a different stock of the same secondary sludge sample. The effect of this new feed can be seen on day 35 through hydrolysis phenomenon modification: COD and DM removals fall down sharply (figure 4.1*g-h*). This period 2 (from day 28 to day 70) presented significant variation on micropollutant removals (period 2, figure 4.2*e-h* and figure 4.3). Indeed, micropollutant removals were 63 ± 8%, 28 ± 6% and 38 ± 14% for low molecular weight PAHs (fluorene to pyrene), high molecular weight PAHs and NP, respectively. The drop in removal values increases when hydrophobicity increases (figure 4.3), e.g. the low molecular weight compounds (fluorene, phenanthrene and anthracene) outlet concentration only increase on day 35, whereas the high

molecular weight compounds presented an accumulation during the period (period 2, figure 4.2*e-h*). Indeed, a decrease in the hydrolysis rate may cause a decrease in the micropollutant desorption rate and thus a decrease in the amount of micropollutant available for biological activity. Indeed, modification of sludge composition affects the overall metabolism and thus the micropollutant bioavailability and its co-metabolism. This is more evident for compounds with high hydrophobic character. These findings are in accordance with Barret *et al*. (2010b). It is worth noting that during period 2 the methanogenic inhibition condition still persists. Then, these results suggest that the removal of micropollutants could be related to hydrolysis phenomenon.

On day 70, the *Raa* reactor (period 3, figure 4.1*f-j*) started to recover the methanogenic activity, despite the acid presence. The VFA concentration already decreased since day 56, and reached low level of acetate (0.4 $g.L^{-1}$) on day 91. Between day 70 and 91, the process recovered a neutral pH and the methane production rose. This is probably due to biomass acclimation to the acid load. Besides, hydrolysis phenomenon recovered the removal values of period 1 in terms of COD and DM removals (table 4.1). Similarly, the micropollutant removal varied with hydrolysis behavior (period 3, figure 4.2*e-h*). Indeed, the micropollutant removals were 81 ± 6%, 49 ± 5% and 65 ± 12% for low molecular weight PAHs (fluorene to pyrene), high molecular weight PAHs and NP, respectively.

Apparent first order degradation rates for hydrolysis, COD, VFAs and PAHs were calculated in *Raa* to identify any correlation between these various microbial activities (figure 4.4). The introduction of acetic acid (day 0) and the resulting lower pH induced no variation of the apparent first-order degradation rate for all the micropollutants (period 1, figure 4.4). Hence, a significant micropollutant removal remains despite the total stop of the methanogenic activity (methane production and VFA degradation stop with k_{VFA} equal to 0). The apparent first-order degradation rate for hydrolysis started to decrease at day 14 with a concomitant decrease of the degradation rate of COD to reach low values during the period 2. Such behavior induces with 7 to 14 days delay a sharp decrease in the degradation rate for all the micropollutants but the effect is earlier and stronger for the heaviest one. As both methanogenic and hydrolytic consortia adapted to the environment change, the rest of the experiment is harder to interpret (period 3, figure 4.4). From day 50, VFA degradation restarts until reaching a detectable methane production from day 60. This activity correlates with a restart of the micropollutants removal (the low molecular weight PAHs removals being recovered earlier and higher), implying a possible metabolic link with the Acidogens. From the day 63, hydrolysis rate

increases which correlates also with a further increase of micropollutants removal rate. This tends to show that Methanogens might be involved in micropollutant degradation but that obviously other organisms play a significant role too. For each group, independently of the activity of the other group, a correlation between micropollutant degradation rate and microbial activity can be detected. This tends to "prove" the existence of a PAHs/NP co-metabolic pathway for mainly hydrolytic and Acidogens consortia and to a lower extend for Methanogens consortia.

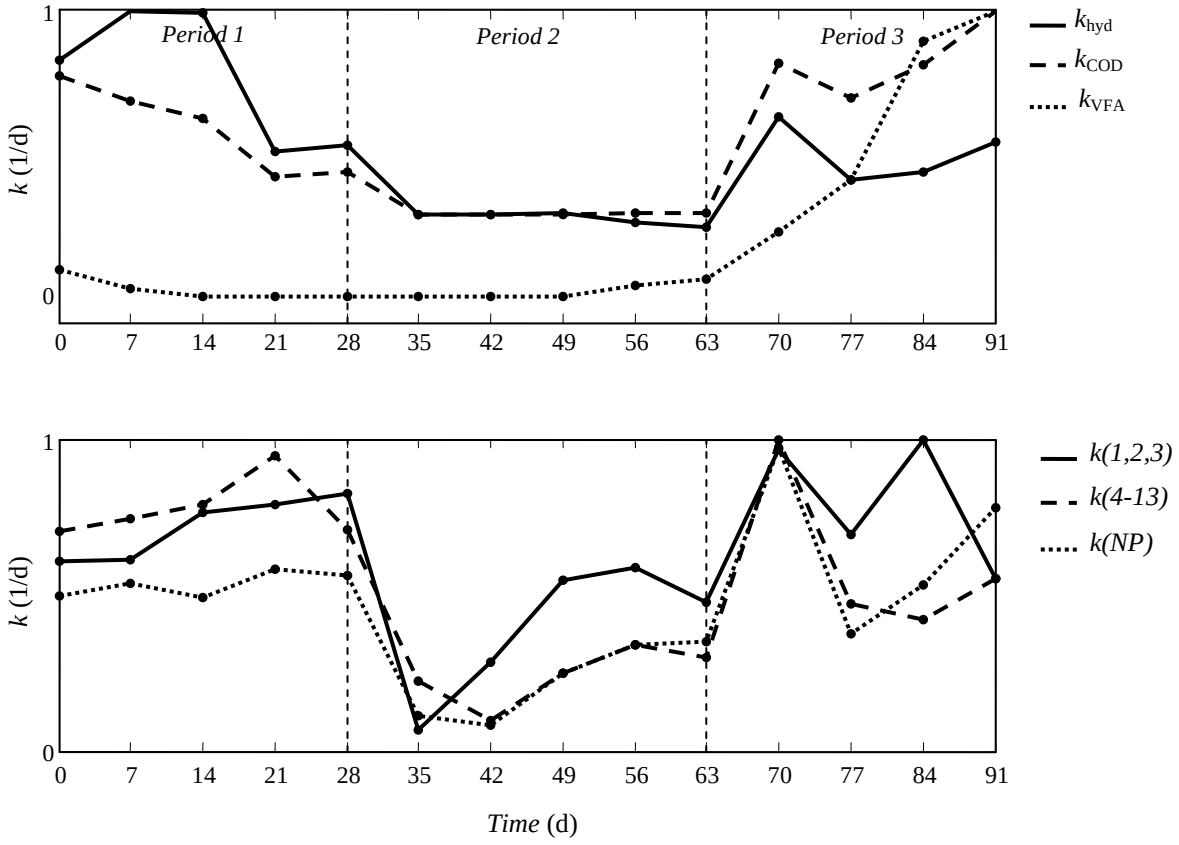

Figure 4.4. The apparent first-order degradation rates in *Raa* reactor. *k(1,2,3)* is the mean of apparent first-order degradation rates for fluorene, phenanthrene, anthracene. *k(4-13)* is the mean of apparent first-order degradation rates for others PAHs and *k(NP)* is the NP first-order apparent degradation rate.

To conclude, in the overall performances of *Raa* reactor, micropollutant behaviors seem to be more correlated to the upper part of the anaerobic pathway (hydrolysis and acidogenesis) than

the methanogenic activity. This could be due to the close link between hydrolysis phenomenon and micropollutant bioavailability, through sorption equilibrium variation. This could also be explained by the direct activation of the overall metabolism (various pathways and versatility of *Bacteria*) that supports the micropollutant co-metabolism.

4.3.3. Inhibition with selenium oxyanion (Rss)

Sodium selenite (1.7 $g.L^{-1}$) was added in the *Rss* reactor and also in the feed. Selenium oxyanions (selenate and selenite) have been utilized as a strong irreversible inhibitor of acetoclastic and hydrogenotrophic Methanogens (Lenz *et al*. 2008). Indeed, methane content in *Rss* reactor is lower than in *Raa* reactor during the inhibition periods (table 4.1). The selenite salt addition produced a sharp decrease in the methane yield in the first day of the inhibition process (figure 4.1*k-o*). In the reactor, toxic soluble selenite is converted into soluble elemental selenium: the formation of a dense red precipitate was visually observed, indicating the precipitation of elemental amorphous selenium, as described by Oreland *et al*. (1989), Fujita *et al*. (2002) and Scheinost and Charlet (2008). During this experiment, VFAs accumulation (acetate, propionate, butyrate and valerate) was observed (figure 4.1*n*) and pH (7.9 ± 0.1) reached alkaline level on days 22 (figure 4.1*o*). Thus, in this condition of complete methanogenic inhibition, micropollutant removals were 58 ± 9%, 32 ± 3% and 28 ± 15% for low molecular weight PAHs (fluorene to pyrene), high molecular weight PAHs and NP, respectively. These removals are statistically different from those of *Rco* but not statistically different from those of *Raa* period 2 (figure 4.3).

It is worth noting that in the inlet (due to salt addition) and outlet (due to selenium precipitation) of *Rss* reactor, the dry matter concentration is significantly higher than that of *Rco* reactor, with a ratio OM to DM of 0.4. It subsequently decreased the micropollutant concentration measured with respect to *Rco* reactor. Nevertheless, the micropollutant removals in *Rss* reactor compared with *Rco* one (table 4.1 and figure 4.3), may suggest that methanogenic populations is involved in micropollutant degradation, as reported by Chang *et al.* (2003 and 2006). However, the inhibition procedure by selenite requires a special attention.

In anaerobic digestion, selenite is reductively transformed into elemental selenium and selenium particles are accumulated inside and outside of the bacterial cells. Fujita *et al.* (2002)

have theoretically expressed the selenium respiration on lactate by some *Bacteria* during anaerobic condition (reaction 4.1). Thus, through inhibition procedure by selenite, the *Bacteria* can grow with sludge as an electron donor and a carbon source while using selenite as electron acceptor. The observed VFAs accumulation (figure 4.1*n*) could confirm this theoretical equation. As a consequence, the new electron flow produced from selenite reduction could affect the co-metabolic micropollutant transformation and decrease their removals.

$$Lactate^- + SeO_3^{2-} + H^+ \rightarrow acetate^- + Se^0 + HCO_3^- + H_2O \quad (4.1)$$

Besides, Viamajala *et al*. (2006) described that the selenium removal can occur under two conditions: (i) selenium precipitates inside cell and/or (ii) selenium forms extracellular precipitates being trapped within the biomass formed in the matrix. These both mechanisms act together and the precipitates retained would modify strongly the matrix structure. Indeed, an increase of dry matter (mainly mineral matter) was observed during the inhibition period caused by the red precipitate (figure 4.1*m*). It should, be mentioned that, in *Rss* reactor, the decrease of micropollutant removals may also be due to a modification of the matrix structure, given that these changes could modify the equilibrium sorption and subsequently the bioavailable fraction of micropollutant.

Hence, the inhibition procedure by selenite involved different aspect: (i) inhibition of methanogenic activity, (ii) probably alteration of micropollutant co-metabolism from a new electron flow and (iii) modification of the bioavailable fraction of micropollutant due to changes of the matrix structure. As a consequence, the observed decrease in micropollutant removal is still subjected to hypothesis.

In order to compare the micropollutant removal performances in each reactor, an analysis of variance was performed for each micropollutant (figure 4.3). For this analysis, the three periods in the *Raa* reactor were considered separately. We considered two groups: the first one grouped the reactors where a methanogenic activity (*Rco* reactor, period 3 of *Raa*) and a complete inhibition of the methanogenic activity (period 1 of *Raa* reactor) were observed; the second one grouped the reactors where complete Methanogens inhibition and hydrolysis changes (period 2 of *Raa* reactor and *Rss* reactor) were observed. For most compounds, removals for each group were statistically similar (figure 4.3), while the removal values

between groups were statistically different (data not shown). This analysis tends to confirm the close link between micropollutant removal and the upper pathway of anaerobic digestion.

In the last decade, several experiments were conducted for better understanding the microbial population involved in hydrophobic micropollutant removal during anaerobic digestion onto sewage sludge. Trably *et al*. (2003) observed the PAH removals in adapted ecosystems (long term PAHs contaminated sludge) and it was shown the PAH removal efficiency correlated well with the methanogenic activity and the biogas yield. Besides, in order to identify specifically the microorganisms potentially involved in micropollutant removal, inhibition procedures were performed from adapted ecosystems (Chang *et al*. 2003, 2005 and 2006). Chang *et al*. (2003 and 2006) reported that the addition of microbial inhibitor (bromoethanesulfonic acid - BES) delayed the PAH degradations, indicating that Methanogens were implied in their degradation in sludge. Similar results were shown for NP in sludge (Chang *et al*. 2005). These results suggested that archaeal Methanogens are very specific of micropollutant removal either by being less inhibited by their presence or, more likely, by favoring the local conditions through specific syntrophic relationships with bacterial degraders. However, in both cases, the microbial inhibitor produced an incomplete inhibition of the hydrophobic micropollutant degradation, this should indicate the presence of other microorganisms involved in their degradation. Additionally, it was found that BES can inhibit also non-methanogenic microorganisms (Chiu and Lee, 2001). This study showed that the BES, used as a methanogen-specific inhibitor, would indeed affect indirectly the *Bacteria* which consume and/or produce substrates for methanogenesis, such as H_2 or acetate. In fact, in the inhibition procedure by BES, the decrease in the micropollutant removal could be due to a partial inhibition of non-methanogenic population. Likely, this may also occur with the selenite addition. As a consequence, data from studies using BES, as well as selenium oxyanions, presented as Methanogen-specific inhibitors should be interpreted with care.

Moreover, Dolfing *et al*. (2009) have evaluated the thermodynamic constraints on methanogenic PAH degradation. They showed that the methanogenic degradation of five PAHs is thermodynamically feasible and suggested that PAH degraders may have evolved towards incomplete oxidation to acetate plus H_2 as the optimal pathway. If acetate is the sole product, methanogenic activity would not be necessary to sustain PAH degradation. This idea supports well our findings that the upper part of the anaerobic pathway is implied in the PAH degradation. But if PAH degradation proceeds via H_2 production, H_2 removal is a prerequisite

to sustain PAH degradation, which implies syntrophy with hydrogenotrophic Methanogens or Homoacetogens.

Moreover, Wang *et al.* (2011), studying the microbial community and PAH biodegradation in sediments with a spiked PAH mixture (fluorene, phenanthrene, fluoranthrene and pyrene), demonstrated that a significant decrease of PAH concentration was achieved in parallel with an increase of fatty acid degraders but a decrease in species diversity. In fact, Ryan *et al*. (2010) have indicated that, during anaerobic digestion, the acetogenic bacteria are capable of a highly diverse range of metabolic transformations: (i) the use of chemolithoautotrophic substrates (H_2/CO_2 or CO/CO_2) as sole sources of carbon and energy, (ii) heterotrophic conversion of certain sugars stoichiometrically to acetate, and (iii) the ability to oxidize methoxylated aromatic compounds via the acetyl-CoA pathway. These three metabolic capabilities are not present in all acetogenic bacteria; however, in micropollutant-adapted ecosystem these capabilities could be present.

4.4. Conclusions

Anaerobic digestion can be considered as a potential biological process to remove hydrophobic micropollutant at low concentration. The thirteen PAHs and NP were degraded by anaerobic populations with different methanogenic inhibition strategies. The results showed that the micropollutant degradation is not coupled only to Methanogens activity. Micropollutant removal values were compared between good methanogenic conditions (*Rco* reactor) and complete methanogenic inhibition periods (*Raa* reactor). These micropollutant removals were statistically similar and it provided evidence for this conclusion: non-methanogenic communities are involved in the micropollutant degradation, perhaps the bacterial populations responsible for acetate and/or H_2 production. Moreover, hydrophobic micropollutant degradation is also affected by hydrolysis changes. Indeed, a decreased in the compounds removals was observed given changes in the sludge source (period 2, *Raa* reactor) and matrix structure (*Rss* reactor). These physico-chemical changes affect either the sorption equilibrium and subsequently the bioavailability or the overall metabolic pathways and subsequently the co-metabolism. Future researches needed to confirm the role of hydrolytic, Acidogens and Methanogens consortia for anaerobic hydrophobic micropollutant degradation.

4.5. General Discussion

This chapter suggests that **the anaerobic degradation of micropollutants mainly involves non-methanogenic microorganisms, which means that it is linked to the upper pathway of the digestion**.

The inhibition procedures applied could produce different levels of co-metabolism and available fraction of micropollutant, due to the imbalance produced. These processes can favor the growth of certain organisms and the production of certain substrates that could be involved in the co-metabolic micropollutant transformation. In particular, the addition of acid acetic (*Raa*) produces an increased in soluble COD, mainly due to the VFAs accumulation. But, the other part of this soluble COD may be due to the hydrolysis of particulate matter and the production of colloidal matter or to other soluble compounds due to physicochemical changes (pH drop). This presence of colloidal/soluble compartment may favor the presence of the micropollutant in a more bioavailable fraction and thus maintain the removal activity whereas the Methanogens are fully inhibited.

On the other hand, the results from inhibition procedure by selenite were shown to be hard to interpret. The selenite reduction into elemental selenium could alter the electron flows in the system and subsequently could affect the co-metabolic micropollutant transformation. Moreover, selenium particles accumulate inside and outside bacterial cells, this fact produce an increase of the mineral matter that could trap the hydrophobic micropollutant within nano and micropores in solids containing little organic matter (Semple *et al*. 2003) and obviously modify the micropollutant availablility.

The findings shown in this chapter suggest the existence of a micropollutant co-metabolism for hydrolysis and Acidogens and to a lesser extend for Methanogens consortia. Thus, these co-metabolisms could be evaluated independently in a model that involved the synthropic relationships between each step for organic matter conversion to methane.

5. Anaerobic digestion steps involved in micropollutant removal: the model part

The chapter 4 proposed a dynamic model with physical exchanges of Polycyclic Aromatic Hydrocarbons (PAHs) between four compartments during anaerobic digestion. The model is based on the assumption that PAH sorption occurs on two different phases: particulate matter (S_p) and dissolved/colloidal matter (DCM) (Barret *et al*. 2010c). The sensitivity analysis of the model demonstrated that sorption kinetics is not the rate-limiting step of the hydrophobic micropollutant removal and suggested that the equilibrium state is sufficient to represent the sorption phenomenon. Besides, the PAH biodegradation is considered to depend on a bioavailable compartment and to proceed via co-metabolism (figure 5.1a). This latter was represented by an adaptation of the Criddle (1993) equation, choosing the soluble substrate (S_S) as the co-metabolite. The PAH biodegradation rate was satisfyingly modeled when considering the aqueous fraction (including free and sorbed-to-DCM of PAHs) as the bioavailable compartment.

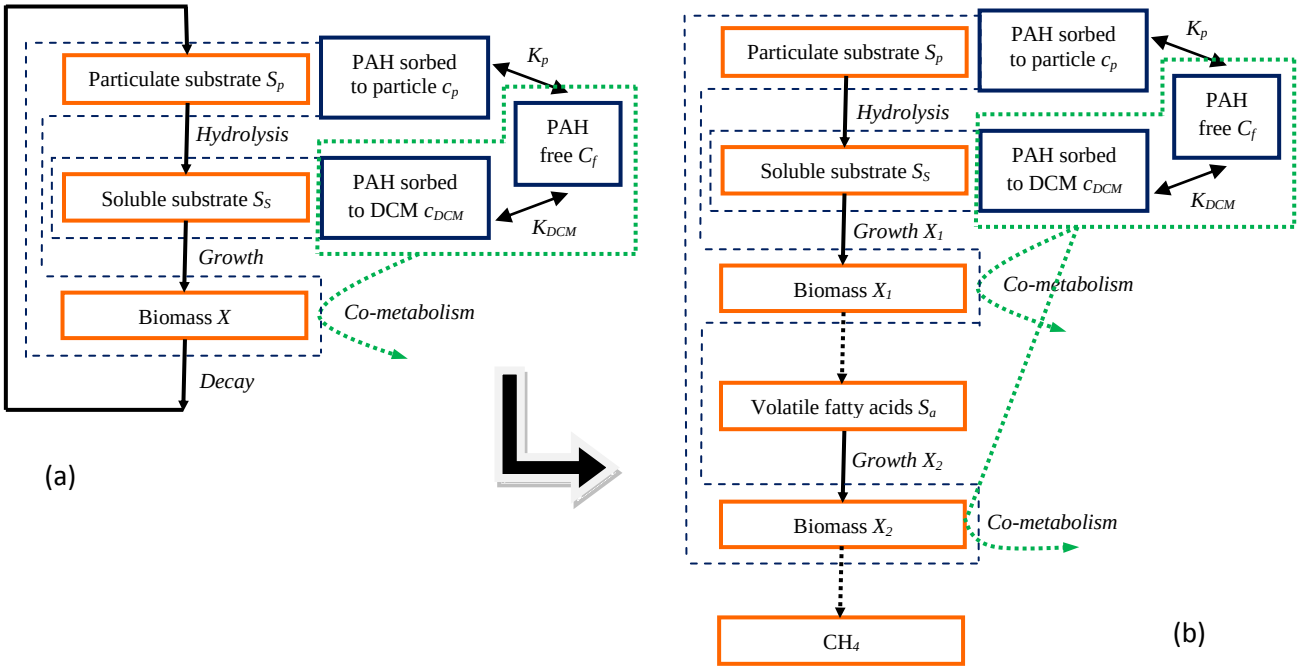

Figure 5.1. Representation of the PAH models (a) Simple approach model (chapter 3), (b) Extended advanced model (chapter 5).

This simple approach involves one biomass that represents the overall anaerobic community responsible for both utilization of soluble substrate and micropollutant transformation by co-

metabolism (figure 5.1a). However, anaerobic digestion of sludge is a complex process that involves syntrophic relationship in which Bacteria and Archaea are juxtaposed, facilitating interspecies transfer of substrates necessary for the complete methanogenesis of the organic matter. The previous experimental part (chapter 4) suggested that hydrophobic micropollutant degradation is closely linked to the upper part of the anaerobic pathway, with a highest implication of the Acidogens versus the Methanogens. In this sense, an advanced model with the two main populations of microorganism (Bacteria and Archaea) and intermediate by-products (as volatile fatty acids – VFAs) is now proposed. This new approach allows the evaluation of micropollutant co-metabolic transformation due to each microorganism community and the determination of new parameter sets (figure 5.1b).

Advanced model of hydrophobic micropollutant fate in anaerobic sludge digestion

Abstract

Anaerobic degradation has been considered as a putative treatment to remediate hydrophobic micropollutant, such as Polycyclic Aromatic Hydrocarbons (PAHs) present in sewage sludge. The aim of this work is to develop a model, which links the PAH co-metabolic transformation with two different microbial communities (Bacteria and Archaea) involved in the anaerobic degradation of sewage sludge. The model was calibrated with data sets from two continuous reactors, fed with adapted-to-micropollutant sludge, in which the degradation of 13 PAHs was observed under mesophilic conditions. The yield coefficients and model parameters were optimized with a mathematical method proposed in the literature and a non-linear least square method, respectively. The results show that overall biomass is involved in the PAH removal, but the PAH acidogenic rate is higher compared to the PAH methanogenic one. This modeling approach might direct further efforts to understand and optimize the PAH biodegradation pathway under anaerobic condition.

5.1. Introduction

Anaerobic digestion of sludge is a biological process in which bacteria transform organic matter in absence of the oxygen to generate renewable energy in form of methane. Currently, anaerobic sludge digestion has been proven a potential treatment for micropollutant depletion, in particular, Polycyclic Aromatic Hydrocarbons, PAHs (Barret *et al*. 2010a, Chang *et al*. 2003, Trably *et al*. 2003). Hence, PAHs are regarded as priority pollutants by environmental and health agencies because of their toxic, mutagenic and carcinogenic effects on living organisms (Samanta *et al*. 2002). The fate and distribution of PAHs in anaerobic digested sludge depend of their physicochemical properties and also the sludge ones (Barret *et al*. 2010b and 2010c, Dionisi *et al*. 2008). Indeed, the hydrophobic character of these compounds favors their sorption onto sludge. In fact, Kordel *et al*. (1997), Dionisi *et al*. (2006) and Barret *et al*. (2010b) have demonstrated that sorption mechanism of PAHs is faster compared to anaerobic process.

Recently, we developed a dynamic model with a four-compartment distribution (sorption to particle, sorption to dissolved-colloidal matter, free dissolved and gaseous compartments) to describe the PAHs fate during anaerobic digestion of sludge (Delgadillo-Mirquez *et al*. 2011). The model includes abiotic and biotic processes such as volatilization, sorption and micropollutant biodegradation by co-metabolism. This model used one biomass to couple the consumption of growth substrate and the PAH co-metabolic transformation. Thus, the simulation carried out showed that sorption kinetics of these hydrophobic compounds is not the rate-limiting step and the equilibrium is sufficient to represent the sorption mechanism. It was also demonstrated that the co-metabolism is the main micropollutant biodegradation mechanism and the aqueous phase (sum of dissolved-colloidal matter and free dissolved compartments) is the bioavailable fraction. Similarly, Barret *et al*. (2010a) experimentally validated the co-metabolism and aqueous available fraction in the anaerobic removal of 13 PAHs and different sludge.

However, anaerobic digestion is a complex process that involves several microbial populations in the organic matter transformation and they or one part of they could be responsible for PAH degradation. Besides, the specific microbial consortium involved in hydrophobic micropollutant depletion during anaerobic digestion is still a crucial research. Nevertheless, it was shown that methanogenic microorganisms were essential to effectively remove PAH. Chang *et al*. (2003 and 2006) observed that the addition of a selective inhibitor

of Methanogens delayed PAH degradation, indicating that methanogenic populations are involved in these pollutants degradation in sludge. Moreover, Trably *et al*. (2003) observed that PAH removal efficiencies seemed to be closely associated to the methanogenic activity, e.g. biogas yield decreased proportionally with the increased of PAH removal. In contrast, in more recent studies Dolfing *et al*. (2009), evaluating the thermodynamic constraints on methanogenic PAH degradation indicated that the thermodynamic is not an impediment of PAHs biodegradation because it is an exergonic process. This thermodynamic study suggested also PAH degraders may have evolved towards incomplete oxidation to acetate plus H_2 as the optimal pathway under thermodynamic standard conditions. Furthermore, results obtained on anaerobic continuous reactors (Delgadillo-Mirquez *et al*. in preparation) have shown that PAH degradation occurs even if Methanogens are inhibited and suggested that the bacterial populations responsible for acetate and/or H_2 production could be involved mainly in the micropollutant degradation pathway. Likewise, observations in batch experiment (Cea-Barcia *et al*. in preparation) suggested that the PAHs anaerobic removal is linked to the first steps of the anaerobic process.

This study proposes to evaluate the link between the PAH co-metabolic degradation and two different microbial communities involved in the anaerobic process. To this end, the dynamic model developed by Delgadillo-Mirquez *et al*. (2011) is extended by considering two main bacterial populations: the acidogenic bacteria and the methanogenic microorganisms. This modeling approach might direct further efforts to understand and optimize the PAH biodegradation pathway under anaerobic condition.

5.2. Materials and Methods

5.2.1. Chemicals

All solvents were purchased from J.T.Baker. This experiment was conducted on measured for 13 Polycyclic Aromatic Hydrocarbons (PAHs). Fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3,c,d)pyrene powders were obtained from Dr Ehrenstorfer GmbH. Each PAH was dissolved in dichloromethane at 1 $g.L^{-1}$. The spiking mix was prepared from these individual concentrated solutions, adding 5 mL of each, evaporating solvent under gentle nitrogen flow

and dissolving in 50 mL of acetonitrile. Final concentrations were 100 $mg.L^{-1}$ for each PAH, except for indeno(1,2,3,c,d)pyrene (20 $mg.L^{-1}$). The 10 $mg.L^{-1}$ standard solutions of PAHs in acetonitrile were provided by Dr Ehrenstorfer GmbH. The standard solutions were diluted to obtain 6 calibration levels from 10 to 1000 $\mu g.L^{-1}$ of PAHs in acetonitrile. Standards were stored at –20°C.

5.2.2. Experimental setup

Studies were carried out in two laboratory-scale continuous tank reactors, *Rnc* and *Raa*, operated under perfectly mixed conditions with a hydraulic retention time of 20 days. Organic loading rate was slightly oscillating around respectively 1.15 $g_{COD}.L^{-1}.d^{-1}$ and 1 $g_{COD}.L^{-1}.d^{-1}$. Reactors were fed with a secondary sludge sampled in an activated sludge domestic plant treating 250 000 PE. This secondary sludge has been previously frozen at -20°C for storage. After defrosting, the sludge contained 52.8 ± 1.1 $g_{COD}.L^{-1}$ and 41.8 ± 1.5 $g.L^{-1}$ of dry matter (DM). The samples were diluted with deionized water to reach 24 ± 5 $g_{COD}.L^{-1}$ and spiked at 5 $\mu g.g_{DM}^{-1}$ for each PAH except for indeno(1,2,3,c,d)pyrene (1 $\mu g.g_{DM}^{-1}$). Both reactors have been inoculated with anaerobic sludge adapted to micropollutants (PAHs and NP). Biogas volumetric production and pH were monitored on line. Seven day composites samples were taken once a week from the feed tank and the outlet tank for further analysis. The composition of the gaseous phase was analyzed twice a week by direct sampling in the gaseous phase. The feed and outlet were stored at 4°C. Observations are performed on stabilized reactors after 2 to 4 HRT of operation.

The inlet of the reactor *Raa* was diluted with acetic acid keeping the same organic load in order to induce a specific inhibition of the Methanogens.

5.2.3. Analytical methods

Particulate and soluble COD, Volatile Fatty Acids and organic and mineral dry matter of the composite samples have been analyzed. The chemical oxygen demand in total and soluble fraction (COD, $g_{O2}.L^{-1}$) was determined thanks to Merck Spectroquant kits, in accordance with the ISO 15 705. The samples were diluted to adjust their COD within the range 150 – 1500 $mg_{COD}.L^{-1}$. The dry matter (DM, $g_{DM}.L^{-1}$) in total and soluble fraction was measured by

weighing the sample after heating at 105°C during 24 h. The volatile fatty acids (VFA) as acetate, propionate, iso-butyrate, butyrate, iso-valerate and valerate concentrations were determined in soluble phase by gas chromatography (GC800, Fisons Instruments). The biogas composition was measured 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).

A fraction of each sample was freeze-dried and ground in order to quantify the micropollutants. Extraction from inlet and outlet sludge samples and quantification in all extracts were performed according to Trably et al. (2004).

5.2.4. Simulation software

Simulations presented in this work have been developed in MatLab®-Simulink. The optimization toolbox for solving non-linear least square problems has been used to estimate the model parameters.

5.3. Model formulation

Delgadillo-Mirquez *et al.* (2011) developed a dynamic model. A physical equilibrium between four compartments was used to describe the distribution of micropollutants between the particulate phase (c_p, $\mu g.g_{COD\text{-}p}^{-1}$), the dissolved/colloidal phase (c_{DCM}, $\mu g.g_{COD\text{-}DCM}^{-1}$), the free phase ($C_f$, $\mu g.L^{-1}$) and the gas phase. The analysis of the model simulation using data from two different reactors and a sensitivity analysis showed that (i) the low volatility of the studied PAHs allows to neglect the exchanges with the gas phase, (ii) sorption and desorption dynamics are fast compared to dilution rate and biodegradation of micropollutants and (iii) concentration of micropollutant is always at equilibrium between the three compartments (particulate matter, dissolved/colloidal matter and free phase). The micropollutant distribution was described by two equilibrium constants: K_p, the equilibrium constant of PAH sorption to particle ($L.g_{COD\text{-}p}^{-1}$) and K_{DCM}, the equilibrium constant of PAH sorption to DCM ($L.g_{COD\text{-}DCM}^{-1}$)

$$K_p = \frac{c_p}{C_f} \quad (5.1), \qquad K_{DCM} = \frac{c_{DCM}}{C_f} \quad (5.2)$$

These equilibrium constants were determined experimentally using the methodology developed by Barret *et al*. (2010c). However, an empirical and linear relation had also been developed to estimate these constants based on physicochemical characteristics of the micropollutant and the sludge (Barret *et al*. 2010d).

Moreover, the concentrations of a micropollutant respectively adsorbed to particulate and adsorbed to dissolved/colloidal matter are given by $C_p = c_p \cdot S_p$ and $C_{DCM} = c_{DCM} \cdot S_S$. As a consequence, we can refer only to the total concentration C_t and use the following relation to evaluate C_f or C_{DCM}.

$$C_t = C_f + C_P + C_{DCM} \qquad (5.3)$$

$$C_t = (1 + K_p S_p + K_{DCM} S_S) \cdot C_f \qquad (5.4)$$

Besides, a co-metabolism reaction rate (Criddle, 1993) was proposed to describe the biodegradation of micropollutants. Degradation kinetic is relative to the bioavailable fraction C_{bio}, and to the biomass quantity and activity of the organism concerned by the co-metabolism. For instance, for a biomass X_i with a growth-rate μ_i, the biodegradation rate of the micropollutant is described by

$$r_C = (T_c Y_i \mu_i + k_c) \frac{C_{bio}}{K_{SC} + C_{bio}} X_i \qquad (5.5)$$

where T_c is the micropollutant transformation capacity ($\mu g.g_{COD\text{-}Si}^{-1}$) standing for co-metabolic interaction between the soluble substrate metabolism and the micropollutant metabolism, k_c is the maximum specific rate of micropollutant biodegradation in absence of primary substrate ($\mu g.g_{COD\text{-}X}^{-1}.d^{-1}$) and K_{SC} is the half saturation constant of micropollutant in the Monod formalism ($\mu g.L^{-1}$).

On the other hand, the organic matter biodegradation by anaerobic digestion can be described by a three-step model. This is an adaptation of the anaerobic model developed by Bernard *et al*. (2001), where the anaerobic bacterial populations are divided into two main groups of homogeneous characteristics, Acidogens and Methanogens. As the substrate is here mainly composed of particulate matter, it is necessary to add a preliminary step of hydrolysis during

which particulate matter (S_p) is hydrolyzed into soluble substrate (S_S). Then during the acidogenesis stage, the acidogenic bacteria (X_1, $g_{COD}.L^{-1}$) degrade the soluble substrate into volatile fatty acids (S_a, $mmol.L^{-1}$). Next, the population of methanogenic microorganisms (X_2, $g_{COD}.L^{-1}$) uses the volatile fatty acid as growth substrate and produces carbon dioxide and methane (S_M, $g_{COD}.L^{-1}$). Organisms' growth-rates (μ_1 and μ_2) are described respectively by Monod-type and Haldane-type kinetics.

It is important to notice that the model can be separated in two sections: the first one is only describing the anaerobic digestion of the organic matter (S_1); the other one is describing the fate of micropollutants within this specific biochemical environment (S_2). From a model identification point of view, the first section is independent of the second one and hence can be identified regardless of the micropollutants.

$$S_1\begin{cases} \frac{dS_p}{dt} = D(S_p^{in} - S_p) - k_{hyd}S_p \\ \frac{dS_S}{dt} = D(S_S^{in} - S_S) + k_{hyd}S_p - Y_1 r_1 \\ \frac{dS_a}{dt} = D(S_a^{in} - S_a) + Y_2 r_1 - Y_3 r_2 \\ \frac{dS_M}{dt} = Y_4 r_2 - q_M \\ \frac{dX_1}{dt} = -DX_1 + r_1 \\ \frac{dX_2}{dt} = -DX_2 + r_2 \end{cases} \qquad S_2\left\{\frac{dC_t}{dt} = D(C_t^{in} - C_t) - r_C\right.$$

where D is the dilution rate (d^{-1}), k_{hyd} is the first order kinetic of hydrolysis (d^{-1}), q_M is the flow rate of methane production ($g_{COD}.d^{-1}.L^{-1}$), $Y_{1,2,3,4}$ are the yield coefficients and $r_{1,2}$ are the biodegradation rates ($g_{COD}.d^{-1}.L^{-1}$) with $r_1 = \mu_1.X_1$ and $r_2 = \mu_2.X_2$.

5.3.1. Estimation of the yield coefficients

The identification procedure will mainly be based on methods presented in Bernard and Bastin (2005). Therefore, we will not focus on their mathematical justification. The

identification approach relies on identifying separately yield and kinetics parameters with taking care of the identifiability of parameters. S_1 system follows the general form:

$$\frac{d\xi}{dt} = D(\xi_t^{in} - \xi) + Kr(\cdot) - Q(\cdot) \qquad (5.6)$$

where ξ is the vector of state variables, K a matrix of pseudo-stoichiometric coefficients, r the vector of reaction rates and Q the vector of gas flows. Furthermore, we define the terms $u(t)$ and $w(t)$ by

$$u(t) = \xi(t) - \xi(t-T) - \int_{t-T}^{t} D(\xi_t^{in} - \xi)dt + \int_{t-T}^{t} Q(\cdot)dt \qquad (5.7)$$

$$w(t) = \int_{t-T}^{t} r(\cdot)dt \qquad (5.8)$$

According to Bernard and Bastin (2005) and based on the variables really measured in our system, the matrix K corresponding to S_1 is not identifiable. It is hence necessary to reduce the system to variables really observed and to standardize yield parameters as shown:

$$\overline{\xi} = \begin{pmatrix} S_p \\ S_S \\ S_a \\ S_M \end{pmatrix} \qquad \overline{K} = \begin{pmatrix} -1 & 0 & 0 \\ 1 & -1 & 0 \\ 0 & Y_2/Y_1 & -Y_3/Y_4 \\ 0 & 0 & 0 \end{pmatrix} \qquad r = \begin{pmatrix} r_{hyd} \\ r_1/Y_1 \\ r_2/Y_4 \end{pmatrix}$$

It is easy to find a vector belonging to the kernel of $\overline{K}$, such as $\lambda = (\frac{Y_2}{Y_1}\ \frac{Y_2}{Y_1}\ 1\ \frac{Y_3}{Y_4})$. We hence have the property $\lambda \cdot u(t) = \lambda K \cdot w(t) = 0$ which means that the terms Y_2/Y_1 and Y_3/Y_4 can be identified by linear regression. It is worth noticing that at this point, the identification of stoichiometric parameters is independent of the reaction rates which are often the most ill-known parameters.

Moreover from steady-state conditions, we can derive the following conditions:

$$\frac{dS_S}{dt} = 0 \Rightarrow Y_1 = \frac{(S_p^{in} + S_S^{in} - S_p^* - S_S^*)}{X_1^*} \qquad (5.9)$$

$$\frac{dS_a}{dt} = 0 \Rightarrow Y_3 = \frac{(S_a^{in} - S_a^* + k_2 X_1^*)}{X_2^*} \qquad (5.10)$$

At this point, it is obvious that without any estimation of X_1 and X_2, no further identification is possible. A first step is to estimate X_t. Indeed, the particulate substrate and the biomass are both particulate and hence are measured in the same time when measuring organic matter content (*VSS* in $g.L^{-1}$) or the particulate COD (COD_p in $g_{COD}.L^{-1}$). Despite the impossibility to get a direct or indirect measurement of X_t, we can rely on the relation between *VSS* and COD_p to estimate each term.

$$\begin{cases} VSS = \eta S_p + \theta X_t \\ COD_p = S_p + X_t \end{cases} \qquad (5.11)$$

Around a given operating condition and for a reduced amount of time, it is reasonable to assume that the properties of the residual sludge and especially the organic content per gram of COD are stable. Furthermore, a relationship linking mass and COD can be proposed for the biomass based on its putative chemical formulation $C_5H_7O_2N$ (Batstone *et al.* 2002). Let us assume then we have an initial estimation of $\hat{X}_t$. Hence, we can produce a first estimation $\hat{\eta}_t^0$ by linear regression between $VSS - \theta \cdot \hat{X}_t^0$ and $COD_p - \hat{X}_t^0$. From there, we can build a new estimation of X_t. This initiates a recursive computation of $\hat{\eta}_t$ and $\hat{X}_t$ which converges.

$$\hat{\eta}_t^i = \frac{VSS - \theta \cdot \hat{X}_t^i}{COD_p - \hat{X}_t^i} \qquad (5.12)$$

$$\hat{X}_t^{i+1} = \frac{VSS - \hat{\eta}_t^i (COD_p - \hat{X}_t^i)}{\theta} \qquad (5.13)$$

Table 5.1. Identification result for X_t and η

Parameter	***Reactor***	***Value***	***X_t avg g_{COD}/L***
Heta ($g.g_{COD}^{-1}$)	*Rnc*	0.51	1.64
Heta	*Raa*	0.46	1

Furthermore, using an extra assumption on the relative distribution of X_1 and X_2 (Bernard *et al*. (2001) propose a ratio of 20% of acidogenic and 80% of methanogenic microorganisms),

estimations of X_1 and X_2 can be proposed. Hence, on the basis of relations (5.8) and (5.9) determined from steady-state conditions, Y_1 and Y_3 are identified; finally by combining these values with the values estimated for Y_1/Y_2 and Y_3/Y_4, we obtain values of all stoichiometric coefficients. Table 5.2 summarizes estimated parameters for reactors *Rnc* and *Raa*.

Table 5.2. Estimates of the yield coefficients.

		Rnc		*Raa*	
Parameter		***Value***	***Interval***	***Value***	***Interval***
Y_2/Y_1		0.143	-0.24; 0.52	0.266	-0.86; 1.39
Y_3/Y_4		0.56	-0.45; 1.57	0.534	0.395; 0.6745
Y_1	$g_{COD\text{-}Ss}.g_{COD\text{-}X1}^{-1}$	42.1	Std = 2.76	45	Std = 9.8
Y_2	mmol $S_a.g_{COD\text{-}X1}^{-1}$	93.9		187.5	
Y_3	mmol $S_a.g_{COD\text{-}X2}^{-1}$	36.9	Std = 6.51	98.4	Std = 7.81
Y_4	mmol $CH_4.g_{COD\text{-}X2}^{-1}$	65.8		171.8	

5.3.2. Identification of kinetic parameters

Relying on the former identification of S_p within the particulate COD (e.g. separating the particulate COD stemming from the active biomass from the particulate COD stemming from the substrate), it is also possible to estimate k_{hyd} by a linear regression. Indeed, if we integrate between *t-T* and *t* the first differential equation of S_1, we obtain a linear relationship between measured variables:

$$S_p(t) - S_p(t-T) = \int_{t-T}^{t} D(S_p^{in} - S_p)dt - k_{obs}\int_{t-T}^{t} S_p dt \qquad (14)$$

Table 5.3. Kinetic coefficients.

Parameters	***Rnc***	***Raa***
k_{hyd} (day^{-1})	0.1293	0.0834
μ_{max1} (day^{-1})	0.7	0.55
K_{S1} ($g_{COD}.L^{-1}$)	11	8.9
μ_{max2} (day^{-1})	0.85	0.42
K_{S2} ($mmol.L^{-1}$)	38.9	55.1
K_{i2} ($mmol.L^{-1}$)	5.62	11.87

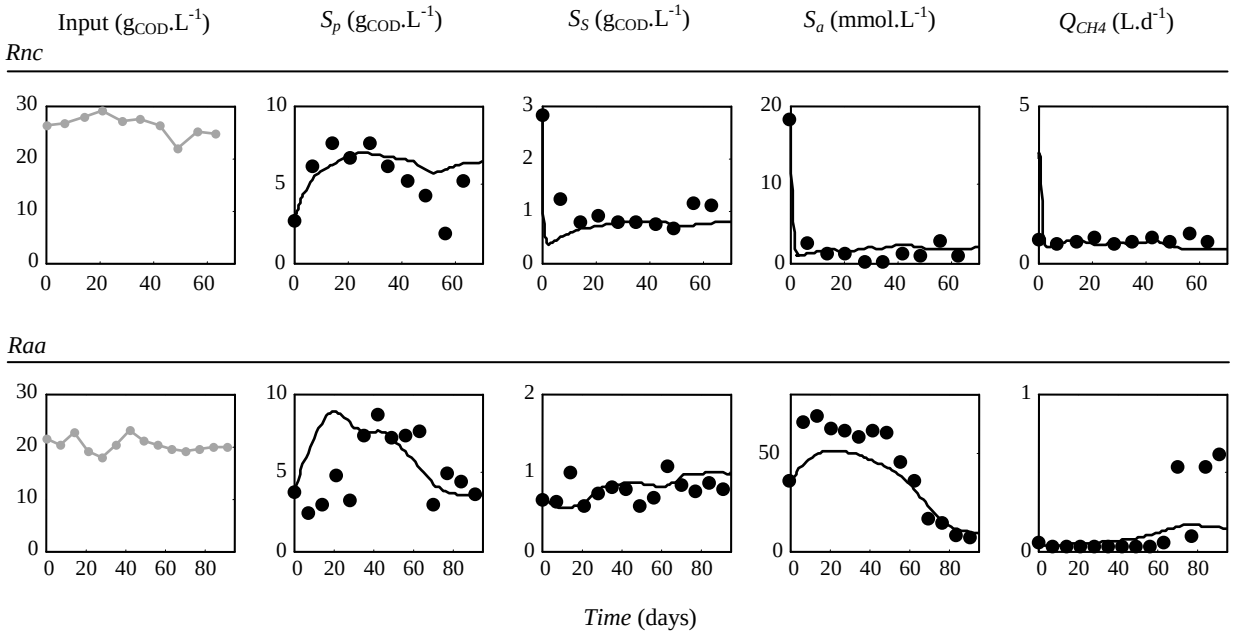

Figure 5.2. Behavior (black points) and model simulation (solid line) of *Rnc* and *Raa* reactors.

Figure 5.2 shows the model simulation of the first differential equation (S_1) for both *Rnc* and *Raa* reactors. The simulation closely follows the dynamical evolution of soluble substrate (S_S) and VFAs (S_a) concentrations. However, the particulate substrate (S_p) is not well predicted by the model, particularly for the first 30 days of *Raa* reactor. This may be due to the first order kinetic used to describe the hydrolysis step which may be inaccurate to represent the solubilisation of complex substrate as sewage sludge (Ramirez *et al.* 2009, Vavilin *et al.* 2008). Moreover, methane gas production (Q_{CH4}) into *Raa* reactor was harder to predict. The acid addition into *Raa* implied the VFAs accumulation and the decrease of methane production i.e. the inhibition of methanogenic microorganisms. However, at day 70, the biomass seems to acclimate to the acid load and the *Raa* reactor recovered its methanogenic activity. In this period, the model could hardly to follow the methane production dynamics.

5.3.3. Co-metabolism parameters estimation

The simulations carried out and experiments of hydrophobic compounds have demonstrated that the aqueous phase is the bioavailable fraction and the co-metabolism is the driver mechanism for their transformation (Delgadillo-Mirquez *et al*. 2011, Barret *et al*. 2010a). Co-

metabolism model parameters (T_c, k_c and K_{SC}) of PAHs were estimated using a non-linear least square method between simulated values and measurements.

By combining equation of S_2 and the co-metabolism rate description (equation 5), we can test two hypotheses. Hypothesis 1, the PAH co-metabolic degradation by acidogenesis step is evaluated: the micropollutant aqueous fraction ($C_{bio} = C_f + C_{DCM}$) is linked to soluble substrate (S_S) consumption for growth of acidogenic bacteria population (X_1). Hypothesis 2 tests the PAH co-metabolic degradation by methanogenic microorganisms: the micropollutant aqueous fraction is coupled to volatile fatty acid (S_a) degradation from methanogenic biomass growth (X_2).

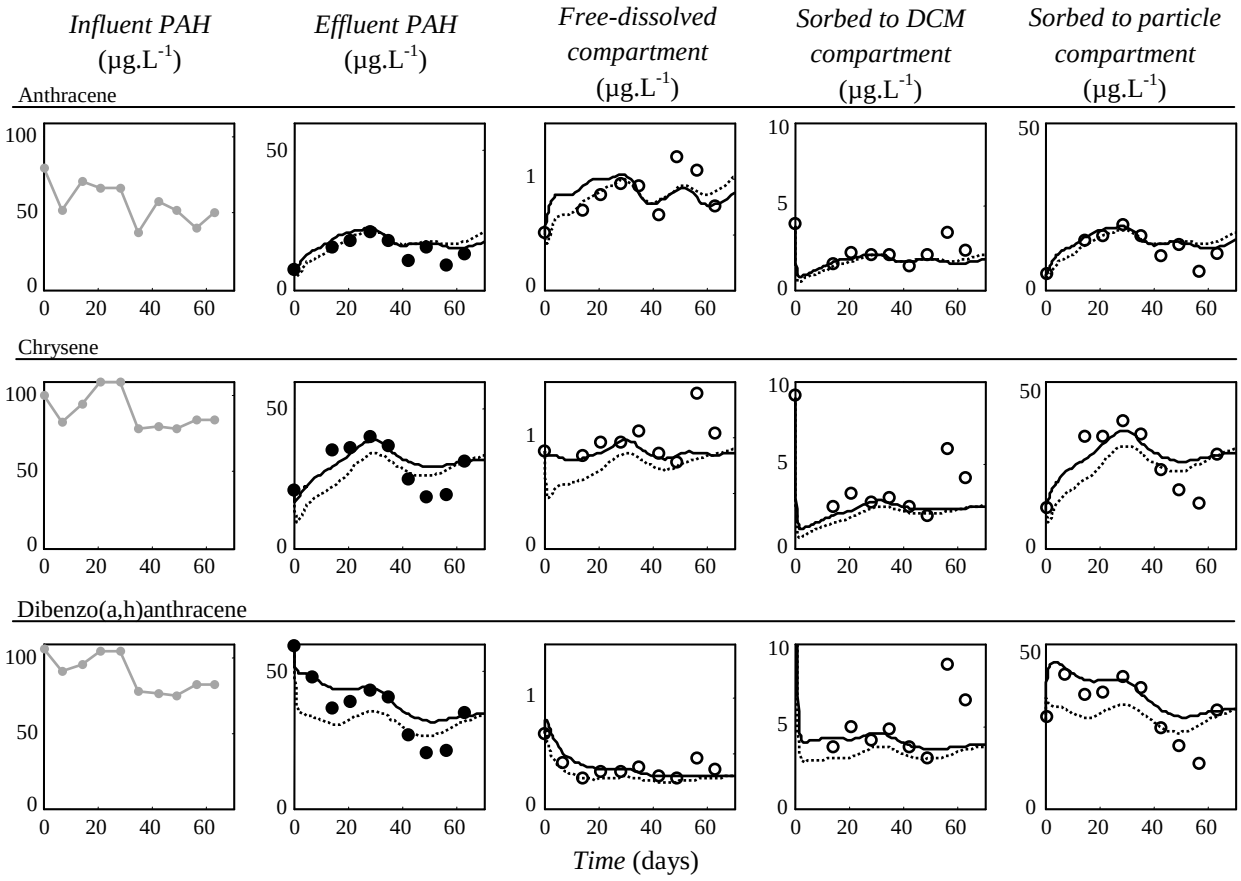

Figure 5.3. PAHs behavior in *Rnc* reactor: (gray line) influent concentration, (black point) experimental data, (white point) values estimated from equilibrium constants, (solid line) model with hypothesis 1 and (dashed line) model with hypothesis 2.

The experimental results of *Rnc* and model simulation for both hypotheses are shown on figure 5.3. The simulations closely follow the dynamic evolutions of the PAHs at total concentration and the different compartments for both hypotheses. Unfortunately the residual values taken up to quantify the best fitting present a trifling variation between hypotheses e.g. the residual values for anthracene in free dissolved concentration are 0.599 and 0.592, respectively. Similar behaviors have been obtained for the other PAHs (data not shown). These results do not allow as to conclude which microbial population is involved in the PAH co-metabolism. This is easily explained by the low dynamic of the system during the experiment which makes any kinetic parameter hard to estimate.

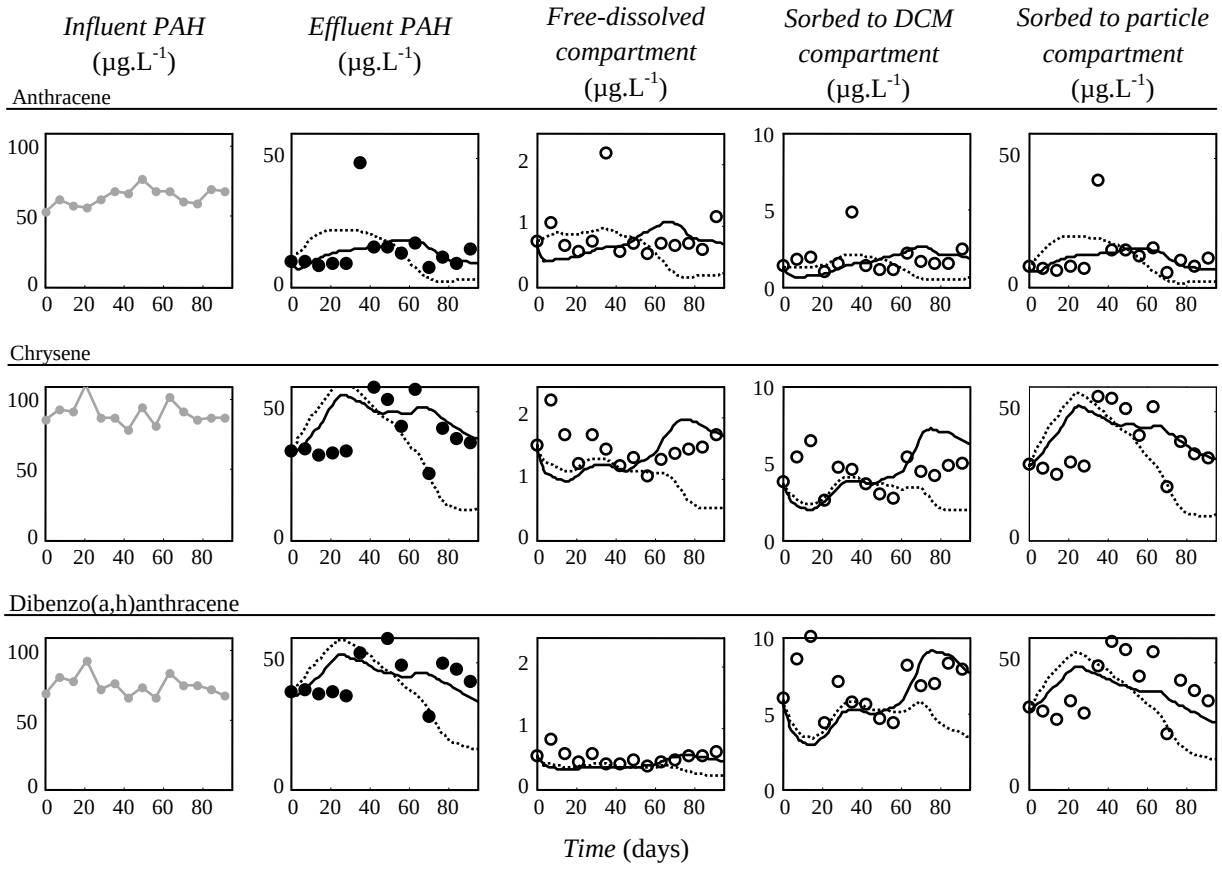

Figure 5.4. PAHs behavior in *Raa* reactor: (gray line) influent concentration, (black point) experimental data, (white point) values estimated from equilibrium constants, (solid line) model with hypothesis 1 and (dashed line) model with hypothesis 2.

Figure 5.4 shows the PAHs behavior in *Raa* for both hypotheses. The model simulations with PAHs co-metabolic transformation during acidogenesis step (hypothesis 1) follow the dynamical evolution of the total PAHs concentrations and their compartments. However, our model overestimates the concentration values measured during the first 30 days of the process. It may be due to the inaccurate reports during the hydrolysis of particulate matter (S_p) into soluble substrate (S_S). In contrast, the simulations of PAHs co-metabolism associated to methanogenic activity show that our model significantly overestimates the PAHs concentration measured and their compartments concentration. Nevertheless, during the performance of *Raa*, clearly the methanogenic microorganisms are inhibited. The strong inhibition of *Raa* during the first phase of the experiment makes the system more identifiable. Thus, the model simulations achieve to explain most of the variability when the co-metabolism is attributed to X_1 rather than to X_2. The residual values for anthracene in free dissolved concentration are 1.47 and 10.63 for hypothesis 1 and 2, respectively. Similar behaviors have been obtained for the other PAHs (data not shown).

Table 5.4. Estimated values of co-metabolic parameters for both reactors.

PAH	***Rnc***						***Raa***		
	Acidogenenic step			***Methanogenic step***			***Acidogenic step***		
	T_c	k_c	K_{SC}	T_c	k_c	K_{SC}	T_c	k_c	K_{SC}
Anthracene	2.51	2.71	1.32	0.72	0.86	2.35	13.3	12.6	0.79
Chrysene	4.62	4.41	4.37	1.59	1.40	3.69	12.8	12.1	3.39
Dibenzo(a,h)Anthracene	4.75	4.58	6.74	1.42	1.31	4.05	12.2	11.4	6.34

Table 5.4 summarizes the PAHs co-metabolic parameters (T_c, k_c and K_{SC}) for both reactors. These results show that the co-metabolism rate in acidogenesis step is higher than methanogenesis one. Futhermore, it was shown that the micropollutant removals (all PAHs) for both *Rnc* (normal methanogenic condition) and *Raa* (inhibition of methanogenic activity) reactors were statistically similar, 70.6±7.8% and 65.8±10.5, respectively.

Given that, the anaerobic degradation pathway involves a complex food web, in which organic matter is sequentially degraded by a wide variety of microorganisms with multiple series and parallel reactions. Obviously, the overall anaerobic ecosystem should be involved in the PAH degradation by complex interactions. To our knowledge, the simulations in this study propose that a great degradation amount occurs at the first steps of anaerobic process. Clearly, acidogenic bacteria and methanogenic microorganisms are involved in the PAH co-

metabolic degradation but with a different rate. Nevertheless, this conclusion is in contrast with some experimental observations. Chang *et al*. (2003) observed that the addition of specific microbial inhibitor delayed PAH degradation, indicating that Methanogens populations were involved in these pollutants degradation in sludge. Similarly, Chang *et al*. (2006) have shown that the addition of inhibitor brought about the partial inhibition of both naphthalene and phenanthrene degradation and suggested that methanogenic organisms were involved in PAH degradation. However, in the same study, the inhibitor addition did not produce a complete inhibition of hydrophobic compounds degradation, which indicate the presence of other species involved in their removal. Additionally, the inhibitor used could affect other species of organism and could produce an imbalance in the overall food web (Chiu and Lee 2001).

However, our conclusions are confirmed with most recently studies. Wang *et al*. (2011) studied the microbial community and PAH biodegradation in sediments with a PAH mixture (fluorene, phenanthrene, fluoranthrene and pyrene) and demonstrated that a significant decreased of PAH concentration was accompanied with an increase of fatty acid degraders but a decrease in species diversity. Moreover, from a thermodynamic point of view, Dolfing *et al*. (2009) have indicated that the most favorable pathway of PAH degradation is into acetate. This conversion is already exergonic at rather high acetate concentrations and can proceed under widely varying acetate concentration. In this sense, the organisms oxidising the organic acid are required to utilize an additional electron acceptor such as carbon dioxide and would simultaneously act as PAH degraders. This could indicate that these bacteria might degrade PAH by co-metabolism but not using them as a carbon and energy source.

5.4. Conclusions

In this paper, a dynamic model of PAH fate was extended by considering two different microbial communities involved in the anaerobic digestion. The assumptions: that aqueous phase is the available fraction and co-metabolism is the driver mechanism for PAH degradations have been shown to hold when comparing experimental data and simulations. The micropollutant co-metabolic transformation was evaluated for two main consortia (Acidogens and Methanogens) independently. The calibrated model shows that the PAH co-metabolism rate in acidogenesis step is higher than methanogenic one. The simulation results

suggest that a great amount of PAH is degraded at the first steps of the anaerobic process but total anaerobic biomass is involved in the PAH removal. Future work could evaluate the overall biomass growth in the PAH anaerobic degradation. However, this approach model might direct specific efforts to understand the PAH biodegradation pathway under anaerobic condition.

5.5. General Discussion

The previous chapters suggest that acidogenic bacteria and methanogenic microorganisms are involved in the PAH co-metabolic transformation but with a different rate. In this last study we demonstrated that **the anaerobic degradation of micropollutants mainly occurs during the first steps of the anaerobic process**. Indeed, the PAH co-metabolism rates in acidogenesis step is higher than methanogenesis one. Similar results were shown at experimental inhibition of methanogenic activity (chapter 4) where the PAH co-metabolic degradations were linked to acidogenic consortium.

The inhibition procedure applied at *Raa* showed that the micropollutrant removal seemed to be not affected in comparison of a control reactor without inhibition (chapter 4). A possible reason for such observation is that the micropollutant biotransformation is supported by acidogenic bacteria. A possible reason for such observation is that the micropollutant biotransformation is supported by the upper anaerobic pathway (hydrolysis, acetogenesis and acidogenesis). Thus, the data set take from strong inhibition of *Raa* provided evidence for these model conclusions. Moreover, the model developed in this section may suggest that the PAH biodegradation pathway is associated to acidogenic consortium under anaerobic condition.

6. Conclusions and Perspectives

The objective of this work was to develop a dynamic model describing the fate of micropollutant during the anaerobic digestion (AD) of contaminated sludge. Simulation results demonstrated the ability of the proposed model to describe and hence to provide a better insight of the complex phenomenon determining the distribution and the fate of hydrophobic micropollutant as Polycyclic Aromatic Hydrocarbons (PAHs) under anaerobic condition.

Fate of micropollutants during AD is the results of interactions between physicochemical equilibriums and complex bioreactions. The proposed model relies on two main traits:

- **The distribution of micropollutants is driven by two equilibrium, between the free phase and respectively the particulate phase and the dissolved/colloidal phase,**
- **The biodegradation of the micropollutant can be described by a co-metabolism reaction.**

A first model applied to the AD of sludge contaminated with PAH showed that because of a very low volatilization, the micropollutant gas compartment is negligible. However, for many other micropollutants the transport from water to air could be an important fate process. Hence most of the contaminant are dissolved in the water phase or adsorbed either to the particulate matter or to the dissolved/colloidal matter (DCM). This double equilibrium could influence the global biodegradation as it can increase the amount of contaminant present in the aqueous phase and also make it dependant on the dissolved organic matter concentration. This three compartment distribution is described by two equilibrium constants: K_p and $K_{DCM,}$ which represent respectively the compound affinity to particle or DCM. Former experimental studies have demonstrated that the sorption of hydrophobic compounds is strictly reversible and very fast in comparison of biological treatment. This statement has been reinforced by the sensitivity analysis of the model, confirming that the sorption-desorption processes of PAHs to particle and to DCM were not dependent of their time constants (k_1 and k_2) but of their equilibrium constants. Thus, it was concluded that **the equilibrium state is sufficient to represent the sorption phenomenon for hydrophobic micropollutants**.

The second originality of the model proposed here is to describe the biodegradation reaction by a co-metabolism. An ecological justification of co-metabolism is that the removal of compounds present only at traces levels ($ng.L^{-1}$ or $\mu g.L^{-1}$) does not result in any significant biomass growth and cannot sustain the maintenance of a specific biomass. Co-metabolism

mechanism has been stated as the ability of an organism to attack the pollutant without assimilating the products of its oxidation. In this model, the co-metabolism is represented by an adaptation of the Criddle (1993) equation. Parameter identifications of a model based on a specific biomass and of a model based on a co-metabolism have shown the better ability of the co-metabolism based model to represent the fate of the PAHs. Moreover, parameter values obtained from different assumptions on the bioavailable fraction lead us to conclude that **the micropollutant aqueous compartment (i.e. free and dissolved/colloidal matter) is bioavailable**.

Furthermore, **the bioavailable concentration of micropollutant could be the limiting factor for the biological transformation**. This study demonstrated that micropollutants located in the free dissolved and sorbed-to-DCM compartments are completely available for biological activity. In the meanwhile the micropollutants sorbed-to-particle can be considered as bioaccessible to bacterial species, because they can be transferred to aqueous phase during the hydrolysis steps of the process. The model simulations show that the increase of readily bioavailable substrate concentration or micropollutant sorbed-to-DCM (affinity to K_{DCM}) could improve the micropollutant removal. In contrast, the increase of particulate concentration or micropollutant sorbed-to-particle (affinity to K_p) favor the transfer to solid phase due to high hydrophobic character of PAHs and thus decrease the removal capacity. This behavior is more pronounced for higher weight PAHs. Considering these observations, biological pretreatments used to enhance the hydrolysis process as an additional stage prior to the main digestion process could be applied in order to improve the micropollutant depletion. Indeed, pretreatment processes can improve the sludge biodegradability, increasing the readily available substrate concentration of sludge, and enhancing the bioavailability of micropollutant by increasing sorbed-to-DCM compartment and thus the activating co-metabolic transformation.

Clearly, **the model developed with co-metabolism was able to represent the removal of hydrophobic micropollutant (as PAHs) even if it was based on the global anaerobic microbial activity** i.e. one biomass is responsible for both utilization of soluble substrate and micropollutant transformation via co-metabolism. However, anaerobic digestion is a complex process that involves several microorganisms and several substrates originating from the organic matter biodegradation and they or one part of they could be responsible for hydrophobic micropollutant depletion. Thus, a new experimental set up was designed in order to elucidate which anaerobic steps are involved in the hydrophobic micropollutant (as PAHs

and NP) removal. The results showed that **the micropollutant degradation is not coupled only to Methanogens activity and suggested that non-methanogenic communities are also involved in the micropollutant degradation**, particularly the bacterial populations responsible for acetate and/or H_2 production may be implied. **For each group, independently of the activity of the other group, a correlation between micropollutant degradation rate and activity can be detected. This lets us to assume the existence of a co-metabolism for both Acidogens and Methanogens consortia**.

A new modeling approach was proposed to represent this new data set. This advanced model involves three steps for the AD of the sludge (hydrolysis, acidogenesis and methanogenesis) and divides the anaerobic consortia into two main groups of homogeneous characteristics i.e. *bacteria* and *archaea*. Indeed, anaerobic digestion of sludge involves syntrophic relationship in which *bacteria* and *archaea* are juxtaposed, facilitating interspecies transfer of substrates necessary for the complete methanogenesis of the organic matter. Thus, the results of the advanced model showed that **the PAH co-metabolism rate in acidogenic step is higher than the methanogenic one**. The simulation results suggest that **a great amount of PAH is degraded during the first steps of the anaerobic process but that the whole anaerobic biomass is involved in the PAH removal**, due to close syntrophic relationship within the anaerobic consortium.

In the course of the work presented in this thesis, several problems and questions that deserve future attention have been encountered. Some of them are summarized below.

- **The four-compartment model developed can be used to describe the fate and behavior of more micropollutants that present a risk for the society** (as the wide list of endocrine disrupters). A large number of these organic micropollutants has been identified and quantified in sludge, each having specific inherent properties and thus behaving differently. However, to apply such model, it is important to determine the micropollutant distribution in the different compartments. The determination of the two equilibrium constants (K_p and K_{DCM}) is required and can be obtained either by experimental methodology (Barret *et al.* 2010b) or by a statistical model based on both micropollutant and sludge characteristics (Barret *et al.* 2010a). However, all these data

were obtained on hydrophobic and neutral compounds. Many of the emerging compounds as pharmaceuticals are less hydrophobic and are charged molecules: their sorption behavior may be largely influenced by the pH which was not taken into account in our model. It is important to get and gather data on such compounds.

- The results of the co-metabolic numerical simulation produced three co-metabolic parameters (T_c, k_c and K_{SC}) that were shown to be molecule-dependent. These estimated parameter values could explain the different biodegradation rates between micropollutant and between sludge sources. Nevertheless, the applied methodology (a non-linear least square method) for the parameters identification may converge toward several values but this study can be considered as a starting point, given that the parameter values comparison with previously published data was hardly feasible. In this sense, **the design of micropollutant removal experiment could be conducted in order to improve the identifiability of co-metabolism parameters. This is important also to improve our understanding of the microbial pathway of micropollutant degradation.**

- **A limitation in this model is that it does not include the micropollutant competitive inhibition and the toxic effect**; despite of that both mechanisms are often concomitant with the co-metabolism of recalcitrant compounds. It is indeed also possible that the formulated model is too simple and neglects these mechanisms, which from our data were not possible to establish. The competitive inhibition is commonly referred to enzyme competition during simultaneous degradation of multiple substrates (growth and co-metabolic substrates), resulting in the decrease of the degradation rates for each substrate. In the meanwhile, the toxic effect is the attack of toxic substrate and/or product on the enzyme and/or cellular materials has resulted in a decrease of activity and viability. Both terms are very different and they can be included into co-metabolism equations (Criddle, 1993). However, the consideration of both mechanisms implied the identification of more kinetic parameters that could complicated the modeling effort. Hence, the design of a co-metabolic micropollutant transformation experiment can help to clarify the need of **the introduction of both mechanisms (inhibition and toxic effect) for better detail to elucidate the hydrophobic micropollutant degradation during anaerobic digestion**.

- **The model based on co-metabolic transformation of micropollutant could be integrated to more complex models that describe the mineralization of organic matter**, such as ADM1 (Anaerobic Digestion Model No.1 from IWA) for anaerobic digestion and/or ASMx (Activated Sludge Models, also depend by IWA) for aerobic treatment. These biological treatments are complex processes that involve several microbial syntrophic relationships that could modify the micropollutant degradation. Moreover, the evaluation of other organic matter, as manure and urban waste, allowed the extrapolation of co-metabolic mechanism. However, several key points must be taken into account, such as: the soluble fraction that corresponds to dissolved/colloidal matter, the microbial diversity and the growth substrates involved in the co-metabolic micropollutant transformation.

- Organic wastes are often stabilized using the composting and it is also one of the convergence points for micropollutant removal. Composting has emerged as a valuable route for the disposal of urban waste, with the prospect of applying composts on arable fields as organic amendments. Hence, **the co-metabolism model proposed could be evaluated in this bioprocess in order to predict their fate under composting process**.

- **The co-metabolism model could be merged with the integrated urban wastewater system to increase the understanding of their fate patterns**. Therefore, in order to model the origin, transport and transfer process of micropollutant within the urban water system in full detail, it is necessary to expand existing biological and urban water models with state variables representing these micropollutants.

References

Ahel M., Giger W. and Koch M. (1994). Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment—I. Occurrence and transformation in sewage treatment. Water Research 28(5), 1131-1142.

Ahring B. K., Sandberg M. and Angelidaki I. (1995). Volatile fatty acids as indicators of process imbalance in anaerobic digestors. Applied Microbiology and Biotechnology 43(3), 559-565.

Alder A. C., Siegrist H., Fent K., Egli T., Molnar E., Poiger T., Schaffner C. and Giger W. (1997) The fate of organic pollutants in wastewater and sludge treatment: significant processes and impact of compound properties. CHIMIA International Journal for Chemistry 51(12), 922-928.

Alvarez-Cohen L. and Speitel G.E.Jr. (2001). Kinetics of aerobic cometabolism of chlorinated solvents. Biodegradation 12(2), 105-126.

Artola-Garicano E., Borkent I., Damen K., Jager T. and Vaes W.H.J. (2003). Sorption kinetics and microbial biodegradation activity of hydrophobic chemicals in sewage sludge: model and measurements based on free concentrations. Environmental Science and Technology 37(1), 116-122.

Bamforth S.M. and Singleton I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. Journal of Chemical Technology and Biotechnology 80(7), 723–736.

Barret M. (2009). Fate of endocrine disruptors during the anaerobic digestion of contaminated sewage sludge: involvement of bioavailability and cometabolism. Montpellier II University.

Barret M., Carrere H., Latrille E., Wisniewski C., and Patureau D. (2010a). Micropollutant and sludge characterization for modeling sorption equilibria. Environmental Science and Technology 44(3), 1100–1106.

Barret M., Cea Barcia G., Guillon A., Carrere H. and Patureau D. (2010b). Influence of feed characteristics on the removal of micropollutants during the anaerobic digestion of contaminated sludge. Journal of Hazardous Materials 181(1-3), 241-247.

Barret M., Patureau D., Latrille E., and Carrère H. (2010c). A three-compartment model for micropollutants sorption in sludge: Methodological approach and insights. Water Research 44(2), 616-624.

Barret M., Carrère H., Delgadillo L. and Patureau D. (2010d). PAH fate during anaerobic digestion of contaminated sludge: do bioavailability and/or cometabolism limit their biodegradation? Water Research 44(13), 3797-3806.

Barret M., Carrère H., Patau M. and Patureau D. (2011). Kinetics and reversibility of micropollutant sorption in sludge. Journal of Environmental Monitoring 13(10), 2770-2774.

Batstone D. J., Keller J., Angelidaki I., Kalyuzhnyi S. V., Pavlostathis S. G., Rozzi A., Sanders W. T. M., Siegrist H. and Vavilin V. A. (2002). Anaerobic digestion model no. 1 (ADM1). IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes. London: IWA.

Belfroid A., van Velzen M., van der Horst B. and Vethaak D. (2002). Occurrence of Bisphenol A in surface water and uptake in fish: evaluation of field measurements. Chemosphere 49(1), 97-103.

Benabdallah El-Hadj, T., Dosta, J. and Mata-Alvarez, J. (2006). Biodegradation of PAH and DEHP micro-pollutants in mesophilic and thermophilic anaerobic sewage sludge digestion. Water Science and Technology 53(8), 99-107.

Bernal-Martinez A., Patureau D., Delgenes J. P. and Carrere H. (2009). Removal of polycyclic aromatic hydrocarbons (PAH) during anaerobic digestion with recirculation of ozonated digested sludge. Journal of Hazardous Materials 162(2-3), 1145-1150.

Bernard O., Hadj-Sadok Z., Dochain D., Genovesi A. and Steyer J.P. (2001). Dynamical model development and parameter identification for an anaerobic wastewater treatment process. Biotechnology and Bioengineering 75(4), 424-438.

Bernard O. and Bastin G. (2005). Identification of reaction networks for bioprocesses: determination of a partially unknown pseudo-stoichiometric matrix. Bioprocess and Biosystems Engineering 27(5), 293-301.

Broholm K., Christensen T.H. and Jensen B.K. (1992). Modelling TCE degradation by a mixed culture of methane-oxidizing bacteria. Water Research 26(9), 1177-1185.

Byrns G. (2001). The fate of xenobiotic organic compounds in wastewater treatment plants. Water Research 35(10), 2523-2533.

Cailleaud K., Forget-Leray J., Souissi S., Hilde D., LeMenach K. and Budzinski H. (2007). Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to a planktonic species Eurytemora affinis (Calanoïda, copepoda). Part 1: PCBs and PAHs. Chemosphere 70(2), 270-280.

Cea-Barcia G., Carrère H and Patureau D.. Evidence for PAH and NP removal coupled to the first steps of anaerobic digestion in sewage sludge. In preparation.

Cerniglia C.E. (1992). Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3(2-3), 351-368.

Chang M. K., Voice T. C. and Criddle C. S. (1993). Kinetics of competitive inhibition and cometabolism in the biodegradation of Benzene, Toluene, and pXylene by two Pseudomonas isolates. Biotechnology and Bioengineering 41(11), 1057-1065.

Chang W.K. and Criddle C.S. (1997). Experimental evaluation of a model for cometabolism: Prediction of simultaneous degradation of trichloroethylene and methane by a methanotrophic mixed culture. Biotechnology and Bioengineering 56(5), 492-501.

Chang B.V., Chang S.W. and Yuan S.Y. (2003). Anaerobic degradation of polycyclic aromatic hydrocarbons in sludge. Advances in Environmental Research 7(3), 623-628.

Chang B.V., Yu C.H. and Yuan S.Y. (2004). Degradation of nonylphenol by anaerobic microorganisms from river sediment. Chemosphere 55(4), 493-500.

Chang B.V., Chiang F. and Yuan S.Y. (2005). Anaerobic degradation of nonylphenol in sludge. Chemosphere 59(10), 1415-1420.

Chang, W., Um, Y. and Holoman, T. R. P. (2006). Polycyclic aromatic hydrocarbon (PAH) degradation coupled to methanogenesis. Biotechnology Letters 28(6): 425-430.

Chang, B. V., Chang, I. T. and Yuan, S. Y. (2008). Anaerobic degradation of phenanthrene and pyrene in mangrove sediment. Bulletin of Environmental Contamination and Toxicology 80(2): 145-149.

Chen J.F., Wong M.H., Wong Y.S. and Tam N.F.Y. (2008). Multi-factors on biodegradation kinetics of polycyclic aromatic hydrocarbons (PAHs) by *Sphingomonas sp* a bacterial strain isolated from mangrove sediment. Marine Pollution Bulletin 57(6-12), 695-702.

Chen Y.G., Jiang S., Yuan H.Y., Zhou Q. and Gu G.W. (2007). Hydrolysis and acidification of waste activated sludge at different pHs. Water Research 41(3), 683-689.

Chiu P.C. and Lee M. (2001). 2-bromoethanesulfonate affects bacteria in a trichloroethene-dechlorinating culture. Applied and Environmental Microbiology 67(5), 2371-2374.

Chung J., Nerenberg R. and Rittmann B.E. (2006). Bioreduction of selenate using a hydrogen-based membrane biofilm reactor. Environmental Science and Technology 40(5), 1664-1671.

Clara M., Kreuzingera N., Strenna B., Gansb O. and Kroissa H. (2005). The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Research 39(1), 97-106.

Coates, J., Woodward, J., Allen, J., Philp, P. and Lovley, D. (1997). Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. Applied and Environmental Microbiology 63(9), 3589-3593.

Corvini P.F.X., Schäffer A. and Schlosser D. (2006). Microbial degradation of nonylphenol and other alkylphenols-our evolving view. Applied Microbiology and Biotechnology 72(2), 223-243.

Couillard C.M., Macdonald R.W., Courtenay S.C. and Palace V.P. (2008). Chemical-environment interactions affecting the risk of impacts on aquatic organisms: A review with a Canadian perspective - interactions affecting exposure. Environmental Reviews 16, 1-17.

Criddle C. S. (1993). The kinetics of cometabolism. Biotechnology and Bioengineering 41(11), 1048-1056.

Christensen N., Batstone D. J., He Z., Angelidaki I. and Schmidt, J. E. (2004). Removal of polycyclic aromatic hydrocarbons (PAHs) from sewage sludge by anaerobic degradation. Water Science and Technology 50(9), 237-244.

De Keyser W., Gevaert V., Verdonck F., Nopens I., De Baets B., Vanrolleghem P.A., Mikkelsen P.S. and Benedetti L. (2010). Combining multimedia models with integrated urban water system models for micropollutants. Water Science and Technology 62(7), 1614-1622.

Delgadillo-Mirquez L., Lardon L., Steyer J.P. and Patureau D. (2011). A new dynamical model for bioavailability and cometabolism of micropollutant during anaerobic digestion. Water Research 45(15), 4511-4521.

Dionisi D., Bertin L., Bornoroni L., Capodicasa S., Petrangeli Papini M. and Fava F. (2006). Removal of organic xenobiotics in activated sludges under aerobic conditions and anaerobic digestion of the adsorbed species. Journal of Chemical Technology and Biotechnology 81(9), 1496-1505.

Dionisi D., Bornoroni L., Mainelli S., Majone M., Pagnanelli F. and Papini M.P. (2008). Theoretical and experimental analysis of the role of sludge age on the removal of adsorbed micropollutants in activated sludge processes. Industrial and Engineering Chemistry Research 47 (17), 6775–6782.

Dolfing J., Xu A, Gray N.D., Larter S.R. and Head I.M. (2009). The thermodynamic landscape of methanogenic PAH degradation. Microbial Biotechnology 2(5), 566–574.

Ejlertsson J., Nilsson M.L., Kylin H., Bergman A., Karlson L., Öquist M. and Svensson B.H. (1999). Anaerobic Degradation of Nonylphenol Mono- and Diethoxylates in Digestor Sludge, Landfilled Municipal Solid Waste, and Landfilled Sludge. Environmental Science and Technology 33 (2), 301-306.

Ely R.L., Hyman M.R., Arp D.J., Guenther R.B. and Williamson K.J. (1995). A cometabolic kinetics model incorporating enzyme inhibition, inactivation, and recovery: II. Trichloroethylene degradation experiments. Biotechnology and Bioengineering 46(3), 232–245.

Ely R.L., Williamson K.J., Hyman M.R. and Arp D.J. (1997). Cometabolism of chlorinated solvents by nitrifying bacteria: Kinetics, substrate interactions, toxicity effects, and bacterial response. Biotechnology and Bioengineering 54(6), 520-534.

Eriksson E., Christensen N., Schmidt J.E. and Ledin A. (2008). Potential priority pollutants in sewage sludge. Desalination 226(1-3), 371-388.

Feng Y., Park J.H., Voice T. and Boyd S. (2000). Bioavailability of soil-sorbed Biphenyl to Bacteria. Environmental Science and Technology 34(10), 1977-1984.

Fernández-Sanjuan M., Rigol A., Sahuquillo A., Rodríguez-Cruz S. and Lacorte S. (2009). Determination of alkylphenols and alkylphenol ethoxylates in sewage sludge: effect of sample pre-treatment.Analytical and Bioanalytical Chemistry 394(6), 1525-1533.

Fountoulakis M.S., Stamatelatou K., Batstone D.J. and Lyberatos G. (2006). Simulation of DEHP biodegradation and sorption during the anaerobic digestion of secondary sludge.Water Science and Technology 54(4), 119–128.

Freedman D.L. and Gossett J.M. (1989). Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Applied and Environmental Microbiology 55(9), 2144-2151

Fu M., Li Z. and Gao H. (2007). Distribution characteristics of nonylphenol in Jiaozhou Bay of Qingdao and its adjacent rivers. Chemosphere 69(7), 1009-1016.

Fujii K., Urano N., Ushio H., Satomi M. and Kimura S. (2001). *Sphingomonas cloacae sp*. nov., a nonylphenol-degrading bacterium isolated from wastewater of a sewage-treatment plant in Tokyo. International Journal of Systematic Evolutionary Microbiology 51(2), 603-610.

Fujita M., Ike M., Kashiwa M., Hashimoto R. and Soda S. (2002). Laboratory-scale continuous reactor for soluble selenium removal using selenate-reducing bacterium, Bacillus sp SF-1. Biotechnology and Bioengineering 80(7), 755-761.

González M.M., Martín J., Santos J.L., Aparicio I. and Alonso E. (2010).Occurrence and risk assessment of nonylphenol and nonylphenol ethoxylates in sewage sludge from different conventional treatment processes. Science of the Total Environment 408(3), 563-570.

Hadibarata T., Tachibana S. and Itoh K. (2009). Biodegradation of chrysene, an aromatic hydrocarbon by *Polyporus sp.* S133 in liquid medium. Journal of Hazardous Materials 164(2-3), 911-917.

Harris C.A., Hamilton P.B., Runnalls T.J., Vinciotti V., Henshaw A., Hodgson D., Coe T.S., Jobling S., Tyler C.R. and Sumpter J.P. (2011). The consequences of feminization in breeding groups of wild fish. Environmental Health Perspectives 119(3), 306-311.

Haws N.W., Ball W.P. and Bouwer E.J. (2006). Modeling and interpreting bioavailability of organic contaminant mixtures in subsurface environments. Journal of Contaminant Hydrology 82(3-4), 255-292.

Hayes L.A., Nevin K.P. and Lovley D.R. (1999). Role of prior exposure on anaerobic degradation of naphthalene and phenanthrene in marine harbor sediments. Organic Geochemistry 30(8), 937-945.

Hernandez-Raquet G., Soef A., Delgenes N. and Balaguer P. (2007). Removal of the endocrine disrupter nonylphenol and its estrogenic activity in sludge treatment processes. Water Research 41(12), 2643-2651.

Horvath, R. S. 1972. Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriological Reviews 36(2), 146-145.

Ince N. H., Gultekin I. and Tezcanli-Guyer G. (2009). Sonochemical destruction of nonylphenol: effects of pH and hydroxyl radical scavengers. Journal of Hazardous Materials 172(2-3), 739-743.

Joss A., Andersen H., Ternes T., Richle P. R. and Siegrist H. (2004). Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic condition: consequences for plant optimization. Environmental Science and Technology 38(11), 3047-3055.

Joss A., Zabczynski S., Göbel A., Hoffmann B., Löffler D., McArdell C. S., Ternes T.A., Thomsen A. and Siegrist H. (2006). Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing and classification scheme. Water Research 40(8), 1686-1696.

Kavlock R.J., Daston G.P., DeRosa C., FennerCrisp P., Gray L.E., Kaattari S., Lucier G., Luster M., Mac M.J., Maczka C., Miller R., Moore J., Rolland R., Scott G., Sheehan D.M., Sinks T. and Tilson H.A. (1996). Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the US EPA-sponsored workshop. Environmental Health Perspectives 104(4), 715-740.

Kordel W., Hennecke D. and Franke C. (1997). Determination of the adsorption-coefficients of organic substances on sewage sludges. Chemosphere 35(1-2), 107-119.

Larsen S. B., Karakashev D., Angelidaki I. and Schmidt J. E. (2009). Ex-situ bioremediation of polycyclic aromatic hydrocarbons in sewage sludge. Journal of Hazardous Materials 164(2-3), 1568-1572.

Latorre A., Lacorte S. and Barceló D. (2003). Presence of nonylphenol, octyphenol and bisphenol A in two aquifers close to agricultural, industrial and urban areas. Chromatographia 57(1-2), 111-116.

Lenz M., Janzen N. and Lens P.N.L. (2008). Selenium oxyanion inhibition of hydrogenotrophic and acetoclastic methanogenesis. Chemosphere 73(3), 383-388.

Le Van T.D., Robinson J.A., Ralph J., Greening R.C., Smolenski W.J., Leedle J.A.Z. and Schaefer D.M. (1998). Assessment of Reductive Acetogenesis with Indigenous Ruminal Bacterium Populations and Acetitomaculum ruminis. Applied and Environmental Microbiology 64(9), 3429-3436.

Lindblom E., Press-Kristensen K., Vanrolleghem P.A., Mikkelsen P.S. and Henze M. (2009). Dynamic experiments with high bisphenol-A concentrations modelled with an ASM model extended to include a separate XOC degradation microorganism. Water Research 43(13), 3169-3176.

Löffler F.E., Ritalahti K.M. and Tiedje J.M. (1997). Dechlorination of Chloroethenes Is Inhibited by 2-Bromoethanesulfonate in the Absence of Methanogens. Applied and Environmental Microbiology 63(12), 4982-4985.

Macy J. M. and Lawson S. (1993). Cell yield (YM) of *Thauera selenatis* grown anaerobically with acetate plus selenate or nitrate. Archives of Microbiology 160(4), 295-298.

Maeng S.K., Ameda E., Sharma S.K., Grützmacher G. and Amy G.L. (2010). Organic micropollutant removal from wastewater effluent-impacted drinking water sources during bank filtration and artificial recharge. Water Research 44(14), 4003-4014.

Marquardt, D. (1963). An algorithm for least-squares estimation of nonlinear parameters. SIAM Journal of Applied Mathematics 11(2), 431-441.

Martin S.A. and J.M. Macy. (1985). Effects of monensin, pyromellitic diimide and 2-bromoethanesulfonic acid on rumen fermentation *in vitro*. Journal of Animal Science 60(2), 544-550.

Mihelcic J.R. and Luthy R.G. (1988). Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. Applied and Environmental Microbiology 54(5), 1182-1187.

Morasch B., Bonvin F., Reiser H., Grandjean D., de Alencastro L.F., Perazzolo C., Chevre N. and Kohn T. (2010). Occurrence and fate of micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part II: micropollutant removal between wastewater and raw drinking water. Environmental Toxicology and Chemistry 29(8), 1658-1668.

Myint M., Nirmalakhandan N. and Speece R.E. (2007). Anaerobic fermentation of cattle manure: Modeling of hydrolysis and acidogenesis. Water Research 41(2), 323-332.

Musolff A., Leschik S., Reinstorf F., Strauch G. and Schirmer M. (2010). Micropollutant loads in the urban water cycle. Environmental Science and Technology 44(13), 4877-4883.

Nakamura M., Nagoya H. and Hirai T. (2002). Nonylphenol induces complete feminization of the gonad in genetically controlled all-male amago salmon. Fisheries Science 68(6), 1387-1389.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. Microbiology and Molecular Biology Reviews 67(4), 593-656.

Oremland R.S., Hollibaugh J.T., Maest A.S., Presser T.S., Miller L.G. and Culbertson C.W. (1989). Selenate reduction to elemental selenium by anaerobic-bacteria in sediments and culture - biogeochemical significance of a novel, sulfate-independent respiration. Applied and Environmental Microbiology 55(9), 2333-2343.

Patureau D., Delgenes N. and Delgenes J.P. (2008). Impact of sewage sludge treatment processes on the removal of the endocrine disrupters nonylphenol ethoxylates. Chemosphere 72(4), 586-591.

Patureau D., Laforie M., Lichtfouse E., Caria G. Denaix L. and Schmidt J. E. (2007). Fate of organic pollutants after sewage sludge spreading on agricultural soils: a 30-years field-scale recording. Water Practice and Technology 2(1), Article 8.

Patureau, D. and Trably, E. (2006). Impact of anaerobic and aerobic processes on PolyChloroBiphenyl removal in contaminated sewage sludge. Biodegradation 17(1), 9-17.

Plósz B.G., Leknes H. and Thomas K.V. (2010). Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in municipal wastewater treatment. Environmental Science and Technology 44(2), 734-742.

Pojana G., Gomiero A., Jonkers N. and Marcomini A. (2007). Natural and synthetic endocrine disrupting compounds (EDCs) in water, sediment and biota of a coastal lagoon. Environment International 33(7), 929-936.

Pons M.N., Louvet J.N. and Potier O (2009). Implementation of micropollutant fate in wastewater treatment plant benchmark simulation models. XENOWAC International Conference on Xenobiotics in the Urban Water Cycle. 11- 13 March 2009, Cyprus.

Poulsen T.G. and Bester K. (2010). Organic micropollutant degradation in sewage sludge during composting under thermophilic conditions. Environmental Science and Technology 44(13), 5086-5091.

Press-Kristensen K., Ledin A., Schmidt J.E. and Henze M. (2007). Identifying model pollutants to investigate biodegradation of hazardous XOCs in WWTPs. Science of the Total Environment 373 (1), 122-130.

Pulliam Holoman T.R., Elberson M.A., Cutter L.A., May H. D. and Sowers K.R. (1998). Characterization of a Defined 2,3,5,6-Tetrachlorobiphenyl-ortho-Dechlorinating Microbial Community by Comparative Sequence Analysis of Genes Coding for 16S rRNA. Applied and Environmental Microbiology 64(9), 3359-3367.

Ramirez I., Mottet A., Carrère H., Déléris S., Vedrenne F. and Steyer J.P. (2009). Modified ADM1 disintegration/hydrolysis structures for modeling batch thermophilic anaerobic digestion of thermally pretreated waste activated sludge. Water Research 43(14), 3479-3492.

Rockne K.J. and Strand S.E. (1998). Biodegradation of bicyclic and polycyclic aromatic hydrocarbons in anaerobic enrichments. Environmental Science and Technology 32(24), 3962-3967.

Rowsell V.F., Tangney P., Hunt C and Voulvoulis N. (2009). Estimating levels of micropollutants in municipal wastewater. Water Air and Soil Pollution 206(1-4), 357-368.

Ryan P., Forbes C., McHugh S., O'Reilly C., Fleming G.T.A and Colleran E. (2010). Enrichment of acetogenic bacteria in high rate anaerobic reactors under mesophilic and thermophilic conditions. Water Research 44(14), 4261-4269.

Samanta S.K., Singh O.V. and Jain R.K. (2002). Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends in Biotechnology 20(6), 243-248.

Scheinost A.C. and Charlet L. (2008). Selenite reduction by mackinawite, magnetite and siderite: XAS characterization of nanosized redox products. Environmental Science and Technology 42(6), 1984-1989.

Schmidt J.E., Christensen N., Batstone D.J., Trably E., Lyberatos G., Stamatelatou K., Kornaros M., Metzger L., Amellal N., Watson J., García K., Ayuso S. and Patureau D. (2006). Safe recycling of sewage sludge on agricultural land-biowaste. Process Safety and Environmental Protection 84(4), 253-257.

Schnürer A., Zellner G. and Svensson B.H. (1999). Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors. FEMS Microbiology Ecology 29(3), 249–261.

Schriks M., Heringa M.B., Van Der Kooi M.M.E., De Voogt P. and Van Wezel A.P. (2010). Toxicological relevance of emerging contaminants for drinking water quality. Water Research 44(2), 461-476.

Semple, K.T. Morriss, A.W.J. and Paton, G.I. (2003). Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. European Journal of Soil Science, 54 (4), 809-818.

Siegrist H., Joss A., Alder A., McArdell-Bürgisser C., Göbel A., Keller E. and Ternes T. (2003). Micropollutants – New Challange in Wastewater Disposal? EAWAG news, 57, 7-10.

Smith, K.E.C., Thullner, M., Wick, L.Y. and Harms, H (2009). Sorption to humic acids enhances Polycyclic Aromatic Hydrocarbon Biodegradation. Environmental Science and Technology 43(19), 7205-7211.

Staples C.A., Naylor C.G., Williams J.B. and Gledhill W.E. (2001). Ultimate biodegradation of alkylphenol ethoxylate surfactants and their biodegradation intermediates. Environmental Toxicology and Chemistry 20(11), 2450–2455.

Sumpter J.P. and Johnson A.C. (2005). Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment. Environmental Science and Technology 39(12), 4321-4332.

Tanghe T., Devriese G. and Verstraete W. (1998). Nonylphenol degradation in lab scale activated sludge units is temperature dependent. Water Research 32(10), 2889-2896.

Tiehm A. and Fritzsche C. (1995). Utilization of solubilized and crystalline mixtures of polycyclic aromatic hydrocarbons by a *Mycobacterium sp*. Applied Microbiology and Biotechnology 42(6) 964-968.

Trably, E. (2002). Etude et optimisation de la biodégradation d'Hydrocarbures AromatiquesPolycycliques (HAPs) et de Polychlorobiphényls (PCBs) au cours de la digestion anaérobie et aérobie de boues urbaines contaminées. Université de Montpellier II, USTL.

Trably E., Patureau D. and Delgenes J.P. (2003). Enhancement of polycyclic aromatic hydrocarbons (PAHs) removal during anaerobic treatment of urban sludge. Water Science and Technology 48(4), 53-60.

Trably E., Delgenes N., Patureau D. and Delgenes J. P. (2004). Statistical tools for the optimization of a highly reproducible method for the analysis of polycyclic aromatic hydrocarbons in sludge samples. International Journal of Environmental Analytical Chemistry 84, 995-1008.

Trably, E., Patureau, D. and Delgenès, J.P. (2005). Comparison of polycyclic aromatic hydrocarbons (PAHs) removals during anaerobic and aerobic sewage sludge treatments. In Lichtfouse, E., Schwarzbauer, J., Robert, D. (eds.) Environmental Chemistry, Green Chemistry and Pollutants in ecosystems. Springer-Verlag, Heidelberg, Germany, pp 431-440.

Trapp S. and Harland B. (1995). Field test of volatilization models. Environmental Science and Pollution Research 2(3), 164-169.

Urase T. and Kikuta T. (2005). Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process. Water Research 39(7), 1289–1300.

Vavilin V.A., Fernandez B., Palatsi J. and Flotats X. (2008). Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview. Waste Management 28(6), 939-951.

Viamajala S., Bereded-Samuel Y., Apel W.A. and Petersen J.N. (2006). Selenite reduction by a denitrifying culture: batch- and packed-bed reactor studies. Applied Microbiology and Biotechnology 71(6), 953-962.

Vinken R., Hollrigl-Rosta A., Schmidt B., Schaffer A. and Corvini, P.F.X. (2004). Bioavailability of a nonylphenol isomer in dependence on the association to dissolved humic substances. Water Science and Technology 50(5), 277-283.

Wang Y., Zhang Y., Wang J. and Meng L. (2009). Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. Biomass and Bioenergy 33(5), 848-853.

Wang Y.F. and Tam N.F.Y. (2011). Microbial community dynamics and biodegradation of polycyclic aromatic hydrocarbons in polluted marine sediments in Hong Kong. Marine Pollution Bulletin 63(5-12), 424-430.

Xu K., Liu H. and Chen J. (2010). Effect of classic methanogenic inhibitors on the quantity and diversity of archaeal community and the reductive homoacetogenic activity during the process of anaerobic sludge digestion. Bioresource Technology 101(8), 2600-2607.

Xu X.R. and Li X.Y. (2010). Sorption and desorption of antibiotic tetracycline on marine sediments. Chemosphere 78(4), 430-436.

Ye D., Quensen III J.F, Tiedje J.M. and Boyd S.A. (1999). 2-Bromoethanesulfonate, Sulfate, Molybdate, and Ethanesulfonate Inhibit Anaerobic Dechlorination of Polychlorobiphenyls by Pasteurized Microorganisms. Applied and Environmental Microbiology 66(1), 327-329.

Yuan S.Y., Chang J.S., Yen J.H. and Chang B.V. (2001). Biodegradation of phenanthrene in river sediment. Chemosphere 43(3), 273-278.

Appendix

PAH fate during the anaerobic digestion of contaminated sludge: Do bioavailability and/or cometabolism limit their biodegradation?

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Abstract

The anaerobic removal of 13 Polycyclic Aromatic Hydrocarbons (PAHs) was measured in five continuous anaerobic digesters with different feed sludge. These feeds were chosen to generate different levels of PAH bioavailability and co-metabolism within the reactors. Based on a three-compartment approach for PAH sorption in sludge, the aqueous fraction (including free and sorbed-to-dissolved-and-colloidal-matter PAHs) was demonstrated to be bioavailable, which validated a widespread assumption about micropollutants bioavailability in sludge. It was also demonstrated that bioavailability is not the only influencing factor. Indeed, PAHs biodegradation resulted from a combination of bioavailability and co-metabolism. An equation adapted from Criddle (1993, The Kinetics of Co-metabolism. Biotechnology and Bioengineering 41, 1048-1056) that takes into account both mechanisms was shown to fit the experimental data, with dry matter removal rate identified as the criteria for co-metabolism. The existence of a threshold of dry matter co-metabolism was also suggested, below which PAHs removal would not be possible. The parameters of the Criddle equation were demonstrated to depend on PAH molecular structure, and the results suggest that they would also be influenced by substrate composition and microbial population. This research provided original outcomes for the assessment of micropollutants fate. Indeed, the understanding of the driving mechanisms was improved, which has implications for the optimization of micropollutants removal.

Trace organic contaminant removal under anaerobic conditions: 15 years of experience[1].

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Pedosphere, submitted

Abstract

Trace organic contaminants (TOCs) correspond to a broad range of molecules generated either directly or indirectly by human activity. Even though TOCs are found at low concentrations in the environment, they often accumulate by biomagnification and bioaccumulation into biological organisms and cause irreversible damages in biological systems through direct or indirect toxic effects, *e.g.* endocrine disruption, carcinogenic effect... Moreover, increasing amounts of industrial and urban TOCs are worldwide generated every year and effluent-collecting treatment plants are one of the most interesting spots for studying their fate in natural microbiological systems. The present manuscript reports the main findings of fifteen years of research based on the biological removal of various TOC found in sewage treatment plants. A specific focus is made on the anaerobic processes of microbial removal in complex ecosystems. Four families of compounds mostly retrieved in urban plants were studied: the Polycyclic Aromatic Hydrocarbons (PAHs), the PolyChloroBiphenyls (PCBs), the phthalic acid esters (PAEs) and the NonylPhenol Ethoxylates (NPEs). It was observed that the microbial potentiality for removing low amounts of TOCs requires a long adaptation time and is often limited by the bioavailability of these compounds. Technological solutions for removing efficiently these compounds are presented. The overall biodegradation is resulting from the numerous interactions existing between the matrix (organic matter) and the microbial ecosystems according to the physical-chemical properties of sorption of these compounds. Mechanistic aspects were also tackled in depth, and specific models were developed for better understanding the network of interactions between TOC, microorganisms and the organic matter. Finally it was found that microbial cometabolism is essential for TOC removal, and the concept of bioavailability is not only

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dependent of the nature, the level and the sorption properties of TOC but it is also strongly dependent of the nature and the level of the sludge organic matter. Specific parameters are proposed for better evaluating the fate of TOC in microbial anaerobic processes.
