DTU Library

Influence of wastewater characteristics on handling food-processing industry wastewaters

Methane potential and sources of toxicity

Maya Altamira, Larisa ; Schmidt, Jens Ejbye; Baun, Anders; Hauschild, Michael Zwicky

Publication date:
2008

Document Version
Publisher's PDF, also known as Version of record

Influence of wastewater characteristics on handling food-processing industry wastewaters: Methane potential and sources of toxicity

Larisa Maya Altamira

Department of Environmental Engineering

Influence of wastewater characteristics on handling food-processing industry wastewaters: Methane potential and sources of toxicity

Larisa Maya Altamira

Ph.D. Thesis
May 2008

Department of Environmental Engineering
Technical University of Denmark

Larisa Maya Altamira

Influence of wastewater characteristics on handling food-processing industry wastewaters: Methane potential and sources of toxicity

PhD Thesis, May 2008

The thesis will be available as a pdf-file for downloading from the homepage of the department: www.env.dtu.dk

Adress:	Department of Environmental Engineering DTU Environment Technical University of Denmark Miljoevej, Building 113 DK-2800 Kgs. Lyngby Denmark
Phone reception:	+45 4525 1600
Phone library:	+45 4525 1610
Fax:	+45 4593 2850
Homepage:	http://www.env.dtu.dk
E-mail:	reception.env.dtu.dk

Printed by:	Vester Kopi Virum May 2008
Cover:	Torben Dolin
Cover photo:	Julie Camilla Middleton
ISBN:	978-87-91855-50-4

Table of Contents

Preface

This thesis is based on research done for a Ph.D. project undertaken from May 2004 to April 2008 at DTU Environment (former E&R). The research was fully funded by the National Council of Science and Technology of Mexico (CONACYT). The supervisors were Senior Researcher Jens Ejbye Schmidt and Associate Professor Anders Baun from DTU Environment. The co-supervisor was Associate Professor Michael Hauschild from the Department of Manufacturing Engineering and Management, DTU.

The thesis consists of two parts. The first part is a dissertation providing backgrounds for understanding the important aspects of anaerobic biological digestion of organic waste(water)s, and also an updated knowledge of the characteristics of food-processing industry (FPI) wastewaters and the potential environmental impacts caused by their handling. Moreover, the specific effects of the wastewaters' characteristics from six FPI used as case studies are presented and discussed for each of the topics included in the dissertation. The second part presents three journal manuscripts and one conference proceeding as short paper, which are referred in the dissertation by their roman numerals:

I. Maya-Altamira, L., Baun, A., Angelidaki, I. & Schmidt, J.E. (2008) Influence of wastewater characteristics on methane potential in food-processing industry wastewaters. *Water Research 42 (8-9), 2195-2203.*

II. Maya-Altamira, L., Baun, A., Gernaey, K. & Schmidt, J.E. Anaerobic biological digestion and co-digestion of six food-processing industry wastewaters. *Manuscript.*

III. Maya-Altamira, L., Eriksson, E. & Baun, A. Source analysis and hazard screening of xenobiotic organic compounds in wastewater from food-processing industries. *Accepted in Water, Air & Soil Pollution: Focus.*

IV. Maya-Altamira, L., Batstone, D.J., Baun, A. Hauschild, M. & Schmidt, J.E. (2005). Use of life cycle assessment and environmental risk assessment as tools for design and optimization of a wastewater treatment system for effluents from the food-processing industry. 2nd International Conference on Life Cycle Management, September 5-7, Barcelona, Spain, *volume II, p.402-405.*

The papers are not included in this www-version but can be obtained from the library at the Department of Environmental Engineering, Miljoevej, Building 113, Technical University of Denmark, DK-2800 Kgs. Lyngby (library@env.dtu.dk).

In addition, two abstracts have been published in conference proceedings which are not enclosed in this thesis, however a discussion of their results is included in the dissertation and referred by their roman numerals:

V. Maya-Altamira, L., Baun, A., Hauschild, M. & Schmidt, J.E. (2006). A design-based model for the life cycle assessment of different process configurations for treatment of food-processing wastewater. 16th Annual Meeting SETAC Europe, May 7-11, The Hague, The Netherlands. *Abstracts volume*, *p.118*.

VI. Maya-Altamira, L., Baun, A., Hauschild, M. & Schmidt, J.E. (2007). Using a life cycle assessment methodology for the analysis of two treatment systems of food-processing industry wastewaters. 3rd International Conference on Life Cycle Management, August 27-29, Zurich, Switzerland. *Abstract book, p.97*.

Acknowledgements

My greatest gratitude goes to my family; my parents for giving me the wings to fly, my brother whose example made me stronger, and to the memory of Ivette Morga Altamira. Also, to those who have contributed to the completion of this thesis work in one way or another:

- The National Council of Science and Technology of Mexico for their funding to my PhD studies.
- My main supervisors, Jens E. Schmidt for opening the door to a person with no experience but a big dream, and for his unique way of provoking and endless curiosity in me to learn more about anaerobic digestion. To Anders Baun for his *invaluable* support and guidance during the past two years and for having always a smile on his face even at complex situations.
- My co-supervisor Michael Hauschild for the opportunities and learnings and for being always open for interesting discussions.
- Assoc. Prof. Krist Gernaey for being always open to share his modeling knowledge and experience and for having a great positive approach to complex problems.
- Prof. Irini Angelidaki, Dr. Jean-Phillipe Steyer, MSc. Ivan Ramirez, Dr. Damien Batstone, Lab Tech. Hector Garcia, and MSc.Guido Tosello, all for sharing their knowledge with me each in their specific areas and topics, and for the encouragement I had from them either directly or indirectly.
- Margrethe Sørensen, Birthe Ebert, Helle Offenberg, Grete Hansen, and Torben Dolin, for their efficient service support in all laboratory, library, and graphics activities.
- Guido Tosello for always believing in me, even when I did not.
- Esteve Casóliba and Simone Manfredi for their enormous support and patience.
- My dearest friends at former E&R and at DTU for their support and fun times.
- My PhD colleagues for a pleasant company and fun times.
- My Kombi-bo mates for being my simulated little family in Denmark.
- My friends at Copenhagen, Europe and Mexico, for being present at difficult moments.
- Guido, Anders, Kim, 12 tonár, and all music available on the net for providing me with wonderful music tunes that filled my soul with joy, love, and peace.
- Last but not least, to all the joyful students like me, squirrels, and birds, that gave me company during holidays, late hours, and weekends, that I spent working in this department.

"A microwavable dinner is programmed for a shelf life of maybe six months, a cook time of two minutes and a landfill dead-time of centuries". ~David Wann, Buzzworm, November 1990

"The aim of education must be the training of independently acting and thinking individuals who, however, can see in the service to the community their highest life achievement." Albert Einstein.

Summary

Food production activities consume more than two thirds of the total fresh water abstraction in the world. Industrialized food production, i.e. food-processing industry, discharges wastewaters containing organic matter in concentrations and volumes that fluctuate from 4 to 40 gCOD/L, and from 500 to 20 000 P.E. respectively. Due to the wastewaters' organic nature, they become suitable for biological treatment, however these fluctuations create problems for their handling, particularly for sewage treatment plants which find difficult to cope with them.

Anaerobic digestion is an alternative for treating food-processing industry wastewaters, providing the benefits of methane production as a renewable energy source, and the reduction of sludge produced. In addition, the microorganisms involved in the process work in symbiotic bacteria groups which can cope better with highly concentrated wastewaters. In the other hand, these bacteria are very sensitive to the type of organic fractions present in the waste(water), as carbohydrates-proteins-lipids. Furthermore, bacteria not always achieve a full reduction/oxidation of these different fractions to methane and carbon dioxide, and it has been noticed in several studies that, apart from the operational practices during treatment, the distribution of these organic fractions may influence the main outputs of the anaerobic digestion process.

The main scope of this thesis is to investigate the effects of the characteristics of wastewaters generated by the food-processing industry, on their assessment for methane potential, anaerobic biodegradability, and potential environmental impacts. The investigation is done by a literature review and six case studies which comprised a number of composite wastewater samples from six food-processing industries located in Denmark. These samples have been taken from different sampling points and during different processing activities. Information provided by the industries is also used for the investigation. The findings were as follows:

On assessing the methane potential and the anaerobic biodegradability of a waste(water), the biological methane potential (BMP) assays provide valuable information regarding the specific effects of the waste(water)s' characteristics. Studies have shown that the substrate:inoculum ratio affects the methane yield and specific methane production in different ranges (from 0.5 to 67 waste to biomass), this depending on the specific interaction of the waste(water) applied and the inoculum. Waste(water) characteristics assessed vary from lumped COD or VS effect, to specific nitrogen, sugars, alkalinity, or lipids effects. Statistical correlation has not been found in most of the studies so the effects have been identified mainly from empirical observations. Since the degradation dynamic patterns of organic waste(water)s present

rather complex intermediaries' profiles, studies have focused on assessing either the characteristics' effects on the hydrolysis step, or on the overall biodegradability of the waste(water). The most frequent observed fractions have been identified as the organic particulates, proteins, and lipids. Carbohydrates and pH have also been assessed in a lesser extent.

The methane potential assessment using BMP assays of ten composite wastewater samples from five food-processing industries was carried out in four wastewater dilution levels. The theoretical methane yields were used as reference values to estimate their methane based biodegradability. The optimal dilution showing the maximum achieved experimental yield was identified for each wastewater. A detailed physico-chemical characterization of the wastewaters was used to estimate their elemental chemical composition and calculate these yields. Statistical correlation of the different waste(water) fractions was tested to find which characteristics affected the experimental methane yields. Normalization of the theoretical yields to COD units showed that the VS analysis did not represent accurately enough the whole organic fraction neither the oxidation state of the wastewaters as the COD analysis did. From the experiments, a statistical analysis proved a significant effect (95% confidence) of wastewaters' acetate concentration on their ultimate methane yields when they were not diluted, and the effect of acetate fraction as % of total COD in undiluted and 75% diluted wastewaters. In the contrary, the analysis showed that bicarbonate alkalinity measured as inorganic carbon enhanced these yields but only when wastewaters were 25% and 50% diluted. Carbohydrates and proteins fractions showed a less significant effect (90% and 92% confidence), and only on the maximum achieved methane yields, i.e. yields at optimal dilutions.

BMP assays were carried out again to assess the anaerobic biodegradability of six individual and five co-digested composite wastewater samples from four industries. Attention was paid to the effect of wastewaters' organic fractions distribution on their experimental free ammonium nitrogen, pH, volatile fatty acids, and methane dynamic profiles. The wastewaters' overall biodegradable fraction, i.e. substrate, was calculated based on these data. The application of the Anaerobic Digestion Model No.1 (ADM1) to experimental data was done by a sensitivity analysis to identify the rate-limiting steps of the wastewaters' anaerobic digestion. Acetate fractions of 15-25% substrate-COD in a vegetable fats and oils wastewater coupled negatively with the presence of readily degradable carbohydrates in individual and co-digested experiments, inhibiting hydrolytic and methanogenic activities. Notably, this inhibiting wastewater contained a nul fraction of lipids, and when co-digested with others containing higher lipids fractions (32-67% substrate-COD), the VFA accumulation switched from propionate and butyrate accumulation to only acetate accumulation. In addition, an adaptation of methanogenic bacteria was observed since co-digestion experiments presented methane production, which did not happen at the individual experiment. When lipids were present in the wastewaters, it enhanced the overall anaerobic biodegradation, particularly at lower organic particulates fractions. Hydrolysis slowed down the overall process when organic particulates in the wastewaters were higher than 50%. The results of the sensitivity analysis, however, revealed that hydrolysis of carbohydrates was also rate-limiting for a more diluted wastewater presenting a smaller fraction of particulates, but a significant amount of carbohydrates (i.e. 54% substrate-COD). Notably, when the experiments showed typical patterns of VFA accumulation-degradation, and a subsequent methane production under stable pH conditions, the ADM1 was rather unsensitive in all processes except for the acetate uptake. In the contrary, when experimental data showed lag-phases, hydrogen inhibition, and VFA accumulations, model outputs were sensitive to nine kinetic parameters, making the analysis interpretation very difficult.

The presence of hazardous trace compounds in wastewaters is nowadays a cause of concern due to the inclusion of priority substances in the Wastewater Framework Directive. Due to the fact that many activities in food-processing industries consume water and that this sector faces strict requirements of equipment's cleaning and disinfection, hazardous trace compounds such as xenobiotics organics (XOC) may be present in their wastewaters discharged. The degree of impact on the environment from these discharged compounds, depends on how the wastewaters are handled and on their removal at the wastewater treatment plant.

Source analysis combined with an environmental hazard screening was carried out to identify the presence of xenobiotic organic compounds (XOC) in four composite wastewater samples from three industries. The source analysis was based on a literature survey of potentially present compounds in raw materials, cleaning agents, and packaging products involved at activities in contact with water. The hazard screening was based on the environmental risk assessment methodology. 29 hazardous XOC were found to be potentially present in the aqueous phase of the wastewater samples, whilst 102 XOC were in the solid phase. This indicated that the majority of the XOC identified would end up in the sludge. 13 XOC were detected by chemical analyses in the samples, from these 5 were typical migrating compounds usually found at background concentrations in the environment, whilst 8 were coming from cooking, packaging and disinfection practices. During the hazard screening, it was noticed a lack of anaerobic biodegradability data at the scientific literature and databases. 91% of the compounds could not be screened for anaerobic biodegradability, making this a drawback for the assessment of anaerobic digestion as a potential treatment option.

Resumé

Fødevareindustrien forbruger mere end to tredjedele af den samlede ferskvandindvinding i verden. Fødevareindustrien udleder spildevand med et indhold af organisk materiale i koncentrationer der kan variere fra 4 til 40 gCOD/L og med et volumen fra 500 til 20 000 P.E. (person ækvivalent). Det organiske stof i spildevandet er egnet til at blive fjernet biologisk, men variationer i sammensætning og mængde kan skabe problemer for håndtering, især for spildevandsanlæg, som har svært ved at håndtere disse.

Anaerob nedbrydning er et alternativ til aerob behandling af spildevand fra fødevareindustrien, idet det organiske stof kan omsættes til biogas samtidig med at mængden af slam reduceres. De nedbrydende mikroorganismer findes i symbiotiske bakteriegrupper, som gør det muligt at behandle det koncentrerede spildevand bedre. Disse bakterier er dog følsomme overfor ændring af forskellige typer af organisk stof i spildevandet, så som kulhydrater, proteiner og fedt. Endvidere kan bakterierne ikke altid opnå fuld reduktion/oxidation af disse forskellige organiske fraktioner til metan og karbondioxid, og i flere studier er det vist at ud over driftspraksis under behandlingen kan også fordelingen af disse organiske fraktioner have indflydelse på nedbrydnings-produkterne fra den anaerobe proces.

Hovedformålet med denne afhandling er at undersøge hvilke effekter sammensætningen af spildevandet fra fødevareindustrien har for vurderingen af metanpotentiale, den anaerobe nedbrydelighed og potentielle miljøeffekter. Undersøgelsen er udført på baggrund af et litteraturstudie og seks case studier, som omfatter et antal spildevandsprøver fra seks fødevareindustrier i Danmark. Disse prøver er blevet udtaget fra forskellige prøvetagningspunkter og under forskellige procesaktiviteter. Information stillet til rådighed fra industrien er også anvendt i undersøgelserne. Følgende resultater er opnået:

Undersøgelsen af det biologiske metan potentiale (BMP) gav værdifuld information ved vurdering af indflydelsen af spildevandets sammensætning på metanpotentialet og den anaerobe bionedbrydelighed af spildevandet. Studierne viste at substarte:podematerialet forholdet har indflydelse på metanudbyttet og den specifikke methaproduktion i forskellige intervallet fra 0,5 til 67 substrate:podemateriale forhold, alt afhængigt af de forskellige interaktioner i det benyttede spildevand og podematerialet. De karakteristika i spildevandet som blev vurderet varierede fra total COD eller VS effekter til specifikke effekter som nitrogen, sukker, lipid eller alkalinitet. Der blev ikke fundet statistisk korrelation i de fleste af de undersøgte tilfælde, så effekterne er primært identificeret fra empirisk observationer. Da det dynamiske mønster for den anaerobe nedbrydning er

relativ kompleks, har studiet fokuseret på at vurdere spildevandets karakteristika på det hydrolytiske trin eller på den overordnede bionedbrydelighed. De fraktioner i spildevandet som oftest blev identificeret var partikulært organisk materiale, proteiner og lipider. Kulhydrater og pH blev også vurderet i mindre omfang.

Metan potentialet blev vurderet ved at lave BMP undersøgelser på ti spildevandsprøver fra fem fødevareindustrier i fire forskellige koncentrationer. Det teoretiske metanudbytte blev brugt som referenceværdi til estimering af spildevandenes metanogene bionedbrydelighed. Den optimale fortynding som gav det højeste eksperimentelle bytte blev identificeret for hver spildevandstype. En detaljeret fysik-kemisk karakterisering af spildevandene blev brugt til at estimere deres kemiske sammensætning og udregne deres udbytte. Statistisk korrelation af de forskellige fraktioner i spildevandet blev testet for at identificere de karakteristika som havde indflydelse på det eksperimentelle metanudbytte. Normalisering af det teoretiske udbytte til COD enheder viste at VS analyse ikke i tilstrækkelig grad repræsenterede hele den organiske fraktion eller oxidationstrinnet for spildevandet, som COD gjorde. Ud fra forsøgene viste den statiske analyse en signifikant effekt af acetatekoncentrationen i spildevandet på det endelige metanudbytte, når spildevandet var ufortyndet. Effekten af acetatfraktionen, i procent af det totale COD i spildevandet, viste imidlertid også en negativ indbyrdes sammenhæng med det endelige metanudbytte for ufortyndet og 75% fortyndet spildevand. I modsætning til dette forøgede bikarbonat alkalinitet, målt som uorganisk kulstof, disse udbytter - men kun når spildevandet var 25% og 50% fortyndet. Kulhydrat- og proteinfraktioner viste en mindre signifikant effekt (90% og 92% konfidens) på det maksimalt opnåede metanudbytte dvs. udbytte ved optimale fortyndinger.

BMP undersøgelser blev lavet for at finde den anaerobe bionedbrydelighed af seks individuelt og fem sammensatte spildevandsprøver fra fire industrier. Specielt fokus var der på effekterne af spildevandenes organiske fraktioner på de dynamiske profiler af ammoniak, pH, flygtige fede syre (VFA) og metan. Spildevandets overordnede bionedbrydelige fraktion, dvs substrat, blev beregnet baseret på disse data. Sensitivitetsanalysen ved brug af Anaerobic Digestion Model No.1 (ADM1) blev brugt til at identificere det hastighedsbegrænsende trin i den anaerobe nedbrydning af spildevandet. Acetate fraktioner på 15 – 25 % af substrat COD i grønsagsolie og fedt spildevand var negativ korreleret med let nedbrydelige kulhydrater i de enkelte forsøg og samudrådningsforsøgene – da der skete en hæmning af det hydrolytiske og methanogene aktivitet. Bemærkelsesværdigt indeholdt dette hæmmende spildevand ikke nogen lipider og når det blev samudrådnet med andre spildevand med højt indhold af lipider (32-67% af substrat COD), ændrede VFA akkumuleringen sig fra propionate og butyrate til acetate. Desuden blev der observeret en adaptation af de metanogene bakterier idet der ved samudrådning blev observeret en metanproduktion, som ikke blev observeret når de enkelte spildevand blev udrådnet. Når der var lipid i spildevandet blev der observeret en forøget bionedbrydelighed af spildevand, især hvis dette indeholdt lidt organisk partikulært materiale. Hydrolysen nedsatte den overordnede proces, når indholdet af organisk partikulært stof i spildevandet var højere end 50%. Resultaterne fra sensitivitetsanalysen viste dog, at hydrolysen af partikulære kulhydrater også var hastighedsbegrænsende for mere fortyndede spildevand, hvor det udgjorde en mindre del af det partikulære materiale, men en signifikant del af kulhydraterne (i.e. 54% substrat COD). Det skal også bemærkes, at når forsøgene viste typisk VFA ophobning og nedbrydning og samtidig metanproduktion under stabile pH forhold, var alle processerne i ADM1 ikke sensitive på nær optagelse af acetate. Modsat dette, viste forsøgene at når de eksperimentelle data havde lag-fase, hydrogen hæmning og VFA ophobning, var output fra modellen sensitive til ni kinetiske parameter, hvilket gjorde datafortolkning meget vanskelig.

Nu til dags giver tilstedeværelsen af farlige sporstoffer i spildevand grund til bekymring. Mange af disse stoffer er prioritetsstoffer i Spildevandsrammedirektivet fra EU (Wastewater Framework Directive). Idet fødevareindustrien bruger store mængder spildevand og det strikse krav i forhold til rengøring og desinfektion, er det sandsynligt at farlige sporstoffer så som organiske miljøfremmede stoffer er tilstede i industriens spildevand. Dette er også en vigtig faktor at vurdere, inden processen vælges og designes, idet fjernelsesgraden og udledningen af disse stoffer til miljøet, enten i udløbsvand eller i slam, vil afhænge af, hvordan spildevandet bliver håndteret og behandlet.

Kildevurdering kombineret med risikovurdering blev udført for at identificere potentielle farlige organiske miljøfremmede stoffer (XOC) i fire spildevandsprøver fra tre indutrier. Kildevurderingen blev baseret på den tilgængelige information i litteraturen om tilstedeværelsen af XOC i råmaterialer, rengøringsmidler og pakkematerialer som kan være involveret i aktiviteter med vand. Risikovurderingen var baseret på tilgængelig metodik indenfor området. 29 potentielle farlige XOC blev fundet i vandfasen af spildevandsprøverne, mens 102 potentielle farlige XOC var i den faste fase. Dette indikerer at størstedelen af de identificerede XOC vil ende I slammet. 13 XOC blev identificeret analytisk i prøverne. Af disse var 5 typiske stoffer som normalt findes i baggrundskoncentrationer i miljøet, mens 8 stammede fra madlavning, pakning og desinfektion. Det er værd at bemærke, at der ved risikovurderingen blev fundet en mangel på data for anaerob nedbrydelighed i litteraturen og i databaser. 91% af de stoffer der blev vurderet for farlige egenskaber kunne ikke vurderes for anaerob bionedbrydelighed, hvilket var en ulempe for vurderingen af anaerob nedbrydning som en potentiel behandlingsmetode.

1 Introduction

More than two thirds of all fresh water abstraction in the world goes towards food production (Kirby et al. (2003)). Efficient food production generally requires use of artificial fertilizers and pesticides and increases soil erosion and run-off. Industrialized food-production contributes greatly to the high-density production of animal proteins on land and in water, and it is often challenging to adequately dispose of their effluents (Kirby et al. (2003)).

Food-processing industry generates high volumes of wastewater which fluctuate in volume and composition according to the raw materials availability, and the processing, cleaning, and water saving practices. Thus, there is a wide variety of studies where different types of food-processing wastewaters are evaluated for their suitability to chemical and biological treatments. Since these wastewaters contain relatively high concentrations of organics in comparison to inorganic compounds, biological treatment by aerobic or anaerobic technologies results amenable for the microorganisms involved, which can biodegrade these wastewaters without the need for laborious adaptation strategies or high addition of ancillaries.

In Denmark, there exists a well established technology which is already applied for the treatment of food-processing wastewaters, mixing and diluting them with domestic wastewater and still managing to comply with the strict effluent discharge limits. This is the biological nutrients removal treatment, conventionally operated by activated sludge plants managed by the municipalities. On the other hand, Denmark introduced the wastewater tax on 1997 which obligates to large manufacturers, e.g. food-processing industries, to pay the cost of their wastewaters' treatment per each kilogram of organic matter and nutrients treated in the wastewater plant. The problem is that they have to pay large amounts of money since food-processing industry wastewaters have high organic content. Anaerobic digestion then becomes an alternative for treatment, however it is important to assess the benefits of this treatment in comparison with the conventional one.

Anaerobic digestion is a well established technology which is suitable for this type of wastewaters. Many successful implementations of full scale reactors have been reported in literature and elsewhere for treating the streams of different food-processing industries (Bernet (2006);Rosenwinkel et al. (2005);Austermann-Haun et al. (1997)). The interest on treating food-processing industry wastewaters by anaerobic digestion relies not only of the removal of pollutants from the streams, but also on the methane generation as a source of renewable energy. In some countries, such as Denmark, methane combustion is utilized as a replacement for district heating and/or electricity generation. In addition, there is an interest of finding better ways to dispose the residues of this treatment, since they may contain relatively high amounts of nitrogen in comparison with organic carbon, which makes them candidates for replacement of industrial fertilizers.

Since anaerobic digestion functions by symbiotic groups of bacteria which degrade and obtain energy for growth from different substrates, the characteristics of the wastewaters influence each of these communities. Furthermore, in the presence of the products of a bacteria group, others may become inhibited, making the overall process to fail. By knowing the characteristics of the wastewaters, it is possible to have an idea of the overall substances flow and predict their theoretical potential for methane production, a parameter that can be compared amongst different wastes to assess which are suitable to treat by anaerobic digestion. The limitation of this assessment is that it assumes all organic matter is oxidized without considering that a fraction of it goes to build biomass, or that a full oxidation will not occur due to nutrients limitation or the presence of inhibitory or toxic organic compounds.

To overcome these limitations, a practical methane potential can be determined, but it is still important to make a thoroughful characterization of the wastewater to identify which characteristics favor or impede getting closer to its theoretical potential. In this way, strategies such as co-digestion can be suggested to enhance this potential. Co-digestion works as an alternative to make the different organic fractions contained in the wastewater more homogenous and amenable for the different bacteria groups, by dilution or counteraction of nutrients and/or inhibitory compounds, or by mixing a slowly wastewater with a readily degradable.

Since the anaerobic biodegradability of an organic waste depends very much on its characteristics, prediction of its organic carbon flow and methane potential is convenient before the process design and implementation. Advantages and limitations for wastewaters' handling can be defined by process modeling, particularly to determine their biodegradability dynamics, so optimized strategies such as co-digestion or buffering can be identified. In this case, more accurate results will be gotten if a detailed wastewater characterization is done beforehand.

The presence of xenobiotic organic compounds in urban wastewaters is constantly regarded as a source of toxicity with growing attention from international environmental legislation. The attention on these compounds relies on their toxicity to the environment when they are discharged in the wastewaters. Since some of these compounds are persistent, they tend to accumulate through the food chain to finally being discharged in the wastewaters or in the sewage sludge, which prevents these wastes from being reused as alternative fertilizers or other applications. Furthermore, they represent a problem for biological treatment plants as recipients, since some of the compounds may inhibit bacterial activity affecting the degradation of other wastewater fractions. It thus becomes important to include the identification of xenobiotic organic compounds as part of the wastewater characterization practices.

Factors affecting the composition of food-processing industry wastewaters are related to their production and cleaning practices. Water in contact with food must be potable to avoid migration of microorganisms creating a risk for consumers, specially when the products are for human consumption. Cleaning practices are therefore an important part of water use, as well as the addition of chemical agents to decrease time and other resources involved. In addition, the availability of raw materials may be seasonal according to vegetables and animals growing. For each different product, different processing practices may take place and different food waste discharged into the wastewater.

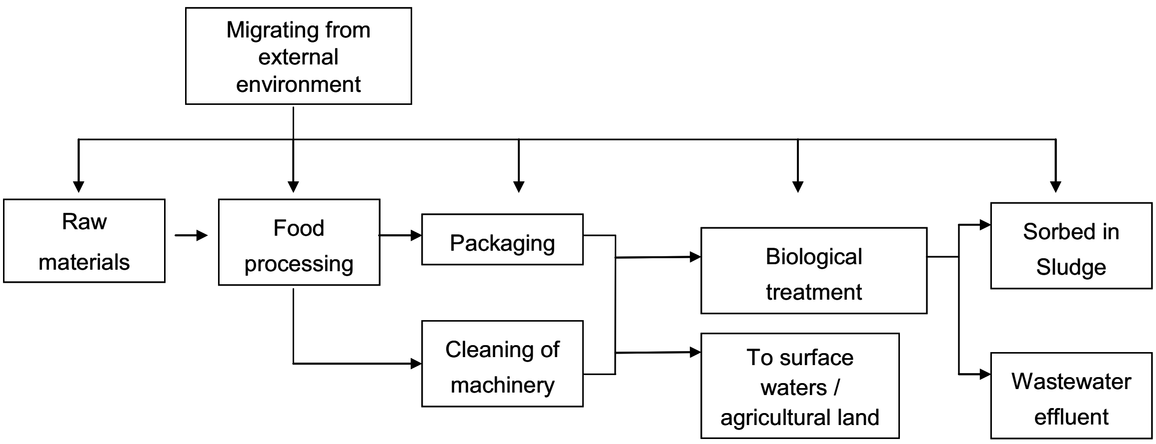


Figure 1. Food-processing industry practices relevant to wastewater discharge and its biological treatment (Paper **III**).

There are thus a series of factors which affect the composition of the wastewater and subsequently its biological treatment in place, particularly anaerobic digestion which is more sensitive to the organic and inorganic components contained in the waste. The relevance of wastewater's different fractions on its biological treatability has not been notably emphasized yet. Their importance can be as high as the process' operational practices during treatment, since concentrations of inhibitors such as fats and xenobiotics can lead to process failure. Furthermore, the consequences of processing practices at the food industries become relevant for identifying sources of pollution and improve wastewater's treatability in place, so environmental impacts arising from the treatment and discharge can be reduced.

The aim of this PhD study was to investigate the effects of the characteristics of the wastewaters from the food-processing industry on their assessment for methane potential, anaerobic biodegradability, and potential environmental impacts. Particular tasks of study were:

- Investigating what physico-chemical characteristics affect methane potential.

- Investigating what organic fractions affect anaerobic biodegradability under batch conditions.

- Investigating the factors affecting food-processing wastewaters' composition, and which are the sources of environmental hazard inherent in these wastewaters.

- Defining relevant indicators for the life cycle assessment of wastewaters' treatment by anaerobic digestion.

Data regarding six food-processing industries located at Denmark were used as case studies. Industry branches were:

1. Vegetable fats & oils processing;

2. Peas, leek & onion processing;

3. Slaughterhouse;

4. Fish meals for aquaculture;

5. Fish ready meals for human consumption;

6. Pet food processing.

A number of composite wastewater samples were taken at different times during the experimental part of this project, and assessed according to their production season, production shift, and sampling point, and to the availability and willingness of the industries for aiding with the sampling. Sampling details can be seen at Papers **I, II,** and **III**.

This thesis is divided in 6 chapters, conclusions, future outlooks, references, and appendices. Chapter 1 is this introduction. Chapter 2 forms a theoretical background for the topics related to anaerobic digestion which are relevant for this study. Chapter 3 is a presentation and discussion of the studies related to methane potential and anaerobic biodegradability, by comparing our findings with others' when assessing organic wastes and wastewaters under anaerobic batch conditions. Chapter 4 gives an overview of the modeling tools applied for the anaerobic biodegradability assessment of organic wastes and wastewaters, and gives the basis for comparison to our study. Chapter 5 highlights the factors affecting food-processing wastewaters' composition, and the sources of environmental hazard during the generation of these streams. Chapter 6 discusses the application of life cycle assessment to wastewater treatment systems, focusing on our studies with food-processing industry wastewaters and anaerobic digestion, and discusses the possibilities of integrating this tool with process modeling and environmental risk assessment. The conclusions summarize the findings of this study, extrapolating them for a general application. Future outlooks identify key areas of research for future studies. Finally, the appendix comprises the three journal manuscripts and one short paper conference proceeding produced from this study.

2 Anaerobic digestion of organic waste(water)s

The anaerobic degradation of organic materials is a complex process which overall rate depends mainly on both the anaerobic microorganisms constraints, e.g. growth and activity, and the physico-chemical characteristics of the waste (Angelidaki and Sanders (2004);Shin and Song (1995)). Some organic pollutants are of simple structure (e.g. simple sugars, acids, and alcohols), and can be metabolized in a matter of minutes, but as the molecular structure increases in size and complexity, the rate of biodegradation commonly decreases (Gosset and Belser (1982)). The biodegradability of a given substrate can be *ultimate, primary,* or *inherent*, depending on the capability of the microorganisms to further degrade the resulting by-products from the anaerobic conversions prior to methanogenesis. *Ultimate* is when substrate is all degraded to methane and carbon dioxide or to intermediaries that microorganisms cannot further degrade, *primary* when it is converted to intermediaries which are still possible to be degraded, and *inherent* when it is potentially degraded only if specific actions are taken (e.g. pre-exposure to substrate, lower S:I ratios) (Rozzi and Remigi (2004)). When the cumulative methane production stabilizes (see Figure 2), it is assumed ultimate biodegradability occurred. However, monitoring the intermediaries and specific methane production can assure by-products biodegradation is not occurring (i.e. primary biodegradability). When assessing unknown waste(water)s' biodegradability, either ultimate or primary, it is the intention to observe how the inoculum responds to the waste(water) by increasing its activity. This activity happens as a consequence of the microorganisms' protein synthetizing system, which increases in relation to the substrates' availability (Grady et al. (1996)). Therefore inherent biodegradability -e.g. low S:I ratios, inoculum acclimation prior to test- should be avoided in the possible extent.

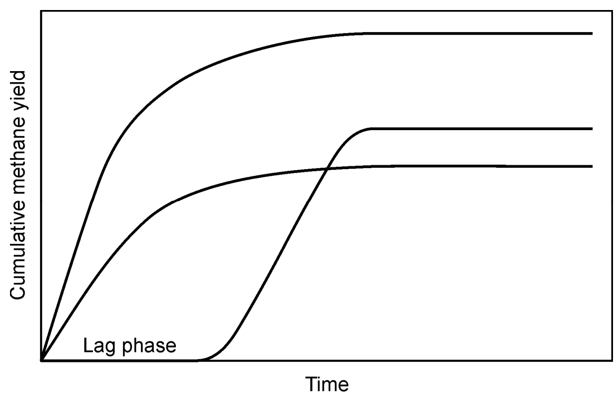


Figure 2. Typical cumulative methane production profiles during anaerobic batch degradation of organic waste(water)s.

2.1 Substrates flow

Anaerobic digestion involves the breakdown of different fractions of organic matter by the action of a bacteria consortium in the absence of oxygen. The process consists of a series of oxidation/reduction reactions where a wide range of microorganisms converts substrate materials available forming a food chain. The end products of these conversions are carbon dioxide in the substrate's most oxidized form, and methane in its most reduced form. For every reaction taking place, chemical energy is released which can be utilized by the organisms, either directly or indirectly by syntrophic relationships. The complete oxidation/reduction of the organic matter contained in a specific waste fed into an anaerobic digestion compartment happens by the accumulation and subsequent degradation of intermediaries which appear based on the waste's organic fractions and the nutrients available for the growth of these bacteria. Figure 3 shows the different substrate conversions occurring during anaerobic digestion.

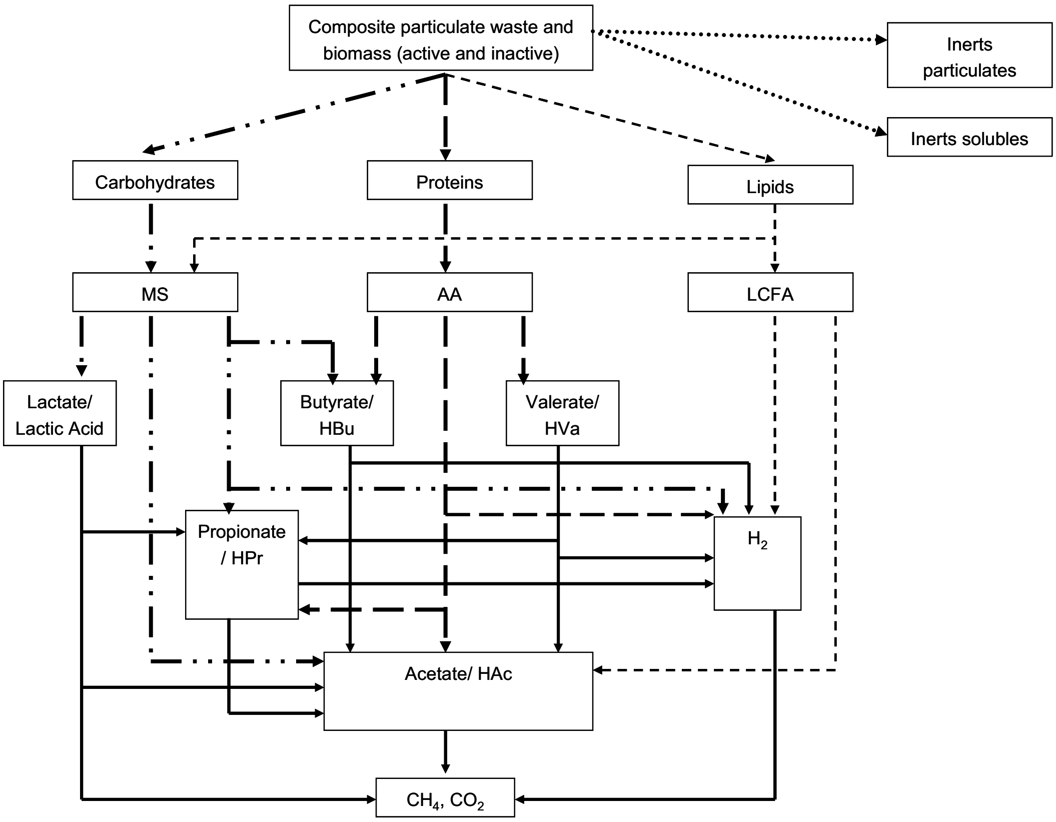


Figure 3. Organic fractions flow during anaerobic digestion. Dotted lines indicate flows regulated by the carbohydrates, proteins, and lipids available. Solid lines indicate flows regulated by the presence of intermediaries (volatile fatty acids, carbon dioxide, and hydrogen). Adapted from, *Gavala and Lyberatos (2001), and Batstone et al (2002)*.

At this Figure we can observe that particulate waste contains composite material which is further disintegrated into carbohydrates, proteins, lipids, and inert material. Some types of wastes can also contain available biomass which is also disintegrated, and non-available biomass which is deposited into the system as inert material without further biodegradation (Batstone et al. (2002);Gosset and Belser (1982)). Carbohydrates, proteins and lipids are hydrolyzed into monosaccarides (MS), aminoacids (AA), and long chain fatty acids (LCFA) respectively, where a fraction of lipids is also hydrolyzed to monosaccarides (MS). More complex conversions are then happening, where MS are converted to lactate/lactic acid, acetate/acetic acid (HAc), propionate/propionic acid (HPr), and/or butyrate/butyric acid (HBu). AA are converted to butyrate/butyric acid (HBu), acetate/acetic acid (HAc), and/or valerate/valeric acid (HVa). LCFA are converted to hydrogen gas (H_2) and/or acetate/acetic acid (HAc) The dissociation of all fatty acids depends on the hydrogen concentrations in the liquid media. Lactate/lactic acid is further converted to propionate/propionic acid (HPr), acetate/acetic acid (HAc), and/or methane (CH_4) and carbon dioxide (CO_2). Propionate/propionic acid (HPr) and butyrate/butyric acid (HBu) are converted to hydrogen and/or acetate/acetic acid (HAc). Valerate/valeric acid (HVa) is converterted only to acetate/acetic acid (HAc). Finally, acetate/acetic acid (HAc) is converted to methane (CH_4). The individual process from composites is called *disintegration*, from carbohydrates, proteins, and lipids is called *hydrolysis*, from monomers (MS and AA) is called *acidogenensis*, from LCFA is called *acetogenesis*, from all volatile fatty acids (VFA) is called also *acetogenesis*, and finally, from acetate and hydrogen gas is called *methanogenesis*. There are other sub-processes happening for each of these conversions, but when the overall digestion from a waste(water) is studied, these are the most common processes assessed.

2.2 Stoichiometry

The stoichiometric equations for the anaerobic conversion of complex substrates are regulated by the energy released during respiration which is required for microbial synthesis. Carbohydrates and proteins conversions yield higher energy coefficients than lipids' and volatile fatty acids' (VFA), since the former substrates release more energy per electron donated during the oxidation/reduction reaction. Thus, the microorganisms mediating the anaerobic conversion of carbohydrates and proteins have more energy available which allows them to grow faster (Pavlostathis and Giraldogomez (1991)).

The hydrolysis, acidogenesis, and acetogenesis conversion rates will control the magnitude of VFA excursions and thus the concentration of alkalinity which must be reserved for such an eventuality (Speece (1996)). Hydrolysis is the rate-limiting process with difficult-to-degrade organics or particulates, and under such operations the VFA concentrations usually remain low. Readily degradable organics, such as sugars and simple organics or proteins, rapidly convert to VFA, so the rate-limiting step may then become methanogenesis, which can result in an accumulation of VFA and an associated need for reserve alkalinity (Rozzi and Remigi (2004);Speece (1996)).

The VFA accumulation is particularly notable when readily biodegradable carbohydrates and/or proteins are anaerobically degraded. The conversion of sugars such as glucose and related, produces either lactate, acetate, propionate, butyrate, or hydrogen (Figure 3). In this conversion the cell puts energy into the system by phosphorylation using ATP and inorganic phosphate. Glucose is then turned into 3-Carbon fragments. Surplus hydrogen is transferred into a carrier called nicotinamide adenine dinucleotide (NAD) by the next reaction (Mosey (1981)):

$$NAD^+ + H^+ \leftrightarrow NADH + H^+ \qquad \text{Eq. 1}$$

A series of energy-yielding reactions take place in which the 3-carbon fragments are eventually converted to pyruvate. Finally, pyruvic acid is converted into acetic acid with the gain of two extra molecules of ATP, one from each molecule of pyruvic acid. So, the overall reaction is (Mosey (1981)):

$$2H_2O + C_6H_{12}O_6 \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \qquad \text{Eq.2}$$

This conversion is energetically unfavourable, so, if this is halted by an accumulation of hydrogen in the digester gas, the bacteria will adopt an alternative strategy and will use pyruvic acid itself as an oxidizing agent and recover their NAD^+ by the formation of propionic acid (Mosey (1981)):

$$CH_3COCOOH + 2NADH + 2H^+ \rightarrow CH_3CH_2COOH + H_2O + 2NAD^+ \qquad \text{Eq.3}$$

The hydrogen producing acetogenic bacteria converts, amongst others, propionic acid into acetic acid. This reaction is fundamental to avoid a digester failure since it prevents the accumulation of acids and pH drop (Mosey (1981)):

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \qquad \text{Eq.4}$$

This cannot proceed whilst there are significant accumulations of acetic acid and hydrogen in the digester. Meanwhile, the continuing accumulation of propionic acid will continue to depress the pH value of the growth medium. No methane bacteria have been found which can ferment propionic, butyric, or higher acids directly to methane.
The formation of methane from carbon dioxide and hydrogen provides a very efficient method for removing gaseous hydrogen, thereby controlling the redox potential of the fermentation raising that of the H^+/H_2 couple (Pavlostathis and Giraldogomez (1991);Mosey (1981)):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad \text{Eq.5}$$

Finally, the formation of methane from acetic acid happens as the methyl group is transferred intact (Mosey (1981)):

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad \text{Eq.6}$$

Growth yields of the aceticlastic methanogens measured experimentally are very low. They cannot even obtain 1 mole ATP/mole methane formed by this route**,** which makes them take longer to become active in the anaerobic degradation. These organisms are the most vulnerable to pH and VFA accumulation, which affects the stability of the whole process (Rozzi and Remigi (2004);Speece (1996)).

2.3 Interspecies hydrogen transfer

The regulatory role of hydrogen in connection to anaerobic metabolism is closely linked to the interspecies hydrogen transfer. Due to the presence of hydrogen consuming bacteria, the hydrogen producing bacteria will form more hydrogen than if the hydrogen consuming bacteria were not present. Two syntrophic bacteria groups of primal importance for methanogenesis to occur are the obligate proton-reducing acetogens co-acting with the hydrogenotrophic methanogens (Schmidt and Ahring (1993)). Due to the unfavourable energetics (see Table 1), the oxidations of propionate and butyrate to acetate are only possible if their products are removed efficiently. Furthermore, the subsequent oxidation of acetate to carbon dioxide in the presence of hydrogen ion is also energetically unfavourable, emphasizing the importance of the hydrogen uptake by the hydrogenotrophic methanogens. In this case, propionate and butyrate become important intermediaries to monitor, since the decrease of free energy for the hydrogenotrophic methanogens and the parallel increase of the free energy for the uptake of propionate and butyrate by acetogens, keep hydrogen partial pressures within a certain range in order to make both reactions exergonic. Outside this range, only one of the hydrogen producing or the hydrogen consuming reactions will occur. In addition to interspecies hydrogen transfer, interspecies formate transfer may also play a role in the syntrophic uptake of fatty acids, although Schmidt and Ahring (1993) and Kleerebezem and van Loosdrecht (2007) found it irrespective in comparison to the hydrogen transfer. The Gibbs free energies of some of the reactions involved in the interspecies hydrogen transfer are shown at Table 1.

2.4 Acid-base reactions

The physico-chemical system including the acid-base reactions is very important for monitoring during anaerobic degradation since it contributes to the inhibition of several bacteria groups and regulates the gas flow and the presence of bicarbonate alkalinity. Anaerobic systems operate in the neutral pH range in which bicarbonate is the dominant species, thus bicarbonate alkalinity is of major relevance.

Table 1. Standard Gibbs free-energy changes for some of the hydrogen releasing/consuming reactions involved in the anaerobic oxidation of organic wastes. Based on *Schmidt and Ahring (1995)* and *Schink (1997)*.

Compound	Reaction	$\Delta G^{0'}$ (kJ/mol)
Glycolic acid	$CH_2OHCOO^- + H^+ + H_2O \rightarrow 2CO_2 + 3H_2$	+19.3
Amino acids	$CH_3CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + 2H_2$	+2.7
Butyrate	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2H_2$	+48.3
	$CH_3CH_2CH_2COO^- + 2HCO_3^- \rightarrow 2CH_3COO^- + 2HCOO^- + H^+$	+45.5[a]
Valerate	$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + H_2$	+25.2
Propionate	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + 3H_2 + H^+$	+76.0
	$CH_3CH_2COO^- + 2HCO_3^- \rightarrow CH_3COO^- + 3HCOO^- + H^+$	+72.2[a]
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6
Formate	$4HCOO^- + H_2O + H^+ \rightarrow CH_4 + 3HCO_3^-$	-130.4[a]
Acetate	$CH_3COO^- + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2$	+94.9
	$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	-31.0[a]
Hydrogen	$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$	-94.9
	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-135.6[a]
	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	-131.0

[a] Estimated at 25°C

pKa values for VFA are of approximately 4.8, for the $CO_{2(aq)}/HCO_3^-$ acid-base pair is 6.35, while for the NH_4^+/NH_3 acid-base pair is 9.25. The base CO_3^{-2} is in very low concentrations as the acid-base pair HCO_3^-/CO_3^{-2} has a pKa of 10.3. The $CO_{2(aq)}$ to HCO_3^- reaction passes through H_2CO_3, a relatively strong acid (pKa=3.5). However, the equilibrium coefficient for $CO_{2(aq)}/H_2CO_3$ is high, meaning that $CO_{2(aq)}>>H_2CO_3$, so $CO_{2(aq)}$ can be taken as the effective acid (Batstone et al. (2002)).

Low pH conditions may be caused by two sources of acidity: H_2CO_3 (or $CO_{2(aq)}$) and VFA, which are both generated in microbial reactions. The major requirement of alkalinity in well-operating anaerobic processes is neutralization of the high or $CO_{2(aq)}$ which results from the high partial pressure of CO_2 gas in the reactor. VFA concentrations are commonly low, and since a very significant fraction of the bicarbonate alkalinity may be allocated to neutralize the CO_2/H_2CO_3, only the excess is available for neutralizing an increase in VFA. In this case the metabolism-generated alkalinity becomes important which is the increase of alkalinity in a wastewater resulting from the metabolism of an organic compound with the release of a cation. Cation-releasing degradable organic components are proteins, salts of organic acids, or soaps. If no cation is released from the organic component during biodegradation, no alkalinity will be generated as it is the case of carbohydrates or sugars (Speece (1996)).

2.5 Inhibition and toxicity

Inhibition and *toxicity* have been distinguished by Speece (1996) as the former denoting an impairment of a particular bacterial function, and the latter adversely affecting the bacterial metabolism as a whole. So, inhibition can occur gradually until the affecting substrate increase in such a way that causes metabolism failure (i.e. toxicity). Rozzi and Remigi (2004);Speece (1996) reported two distinct patterns of toxicity during anaerobic degradation:

- Inhibition increases as the dose of the substrate increases but the relative activity remains constant, meaning that the biomass does not recover from the toxic effect;
- Inhibition occurs in early stages of the test showing a *lag phase*, and later on the biomass adapts to the toxic compound by recovering its base activity.

Alternatively, a progressive decrease in time of the methanogenic activity might be observed. The reason may be that a compound exerts an inhibiting effect on growth however the catabolic conversions recorded as methanogenic activity are not affected. It may be also that this compound accumulates and at a specific moment trespasses the inhibitory level (IWA Task Group (2006)).

Different dynamic patterns have been observed using different organic materials, such as monophasic curves with no inhibition observed using food waste (Zhang et al. (2007)). Others showed different activity periods, e.g. lag phases using plastics (Muller et al. (2004)), and biphasic curves with an initial steady methane production during days using woody biomass (Turick et al. (1991)) and glucose (Chen and Hashimoto (1996)) or during hours using spent grain (Fernandez et al. (2001)), which all later increased to a second steady methane production.

3 Anaerobic biodegradability and methane potential of organic waste(water)s

3.1 Biological methane potential (BMP) assays

In order to assess the activity of an inoculum in regards to a specific waste(water) under anaerobic conditions, experimental data can be gathered from batch or continuous lab-scale reactors where the waste(water) functions as the substrate. However, there is a lack of coherence between the many different methodologies for assessing this activity (Rozzi and Remigi (2004);Speece (1996)).

Anaerobic batch digestion experiments are useful because they can be performed quickly with simple equipment. They are used to determine anaerobic biodegradability, ultimate methane potential, and the rate at which the waste(water) can be digested (Parawira et al. (2004); Angelidaki and Sanders (2004)). The Biological Methane Potential (BMP) assay is a method based on product formation where biogas, methane and/or intermediates production are monitored from closed vials containing the selected waste(water) and methanogenic inoculum incubated at a specific temperature (Angelidaki and Sanders (2004)). The methane potential can be determined as the ultimate specific methane production for indefinite degradation time. The rate of ultimate biodegradation of the waste(water) can also be determined by monitoring methane or intermediaries at pre-set time intervals until specific methane activity is not observed.

Rozzi and Remigi (2004);Speece (1996) distinguished amongst '*biodegradability*' and '*activity*' referring to the former as a property of the tested substance (e.g. wastewater) to its susceptibility to undergoing a biologically mediated degradation and the latter as a property of a microbial population (e.g. inoculum) to undertake the degradation of the test material. In many studies at the literature both concepts are used to refer either to the property of the substrate, or to the inoculum, or to both. Since there is not a consensus yet, in this report it is referred as '*biodegradability*' as the susceptibility of the wastewater(s) to undergoing biological degradation coupled with the initial activity of the inoculum. This initial activity does not depend on the wastewater(s) properties, but rather on the actual microbial population before their protein synthetizing system starts adapting to the substrates (Grady et al. (1996)).

Parameters monitored can be directly involved in the microbial activity or can be a secondary effect of microbial activity (Rozzi and Remigi (2004);Speece (1996)). In Figure 4 are shown the analytical parameters that can be measured from a BMP assay. Microbiological parameters identify the different bacterial groups involved and physico-chemical parameters employed measure the acid-base reactions both happening at the individual degradation processes. Environmental technology applications commonly measure parameters related to the reactants and/or products (Rozzi and Remigi (2004)). For determining individual processes' rates, substrate depletion is measured either as a lumped parameter, e.g. COD, VFA, or as individual parameter, e.g. sugars, acetate. The choice of the analytical parameter measured can affect the assessment of the activity to a considerable extent (Rozzi and Remigi (2004)).

The factors affecting the microbial activity assessed are categorized in seven categories (IWA Task Group (2006); Angelidaki and Sanders (2004);Rozzi and Remigi (2004)):

- Inoculum: Source, characterization, activity, pre-treatment before experiment, amount.
- Waste(water): Physico-chemical characteristics.
- Nutrients: Contained in inoculum/waste(water), or added in solution.
- Buffering capacity: Contained in inoculum/waste(water), or added in solution.
- Equipment: Type and volume of vessels.
- Operating conditions: Gas-to-liquid ratio, temperature, waste(water) dilution, control experiments, sampling frequency, substrate-to-inoculum ratio, others.
- Methods of analysis: Detection principle, measuring devices, variables monitored, inhibition of specific enzymatic pathways.
- Data interpretation and reporting: Length of experiment, correlation to controls, statistical analysis.

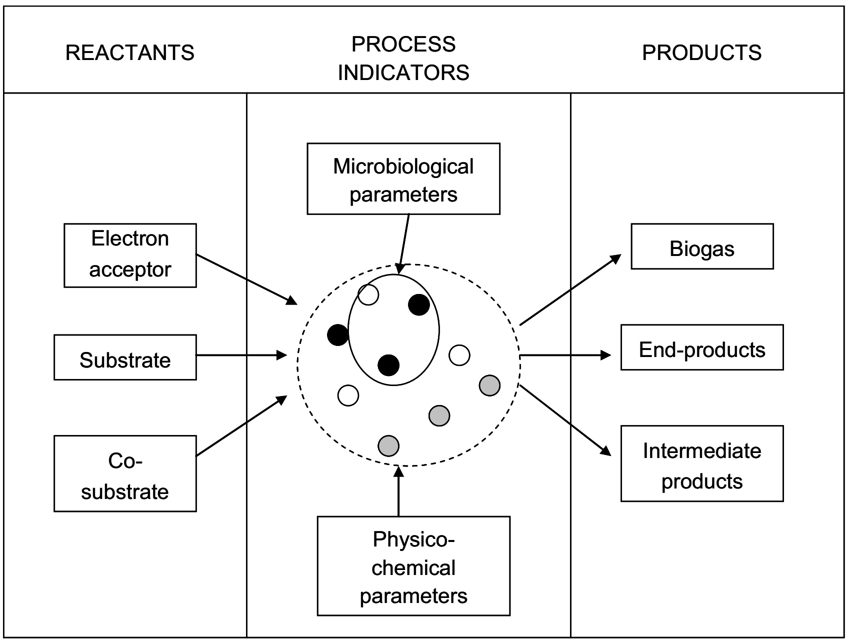


Figure 4. Parameters that can be assessed during a BMP assay. Adapted from *Rozzi and Remigi (2004)*.

3.2 Factors affecting waste(water) methane potential under anaerobic batch conditions

The methane potential of a specific waste(water) is defined as the volume of methane produced per unit of organic matter added into a vessel operated under anaerobic methanogenic activity. Methane potential can be also determined from knowing the characteristics of the waste(water), which is defined as the theoretical methane potential and is based on the oxidation state of the organic carbon present in the organic material of the waste(water). From the elemental composition of a waste(water), Buswell and Neave (1930) defined the allocated theoretical fractions going to methane and carbon dioxide neglecting biomass growth:

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 \qquad \text{Eq. 7}$$

The theoretical methane potential functions only as a reference value for determining the methane potential of a waste(water), since although in a minor percent, a fraction of it goes to biomass growth. Furthermore, the amount of carbon dioxide estimated from this equation is a rather empirical value since the mass balance of inorganic carbon in an experimental vessel depends on several factors rather than only on the waste(water) composition. Examples of such factors are pH, temperature, ammonium released during biodegradation, relative volumes of liquid and gas (Angelidaki and Sanders (2004);Kleerebezem and van Loosdrecht (2005);Rozzi and Remigi (2004)). Another common way to estimate the theoretical methane potential is based on the waste(water) content of carbohydrates, proteins, lipids, acetate and propionate, when known. For each of these components a methane potential coefficient has been determined by Angelidaki and Sanders (2004) based on their elemental compositions. This way of estimation proved to be less reliable in food-processing industry wastewaters due to the lack of representativity of the volatile solids (VS) analytical determination to quantify wastewaters' organic fractions. COD is recommended in the assessment of methane potential for complex wastewaters (Paper **I**).

The practicability of estimating the theoretical methane potential relies on comparing it with the ultimate methane yield obtained in a bioreactor (B_o according to Hashimoto et al. (1981)). The ultimate practical methane yield has been adopted in many studies as an indicator of the suitability of a specific waste(water) to produce methane under anaerobic conditions. Its estimation is done by measuring the methane produced at specific time-intervals during a batch experiment, recording the ultimate cumulative value when no further methane production is observed and expressed per the amount of organic matter added.

3.2.1 Wastewater characterization

Waste and wastewater is often of a complex composition which is difficult to describe in detail (Angelidaki and Sanders (2004)). The relevance and level of detail of waste(water) characterization for methane potential assessments varies widely in the literature. The majority of the studies show only COD and VS characterization to express the ultimate practical methane yield (B_o). In order to determine the elemental composition of the substrate a detailed waste(water) characterization is needed (**I:** Maya-Altamira et al. (2008);Davidsson et al. (2007);Kleerebezem and van Loosdrecht (2005);Speece (1996)). Other authors have determined these elemental fractions directly by an $C_nH_aO_bN_cS_d$ analyzer but details on the analytical method applied are lacking (Buffiere et al. (2006);Raposo et al. (2006)). In spite of the increased amount of work by carrying a detailed characterization, it can be worth to do it since the influence of waste(water) sources and/or pre-treatment technologies on its physico-chemical composition are identified, so strategies can be suggested for handling before and after collection to enhance its methane potential. Furthermore, the analytical determination of proteins, lipids, and fatty acids can also provide valuable information such as potential inhibition of the methanogensis activity (**I:** Maya-Altamira et al. (2008);Davidsson (2007);Hansen et al. (2007);Kleerebezem and van Loosdrecht (2005);Speece (1996)). In the other hand, complex wastes can be problematic to characterize analytically, specially for proteins and carbohydrates fractions since the former is estimated by a standardized conversion factor from total nitrogen measured and the latter is empirically assumed to be part of the residual COD or VS apart from proteins and lipids. Carbohydrates have also been determined taking a reference sugar as a calibration compound but this has led to uncertainties in material balance of the waste (Buffiere et al. (2006)). The advantages and disadvantages of a detailed characterization make this a challenging area for research, particularly for the prediction of methane potential. Statistical analysis of waste(water) sampling and of their analytical characterization aids on the representativity of the results as a solid basis for this prediction (Paper **I**).

3.2.2 Inoculum

The presence of inhibitory substances in the waste becomes of primary importance for the inoculum, because assays can give underestimated results providing that microbial adaptation can improve the methane potential significantly. However, when assessing unknown waste(water)s their inhibitory properties are not known either, so BMP experiments can provide this information. Furthermore, according to Rozzi and Remigi (2004), the reproducibility of the assessment may be improved when a non-specialized inoculum such as sludge from a municipal digester is used (**I:** Maya-Altamira et al. (2008);Parawira et al. (2004)). Other studies have shown that adaptation prior to the experiment either with similar wastes (Buffiere et al. (2006);Hashimoto (1989)), or with synthetic methanogenic substrate (Akram and Stuckey (2008);Raposo et al. (2006))

prevents the assessment from inhibition enhancing the methane potential. The inoculum is an important factor that cannot be easily standardized since its activity depends on its history prior to the experiment and its available enzyme load. Unless it is inoculated with the specific waste(water) to be assessed, it is not known whether the bacteria and enzymes are available for its complete degradation, and when acclimated, the assessment of the waste(water) becomes more specific to the specific characteristics of the waste(water), so results should be carefully interpreted when compared to other waste(water)s' assessments. The IWA Task Group (2006) suggests as general rules that it should be fresh, originated from a reactor operated at the same temperature and with a similar feed composition, and pre-incubated in order to deplete the residual biodegradable organic material.

3.2.3 Substrate concentration and Substrate:Inoculum ratio

The methane potential of food-processing industry wastewaters does not depend solely on the organic matter contained in it (Paper **I**). The effect of initial COD or VS on the methane potential of waste has only been identified in a few studies (Raposo et al. (2006);Gungor-Demirci and Demirer (2004)). Indeed, it is difficult to separate the substrate's COD effect since the inoculum may also contain some COD material which was not degraded during the pre-incubation period. The interaction of the bacteria consortium in the inoculum with a specific waste(water), may be due to other characteristics of the waste(water) besides the substrate COD (Paper **I**).

Substrate:Inoculum (S:I) ratio has been studied with different types of waste(water)s. Hashimoto (1989) found a dramatic increase on methane yield as the ratio decreased from 6.2 to 4.0 $gCOD_w/gCOD_X$ (waste to inoculum), independently of the substrate COD concentration (ball milled wheat straw), using pre-incubated beef-cattle manure as inoculum. Ultimate practical methane yield increased from 0.025 to 0.243±0.01 l-$CH_4/gCOD_{in}$ at standard pressure and 35^{o}C conditions, although it did not increase further at lower observed ratios. Chen and Hashimoto (1996) recommended to work with a minimum ratio of 4.8 $gCOD_w/gCOD_X$ but they used glucose as substrate which can't emulate the complexity of a waste thus this level can differ greatly at other wastes assessed.

Parawira et al. (2004) used potato waste as substrate at 37^{o}C and found optimal ratios at around 13 gVS_w/gVS_X, where higher ratios (i.e. >20 gVS_w/gVS_X) and lower ratios (i.e. <9 gVS_w/gVS_X) decreased methane yield about 40%. However, since methane yield was expressed as a function of VS degraded, it is not known whether the amount of waste added affected its methane potential.

On the other hand, Raposo et al. (2006) did not observe any effect of the ratio on the assessment of maize methane potential. All ratios assessed, i.e. 1 to 3 gVS_w/gVS_X, presented methane yields at around 0.211±0.01 l-CH_4/gVS_{in} at standard conditions of temperature and pressure (STP).

From all these studies, it was noticed that there was a S:I range where the ultimate practical methane yield increased and/or decreased sharply, whereas outside this range S:I did not affect anymore. That may have been the reason why Raposo et al. (2006) did not find any influence, since they applied ratios within a narrow range. The reason for this may be that certain waste(water)'s physico-chemical components affected negatively methanogenesis at a concentration which could have exerted its influence sharply after reaching this level. From assessing 10 food-processing industry wastewaters at different dilutions, it was found at Paper **I** that the influence of S:I ratio on their ultimate practical methane yield, within the range of 0.15-3.75 $gCOD_w/gCOD_X$, reached up to 60% (see Figure 5). The specific influence the S:I ratio range did for each wastewater was very different for three of the wastewaters (VFO, FMS, SPE) where a sharp decrease in B_o was observed in a small range, particularly for FMS and SPE. VFO was the only wastewater inhibiting completely the methanogenic activity at a ratio of 2.5 $gCOD_{ww}/gCOD_X$. The wastewater from the vegetables processing industry (VPE and VLO) affected B_o in a similar way, regardless the product processed and physico-chemical composition was different (see Paper **I**). This was not the case for the wastewater from the fish meals processing industry which affected more B_o during the summer production.

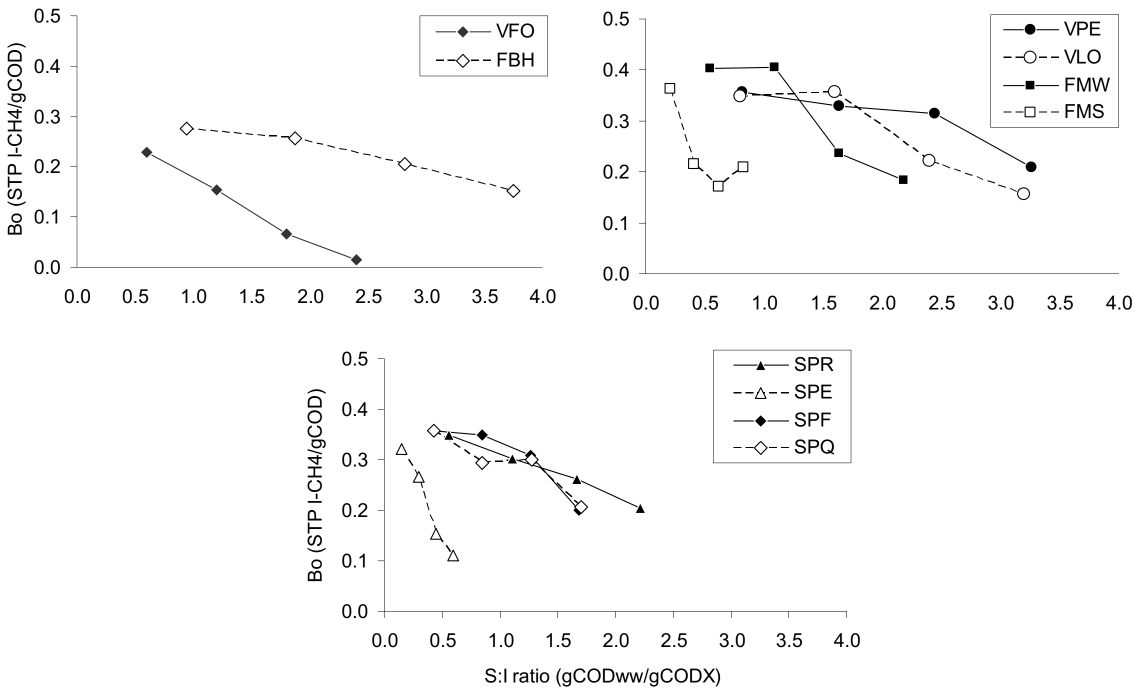


Figure 5. Influence of Substrate:Inoculum ratio on ultimate practical methane yield for wastewaters assessed at Paper **I** (adapted). For abbreviations see Table 1.

The wastewater from the slaughterhouse affected similarly to B_o when sampled before the equalization tank (SPR, SPF, & SPQ), but the S:I ratio range for the equalized effluent (SPE) was quite narrow affecting sharply to B_o. These differences were attributed to the physico-chemical characteristics of the wastewaters rather than to the substrate concentration (COD) or to the S:I ratio.

3.2.4 Waste(water) physico-chemical characteristics

There are numerous studies in the literature which have observed different wastes giving different methane potentials, particularly assessing the organic fraction of municipal solid waste (e.g. Hansen et al. (2007);Davidsson et al. (2007)). However, few of them have identified specific physico-chemical characteristics enhancing/decreasing the ultimate practical methane yield of the waste.

Angelidaki and Sanders (2004) determined from the oxidation state of the elemental composition of carbohydrates, proteins, and lipids, that they should give different methane potentials per gram of molecular weight. This is a theoretical basis to suggest that different organic fractions of the waste have different potentials. However, it doesn't tell anything about the interaction amongst them or with inorganic compounds, i.e. ammonium nitrogen or inorganic carbon, when anaerobically digested.

Ammonium/ammonia is a necessary nutrient for the growth of bacteria involved in the anaerobic digestion process, but when the concentration exceeds a certain concentration limit, it inhibits methanogenesis (Angelidaki and Ahring (1994)). In the cases where nitrogen either in the organic or inorganic form is present in the inoculum or in the waste(water) it also stimulates the growth of microorganisms and buffers the liquid media and prevents from acidification. However, when the concentration level of ammonium exceeds something around 0.7 g-N/l, it can turn inhibitory if pH levels go higher than 8. Industrial waste(water)s are characterized for being highly concentrated in organic carbon and some of them contain low levels of nitrogen. These high Carbon:Nitrogen ratios can make them difficult to treat under anaerobic conditions, particularly when assessing their methane potential since under batch conditions there are higher chances the liquid media turns acidified. Synthetic media can be added as a source of nutrients in the cases where very low concentrations levels are present in the inoculum and in the waste(water). BMP assays which have not reported any nitrogen limitation or inhibition have been around Carbon:Nitrogen ratios of 14 (**I:** Maya-Altamira et al. (2008);Zhang et al. (2007);Davidsson et al. (2007c);Parawira et al. (2004)). pH plays a major part on anaerobic digestion since it influences the activity of the microorganisms such as hydrolytic enzymes, acidogens and specially, methanogens. Anaerobic digestion occurs within a pH range of 6.0-8.3 (Angelidaki and Sanders (2004)), but it is recommended that the buffering capacity inside the batch reactor for methane potential assessments is capable of maintaining the lowest pH of 6.8 (Chen and Hashimoto (1996)). Parawira et al. (2004) observed acidic pH levels, i.e. 5.9 and 4.9, when increased potato waste was fed into co-digestion batch reactors with sugar beet leaves, which led to a decreased ultimate methane yield by more than 60%. They also observed that the initial partial alkalinity was lower in these two cases. The effect of initial partial alkalinity as bicarbonate alkalinity contained in food-processing industry wastewaters over the ultimate practical methane yield (B_o) was studied in Paper **I**. We noticed that the presence of higher concentrations of bicarbonate alkalinity in some wastewaters enhanced ultimate yields for slaughterhouse flocculated wastewater, and maximum achieved yields for all wastewaters assessed. This effect was particularly evident when acetate was also present in the wastewaters. As described by Kyazze (2007), we found a couple effect of bicarbonate alkalinity that contrarested a negative effect of relatively higher acetate concentrations of wastewaters from slaughterhouse production (flocculated liquid fraction), and leek and fried onion production. However, this positive coupled effect was not observed in any of the wastewaters when they presented acetate concentrations higher than 375 mg/l. The presence of acetate in anaerobic batch assays and its effect on biogas production was also studied by Akram and Stuckey (2008). They observed a decreased biogas production when acetate was fed together with glucose, compared with assays fed with glucose only. At our study we concluded that the effect of bicarbonate alkalinity was evident only when wastewaters were diluted at 25% and 50% of their total concentration. When they were at their 75% concentration or were not diluted at all and when acetate was present, a statistical relationship was found showing a negative effect on their ultimate methane yields.

By considering dilution on the methane potential assessment of unknown food-processing industry wastewaters, it provided valuable information on the optimal dilution ranges and influencing phýsico-chemical characteristics for obtaining maximum practical methane yields. This avoids underestimated experimental methane potentials and a more accurate determination of the waste maximum capability (Angelidaki and Sanders (2004)).

3.3 Factors affecting organic waste(water) degradability under anaerobic batch conditions

3.3.1 Waste(water) fractionation

For the treatment of food-processing wastewaters, the hypothesis that all biodegradable COD is soluble and readily biodegradable may not be valid (Bernet (2006)). These wastewaters may contain an organic fraction which is slowly hydrolysable that can influence their overall anaerobic biodegradation. It is important then, to characterize this fraction and consider it in the design because its biodegradation could be the rate-limiting factor (Bernet (2006)). Moreover, the biodegradation of organic materials have relation with its substrate characteristics, and even if the degradation rate of VFA depends on the VFA-hydrogen-CO_2 concentrations and the acetogens/methanogens interactions, the production rate of VFA depends on the physico-chemical characteristics of the waste(water) (Shin and Song (1995)). Several studies have highlighted the importance of characterizing the fractions that are relevant for defining the waste's overall biodegradability. Turick et al. (1991) found inversed correlation amongst lignin content and woody biomass degradation, pointing out the importance of other chemical and physical parameters in the substrate. Biswas et al. (2006) determined the carbohydrates fraction by resting what measured as moisture, ash, crude protein, and fat, and determined the degradable carbohydrates fraction by analytical enzymatic digestion with amyloglucosidase. In this way they assumed that the non-degradable fraction is entirely composed by carbohydrates. Frederic et al. (2007) observed differences on the hydrolysis rate of COD particulate and COD soluble in spent apples, and Davidsson et al. (2007) observed a decreased methanogenic activity by increasing ammonium concentrations in the digestion of organic municipal waste.

The anaerobic biodegradability of a waste(water) can be defined from the methane production according to total organic material fed (Zhang et al. (2007);Speece (1996)), or what is stoichiometrically possible to degrade according to the waste(water) physico-chemical characteristics (Buffiere et al. (2006); Angelidaki and Ahring (1997)). However, this estimation of the biodegradability fraction neglects biomass growth and is carried under optimal conditions, i.e. it is the inherent biodegradability which is measured (e.g. Paper **I**). Although this estimation is comparable with that of the biological oxygen demand (BOD) since this is also carried under optimal conditions, it is more an ultimate value. The biodegradation of an organic waste(water) is rather important to monitor because it gives the dynamics of the substrate depletion and methane production, necessary for the design or starting-up of anaerobic reactors. Three important aspects should be considered when assessing the anaerobic biodegradability of a waste(water) (Batstone et al. (2007);Kleerebezem and van Loosdrecht (2005); Angelidaki and Sanders (2004)):

- Methane production reflects the overall biodegradation only when methanogenesis is the non-limiting process, else, it should be complemented with the monitoring of primary depletion products, i.e. from hydrolysis and acidogenesis;
- The fraction of organic matter incorporated into the biomass should be taken into account;
- The biodegradability fraction defines the inerts fraction, the former being split amongst the other organic fractions determination (i.e. carbohydrates, proteins, lipids).

To define this fraction, Babuna et al. (1998) and Ekama et al. (2007) recognized the presence of biodegradable products together with non-biodegradable fractions in the anaerobic effluent of treated pulp and mill wastewater and activated sludge respectively. The former estimated it upon a glucose control assuming no COD particulates in the

influent, and validated it by monitoring COD particulates and soluble getting quite accurate estimated results. The latter estimated it upon the sludge's yield, age, hydrolysis, and decay in the anaerobic digester. They also validated this model based on COD particulate and soluble experimental data (as hydrolysis substrate and product) which led to COD balances close to accurate (+4%). The difference amongst estimated and measured biodegradability fractions was attributed to an inaccurate COD analytical determination in sludge, which agrees with Huete et al. (2006) referring to biodegradability only as an estimate within a range rather than a concrete value.
In our study (Paper **II**), we determined the degradable fraction assuming all COD in the wastewater was degraded either to VFA, methane, or carbon dioxide, and it was also going to biomass growth. Since there were not analytical measurements for carbon dioxide in the gas phase neither for biomass growth, theoretical coefficients were assumed based on the oxidation state of the substrate (for CO_2), and an average COD coefficient for biomass growth based on Angelidaki and Sanders (2004). This calculation method fitted well to the specific methane production dynamic patterns for ten out of eleven experiments, however it did not to the VFA dynamic patterns where it was underestimated most of the times. This shows the difficulty in setting a concrete value for this fraction, particularly for VFA since their presence and further degradation is regulated by several interconnected complex processes. Further manipulation of the biodegradable fraction can be done by reducing the biomass and/or CO_2 coefficients. However, as an ultimate value it proved useful since results were comparable to other organic waste(water)s assessments in literature.

3.3.2 Inoculum and Substrate:Inoculum ratio

The history of the inoculum is important at assessing the anaerobic degradation of complex organic waste(water)s. The manner in which the culture has been developed determines which species are present (Grady et al. (1996)). As longer the culture is exposed to a specific substrate as more affinity develops for it, thus higher removal potentials are achieved (Grady et al. (1996)), i.e. adaptation occurs. Apart from substrate adaptation, physiological adaptation can also interfere with the growth, e.g. when a continuous culture is deposited into a batch culture. The degree one or the other affects methanogenic activity depends on the history of the inoculum before it is removed from its original environment. Several authors have applied inoculum adaptation to the waste(water) under study (Biswas et al. (2006);Gavala and Lyberatos (2001); Babuna et al. (1998)), however adaptation is better recommended when carrying out specific kinetic studies and an immediate response is awaited (Angelidaki and Ahring (1994)). For biodegradability assessments it would be more important to assure reproducibility by using a standard inoculum, as mentioned before. Nevertheless, it is still important to assure the reactor where it was taken from has methanogenic activity occurring.

Substrate:Inoculum ratio is an important factor of consideration when designing biodegradability assays since its effect has been correlated with specific methane production in many studies in the literature when assessing a specific waste(water). It has been stated that low volumes can blur the methane production since the inoculum will also produce methane, but in the other hand high volumes can lead to acidification (Angelidaki and Sanders (2004)). This agrees with Frederic et al. (2007) and Chen and Hashimoto (1996) that observed acidification with high S:I ratios, although the former did not report which ratios and the latter referred to high ratios those within the range of 33 to 67 $gCOD_w/gCOD_X$ which is much higher than any other study observing the S:I ratio influence. In the other hand, when evaluating the influence of low ratios within a narrow range there was very little (maize-Raposo et al. (2006)), or no difference on specific methane production for spent apples (Buffiere et al. (2006)), and potato with sugar beets (Parawira et al. (2004)). The ratios they investigated, particularly when no difference was observed, were within very narrow ranges (i.e. 0-1 gVS_w/gVS_X), and as it has been reported in our study (Paper **II**) there were differences amongst different ratios but when the range covered was wider (0-5.5 $gCOD_{ww}/gCOD_X$). The effect of inoculum adaptation overcame the effect of Substrate:Inoculum ratio but only at a small range (0.5-0.8 gVS_w/gVS_X) for spent apples (Buffiere et al. (2006)). Finally, the physico-chemical characteristics rather than the ratio, influenced more to the biodegradability of the wastewaters at our study (Paper **II**).

3.3.3 Organic fractions and cumulative methane production

The change in the protein synthetizing system (PSS) in the cells of the inoculum at a batch experiment depends on the amount of substrate that is provided (Grady et al. (1996)). However, as described before, different fractions of the substrate have different uptake dynamics on the basis of the different energy available for the degraders of each fraction, and their interaction. Zhang et al. (2007) observed very little difference on cumulative methane yield based on two initial food waste loadings (6.8 and 10.5 gVS/L) with very similar composition (i.e. low standard deviations from different samples). In the other hand, Biswas et al. (2006) observed increased methane production with higher initial slurry concentration, but notably the carbon dioxide production was higher and they attributed it to the higher carbohydrates and proteins contents in the slurry. Something that was noticed in these two studies is that the curve describing cumulative methane production, presented different dynamic patterns. In the former study, it increased almost linearly during the first five days at a relatively slower rate in comparison to the next nine days. In the latter it also increased linearly but during the first two days and followed a steady line until day 9 when it increased again. Frederic et al. (2007) studied this in more detail and showed that about 60 to 85% of the COD soluble fraction of spent apples was degraded during the first five days, whilst the

COD particulate fraction followed a linear degradation during the same time period, showing increasing biogas production. The shape of the two curves presented, for the soluble fraction, an almost complete methanogenesis whilst for the particulate, it showed the hydrolysis as the rate-limiting step depending only on substrate and biomass concentration. Other authors (Turick et al. (1991) and Parawira et al. (2004)) discussed that the reason for these different two phases may have been due to the rapid degradation of easily degradable compounds such as non-structural carbohydrates during the first phase, and that the second phase was due to the degradation of more complex material. Turick et al. (1991) also attributed this second phase to the shift in the microbial population towards organisms able to degrade less accessible polymers. Biswas et al. (2006) assumed the first phase was due to the degradation of carbohydrates and proteins while the second phase was due to the lipids' until saturation was reached. However, a lack of discussion regarding these differences was noticed.

At Figure 10 (Paper **II**), it is noticed that the three of the wastewaters assessed presenting a more rapid increase of methane production (SCE, FMW, SPQ) had more homogenous proportions of proteins/lipids or carbohydrates/lipids -i.e. about 32-40% total COD-. In the contrary, FBH and SLC had about 20% and also higher solids contents, as it can be seen at the figure as X_I (i.e. particulate inerts calculated on wastewater COD particulates). Thereby affecting their cumulative methane production pattern, yielding less methane per gram of COD. Particularly for FMW, a linear degradation until day 10 was observed meaning that during this period the methane production depended solely on the hydrolysis of its carbohydrates fraction. For VFO, although there was about 40% fraction of carbohydrates/proteins/lipids in the wastewater, the presence of acetate (~10%) inhibited seriously the methanogenic activity in this experiment as it is seen in the figure. This combined with the fact that 80% of this fraction was carbohydrates itself, agrees with Akram and Stuckey (2008), who observed a coupled toxic effect of initial/intermediate acetate during the degradation of glucose in the presence of acetate.

3.3.4 Hydrolysis of organic fractions

According to Pavlostathis and Giraldogomez (1991) the rate of hydrolysis is a function of hydrolytic biomass concentration and type of particulate organic matter, but also of pH and temperature, amongst others. Vavilin et al. (1996) described the hydrolysis of particulate organic matter as a two-phase process where bacteria first attach to the solid particles nearby and after produce monomers which fall into the liquid phase. If those can't find a particle surface to attach again, then are degraded at a constant depth per unit of time. On the other hand, Sanders et al. (2002) described the hydrolysis of dissolved polymers such as gelatine and dissolved starch, as a process dependent only on the enzyme activity and not particles concentration. They both referred to Confer and Logan (1998) and Goel et al. (1998) to relate this hydrolysis activity directly to sludge concentration. Either in one study or the other, they both coincide the rate of hydrolysis depending on solid particles, although the related particles in the sludge should not be inert as they would not release any enzyme. And, Vavilin et al. (1996) did not distinguish what kind of particles are available for attachment.

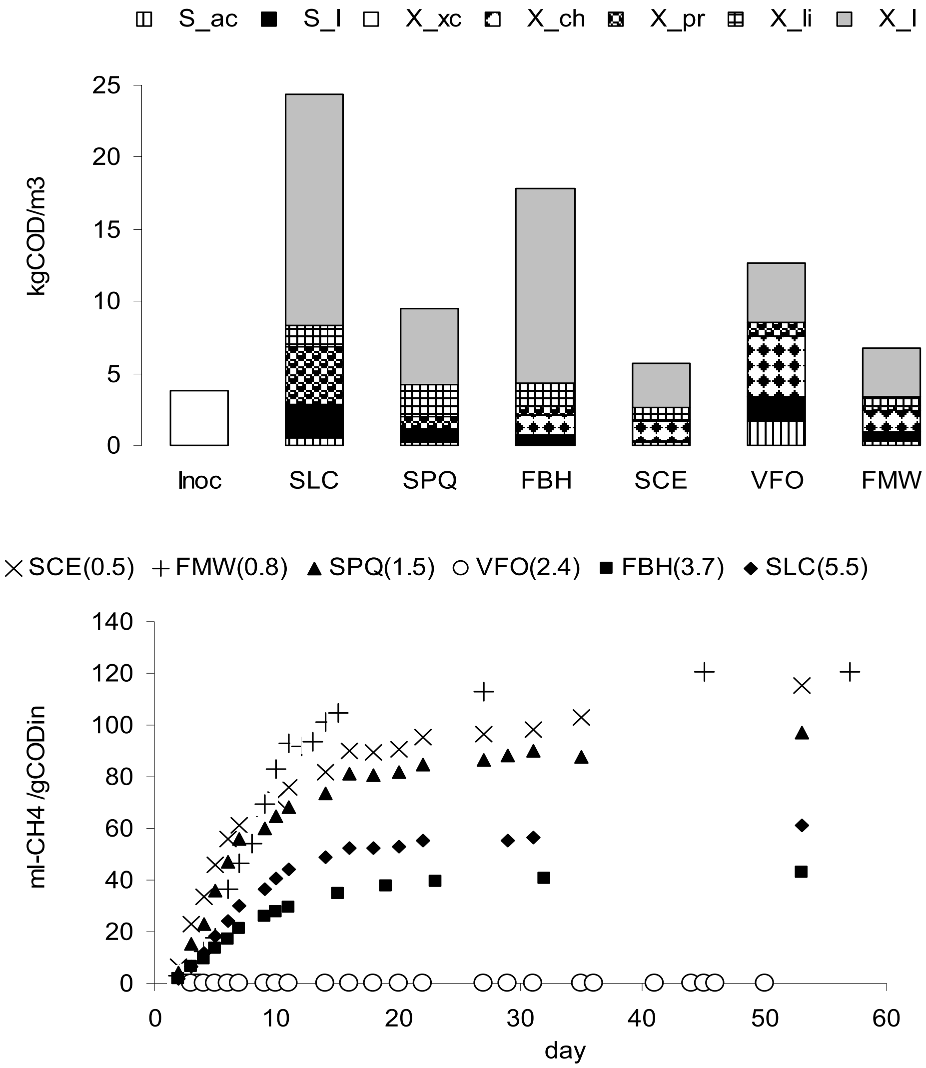


Figure 6. Organic fractions (top) and cumulative methane yield patterns (bottom) for slaughterhouse (SCE, SPQ, and SLC), fish meals for aquaculture (FMW), vegetable fats & oils (VFO), and fish for human consumption (FBH) wastewaters at different S:I ratios (numbers in parenthesis) (*Paper **II***).

In our study (Paper **II**), we found two wastewaters (SLC and FBH) containing high particle fractions which could be available for attachment (Figure 6), thus promoting hydrolysis of both particles and soluble polymers. However VFA did not accumulate as much as for the other wastewaters (Paper **II**), neither the inorganic nitrogen was released (see Figure 7). Actually, the inorganic nitrogen profile reflected a consumption of ammonium thus indicating uptake of sugars and acetogenic/methanogenic activities rather than hydrolytic activity. This indicates that in spite of their particles' availability, it was only the dissolved carbohydrates, lipids, and proteins which were hydrolyzed the first, and that the uptake of nitrogen for growth was higher than the release of free ammonium from the hydrolysis of proteins. Furthermore, after day 10 it was seen an increase in VFA for FBH (Paper **II**), confirming that it was then when particulate proteins were hydrolyzed as it can also be seen in Figure 7. It could also be due to the fact that the inoculum, at the sampling point, was adapted to higher concentrations of ammonium in the substrate making it very active in its uptake (Davidsson et al. (2007)).

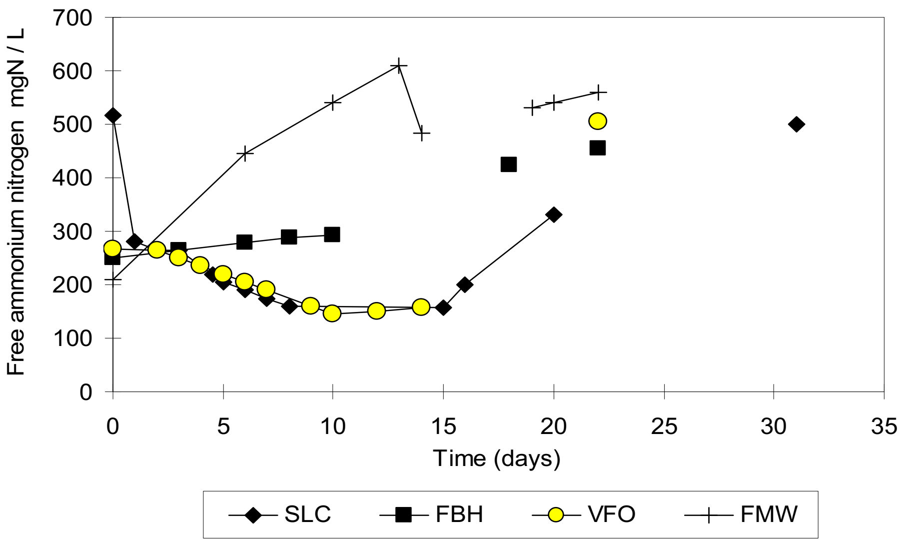


Figure 7. Free ammonium nitrogen for four of the wastewaters assessed by BMP assays during the first 35 days.

Regarding the hydrolysis of lipids, Bernet (2006) argues that the degradation of fat-rich wastewaters is slower than that of fat-poor due to its low hydrolysis rate. In the other hand, Biswas et al. (2006) observed that increasing by double the fat concentration in the substrate did not make any difference on the cumulative methane production rate. This agreed with Pavlostathis and Giraldogomez (1991), who by reviewing hydrolysis studies found that the hydrolysis of lipids and fermentation of long chain fatty acids (LCFA) were undistinguishable for achieving methane production, meaning that the hydrolysis was not the rate-limiting step. Indeed saturated LCFA were found to be less inhibitory to methanogens activity than unsaturated, thus the hydrolysis of lipids and subsequent fermentation of LCFA to VFA may not represent a limiting factor when lipids and LCFA are of saturated composition (Bernet (2006)).

In our study (Paper **II**), we observed that the wastewater presenting the fastest methane production (SPQ) was that which presented the highest lipids fraction. Lipids were rapidly hydrolyzed and fermented to VFA and methane, together with the acetate degradation in the wastewater. This emphasizes what Zeeman and Sanders (2001)

suggested that the hydrolysis of lipids occurs in the presence of methanogenesis. This also reinforced that the inoculum had a healthy methanogenesis activity.
In relation to carbohydrates hydrolysis, it was evident from our study that its presence together with that of acetate in the wastewater, raised the concentration of acetate decreasing pH to inhibitory levels to methanogens since the first day of the experiment. This not only inhibited methanogenesis, but also affected the hydrolysis rate, slowering down the presence of VFA. This is in accordance with He et al. (2006), who also observed decreased VS, carbohydrate, and protein reductions by the hydrolysis of polysaccharide-rich organic waste at low pH and increased acetate concentrations. Similar to the case of free ammonium (Lay et al. (1998)), it was more the effect of dissociated acetate that prevented an initial methanogenic activity rather than that of undissociated acetic acid. This led to a further accumulation of acetate which was not degraded during the first days.

3.3.5 VFA accumulation and degradation

The interdependence of the bacteria is a key factor in the anaerobic digestion process. Under unstable conditions, intermediates such as VFA and alcohols accumulate at different rates depending on the substrate and operational perturbations (Ahring et al. (1995)). If there is an excess supply of fermentable substrate, a drop of pH may occur, inhibiting methanogens activity. If the pH levels decrease down from 6.0, inhibition of the hydrogenotrophic methanogenesis occurs, blocking the uptake of hydrogen accelerating the “turn over” of the system. This translates into accumulation of fermentation products and ceasing of methanogenesis (Schink (1997)).
Raposo et al. (2006) observed that acetate, propionate and iso-valerate were the organics remaining the longest in maize anaerobic batch reactors. They also observed an increase in propionate concentration levels by higher initial substrate loading, and inhibition of its degradation by acetate concentrations higher than 1.4 g/L. In spite it is suspected that maize contains carbohydrates as the biggest organic fraction, they did not make any statement about the organic fractions in the waste. In our study (Paper **II**), we found that the vegetable fats & oils wastewater containing a big fraction of carbohydrates (~60% substrate COD) and a significant fraction of acetate (~25% substrate COD) accelerated the pH drop inhibiting methanogenic activity and very likely increasing the levels of carbon dioxide and hydrogen in the batch reactors. Further hydrolysis of carbohydrates and proteins increased even more the acetate levels and dropped even more the pH. A great amount (i.e.>50%) of the VFA accumulated was propionate and butyrate (~40%) at day 50, which indicates that methanogenesis bacteria may have been absent by then.

3.3.6 Lag-phase

Lay et al. (1998) recognized the importance of bacterial acclimation when a specific toxic component is assessed, by defining the inactivity period that sludge presented in the presence of free ammonium/ammonia at different pH levels. They differentiated physiological adaptation from adaptation to free ammonium, and defined two cases of inhibition effect. Such cases were (i) the initial shock loading when the bacteria were not acclimatized, and (ii) the steady inhibition that occurred for the well acclimatized bacteria. The former effect was possible to define from the lag-phase time, and the latter from the methane production rate. Notably, they found that the latter effect was caused by the free ammonium concentrations whilst the former effect was found from the free ammonia. In this way, pH was an important factor of influence. Chen and Hashimoto (1996) also evaluated the effect of pH and S:I ratio on the duration of the lag-phase period and found optimal pH levels at around 7.0. They noticed that the duration of this period was quite sensitive to small pH changes in the range of 6.4-6.9. At our study (Paper **II**), we noticed also the influence of pH on the lag phase since when levels were lower than 6.4 (VFO), hydrolysis was delayed and methanogenesis did not occur, whilst for Mix 3 and Mix 5, the pH levels>6.4 during the first 20 days making some hydrolysis and methanogenesis happen.

3.3.7 Buffering capacity

Ammonium is often cited in the literature as a buffer for the anaerobic degradation of organic waste(water)s. From our study (Paper **II**) we observed that the ammonium nitrogen dynamics were always lower than 0.5 gN/L, except for FMW where it reached 0.7 gN/L. Since pH levels were not higher than 7.5 it was concluded all ammonium was undissociated thus not inhibiting. Buffering capacity was then provided in most of the cases, except for VFO, Mix 3 and Mix 5. In the period ammonium was consumed (as seen in Figure 7), the rest of the wastewaters' experiments showed an increased bicarbonate production (results not shown), providing enough buffering to keep pH levels inside the methanogenic range.

3.3.8 Co-digestion

Many successful co-digestion studies have been done mixing all sorts of organic waste(water)s together, or mixing them with sewage sludge or manure. As it has been described in these studies, the most common strategy is to treat together nitrogenous with non-nitrogenous organic wastes to compensate the lack of nitrogen for growth and prevent inhibition. However, attention has been drawn on treating available waste(water)s according to their location, from a specific region or area where specific organic waste(water)s are gathered and sometimes mixed with activated sludge in an continuous stirred anaerobic tank reactor (Alatriste-Mondragon et al. (2006)). In this report we focus only on describing the most relevant aspects of our co-digestion studies (Paper **II**) in relation to the influence of their organic fractions in individual and co-digestion experiments on their biodegradation. We observed that Mix 4 increased the specific methane production rate significantly if compared to FMW and SCE individually. There was also a very fast conversion of VFA to methane since VFA were detected in very low concentrations, differently from FMW and SCE experiments. The co-digested organic distribution was very similar to the individual distributions, thus this enhancement was only attributed to a higher COD initial loading, and a slight decrease in the carbohydrates fraction compensated with an increased acetate fraction. SPQ and SCE favored the hydrolysis step when co-digested with SLC and FBH. Particularly for Mix 1, it resulted benefic to increase its lipids fraction if compared to SLC. In the cases of Mix 3 and Mix 5, they both presented similar specific methane production and VFA dynamic patterns. Notably, hydrolysis was enhanced compared to VFO, since pH levels did not drop as fast as they did in the individual experiment (VFO). This produced methane but the further increase of acetate kept the specific methane production very low until day 20. However, there was a switch of VFA accumulated since in these co-digestion experiments it was acetate the main VFA present (~85%). This may have kept hydrogen levels lower thus promoting hydrogenotrophic methanogenesis which was also reflected in higher pH levels (i.e. 6.6-6.9 compared to 6.1). This clearly gave the inoculum the opportunity to adapt to the conditions promoting methanogenic activity. A longer time period for the experiment would may have given increased methane production.

4 Modeling anaerobic digestion of organic waste(water) under batch conditions

The most commonly applied biochemical models to represent anaerobic biodegradation of organic waste(water) are steady state models, 1 or 2 step dynamic models, and fully structured models (Batstone (2006)). Steady state models cannot describe the temporal changes in bacteria and products during transitions from one steady state to another, whilst dynamic models pin-point potentially dangerous operating conditions where accumulation of intermediate degradation products may lead to process inhibition or failure (Bozinis et al. (1996)). The physico-chemical components play an important role, differently from the activated sludge models, since they regulate the gas flow and pH. The former is indeed the most important output variable from a design point of view, and the latter is an extremely influencing process parameter for biological growth and for the carbonate liquid-gas system.

The gas flow is often calculated accordingly to the particular case studied, since it depends not only on the physico-chemical components in the liquid system, but also on the actual temperature, pressure, and mixing conditions, the liquid and headspace volumes in the bioreactor, and liquid-gas transfer constraints. According to Batstone (2006) the different ways of calculating it are summarized as follows:

- From COD conversion across the system and assuming a fixed gas concentration. Used with simple models and failing to predict it outside steady state conditions.
- From the sum of individual gas productions (i.e. CH_4, CO_2, and H_2 -optional-), assuming equilibrium or considering liquid-gas transfer theory.
- From the sum of individual gas productions (i.e. CH_4, CO_2, and H_2 -optional-), but using a pressure differential between headspace and atmosphere.

The pH calculation can be implemented as a differential equations system of the active ionic concentrations of VFA, ammonium/ammonia, bicarbonate and water, plus concentrations of cations and anions added (Batstone et al. (2002)). It can also be calculated as an algebraic system assuming that the hydrogen ion is in equilibrium when system simulations require it (Rosen and Jeppsson (2006)).

Depending on the level of complexity, the model can be defined as an algebraic or differential equation or set of equations. A general conversion process can be assumed to represent the whole digestion, or it can be as detailed as to represent each conversion process by a set of equations. Early models were single state kinetic orientated on homogenous but complicated substrates for evaluating biodegradability and gas flow at steady state, and more recent structured models have been implemented to simulate particulate scenarios. The latter models require a full characterization of kinetic rates and inhibition factors to determine mass balances (Batstone (2006)).

A critical part of mass balance is the rate equation, being the most popularly used in dynamic models, the Monod/Michael-Menton kinetic defined as (Batstone (2006)):

$$\rho = k_m \frac{S}{K_S + S} X \qquad \text{Eq.8}$$

Whereas, in a batch reactor, the sum of time derivatives for each state variable corresponds to the kinetic conversion rates and stoichiometric mass balances (Siegrist et al. (2002)):

$$r_i = \sum_{j=1}^{n} \rho_j v_{j,i} \qquad \text{Eq.9}$$

The overall rate of product formation may depend on the steps preceding the last slow step, but it will not on any of the subsequent more rapid steps. This dictates which conversion process is the rate-limiting, which will cause process failure to occur under imposed conditions of kinetic stress (Pavlostathis and Giraldogomez (1991)). The two often cited slowest steps in anaerobic digestion are either hydrolysis or aceticlastic methanogenesis (Batstone (2006)). Degradation rates of the substrates depend on substrate and biomass concentrations, and on hydrogen and pH inhibitions. Soluble enzymes are assumed to degrade the substrate, which are produced by the bacterial group degrading the specific substrate (Batstone et al. (2000a)), thereby model equations are defined in a way that the concentrations of biomass and, in most of the cases of the substrates, regulate the degradation rates.

Disintegration and hydrolysis are complicated processes since the substrate utilized is not only present in the substrate added, but it can also be an internal product generated from microbial activity (Angelidaki and Sanders (2004)). Furthermore, either the substrate or the internal product can also come from bacterial decay.(Batstone et al., 2002) Batstone et al. (2002) recommended using a first order model for each of these two processes since it showed to fit biogas production as well as a more complicated two-phase model including enzyme adsorption, although the nature of the substrate was not reported. They also suggested using Contois kinetics for the assessment of experimental data at low relative inoculum concentrations in comparison with substrate's (S:I ratios > 1), making hydrolysis a rate-limiting process. Indeed, Pavlostathis and Giraldogomez (1991) considered first order kinetics to be applicable only when substrate concentration was several times smaller than K_S. Angelidaki and Sanders (2004) distinguished hydrolysis of particles from hydrolysis of solubles and claimed that each should be represented by different kinetics. In the case of particles, a surface related expression should be included since surface availability has been reported to be the limiting factor. In the case of solubles, the limiting factor has been reported to be the enzymes concentration which indeed is contained in the biomass. However, when the substrate is a mixture of particles and solubles, i.e. wastewater, first order kinetics may not reflect the whole conversion thus making it difficult to assess whether hydrolysis is the rate-limiting process (Paper **II**). Furthermore, the S:I ratio may exert an influence on the hydrolysis of these kind of substrates. Angelidaki and Sanders (2004) suggested a method to calculate the hydrolysis constant according to the total substrate added (substrate + inoculum) and its total biodegradability expressed as a fraction from experimental data. In this way, the characteristics of both the substrate and the inoculum, and the biodegradability expressed as a function of both, are included in the hydrolysis constant. From our study (Paper **II**) we found that it was more comprehensive to express the biodegradability of the wastewaters also as a function of both the inoculum and the substrate, which goes accordingly to the hydrolysis process where the two are highly interactive and it is difficult to assess the effect of each separately. An important step for determining whether hydrolysis is the rate limiting process in anaerobic digestion, is to characterize the substrate, at least in its soluble and particulate fractions (Pavlostathis and Giraldogomez (1991)). Furthermore, an identification of the biodegradable and inert fractions is important not only for the modeling of hydrolysis, but also to predict anaerobic effluent concentrations (Batstone (2006)).

McCarty and Mosey (1991) developed a model for the anaerobic digestion of carbohydrates. They proposed that the inhibition of the hydrogenotrophic methanogenesis at low pH values is due to the competition between the propionic and butyric acid producing bacteria, when carbohydrates are fermented. Their hypothesis was that both bacterial groups are obligate hydrogen producers, and, that the butyric acid forming bacteria is in advantage to the propionic acid forming since the former is adapted to low substrate concentrations and acid tolerant, and sometimes can produce acetate, carbon dioxide, and hydrogen gas instead, increasing its energy yields and thus permitting faster growth. On the other hand, although the latter unlike its partner group is unaffected by hydrogen partial pressure, it only grows on high substrate concentrations and is affected by low pH values. So therein, there are two bacterial groups which can either adapt to low or high substrate concentrations, being the butyric acid forming which can switch to acetate forming and produces in any case hydrogen and tolerates low pH values. Therefore, accumulation of propionate and butyric acids is the response to an anaerobic digester's inhibition, which can be recovered when the surge load ceases and hydrogen disappears, leading to increased pH values. After this butyric acid decreases, but propionic acid may persist for a time enough to permit the only bacteria able to metabolise it, to grow up from a tiny initial population.

Since complex and syntrotophic processes happen during the anaerobic digestion of organic waste(water)s, still today anaerobic digestion models are not widely applied. Anaerobic digesters are most often designed on a combination of hydraulic and COD mass loading in contrast to activated sludge digesters which are often designed on activated sludge models (Batstone (2006)). In his review, he lists that two of the reasons why this happens is that (i) parameters for anaerobic digestion have not been well estimated or standardized and assessment of parameter variability is limited, and, (ii) the most popular anaerobic models currently are very complex and they don't translate well to simple design rules. More conventional COD mass balance based models do not take into account the characteristics of the waste(water) fed either, which appeals contrary to the fact that the anaerobic biodegradability of a specific waste(water) depends very much on its characteristics more than on a lumped parameter comprising all the organic material content. Indeed, studies have shown that a comprehensive characterization of the substrate allows for a more adequate representation of the biogas flow and concentrations, and a more accurate pH profile (Kleerebezem and van Loosdrecht (2005)), and also provides the basis to assess the effect of co-digesting different wastewaters (Bozinis et al. (1996)).

For the estimation of parameters on the anaerobic biodegradation of organic waste(water)s, the validity of the values obtained are limited to the conditions at which the experiments were conducted, therefore a careful experimental design and a statistical evaluation of the results are necessary (Mosche and Jordening (1999);Bozinis et al. (1996);Pavlostathis and Giraldogomez (1991)). The change of dependent variables on a change in a parameter, i.e. sensitivity coefficient, depends on the initial conditions when batch experiments are carried out. Careful manipulation of the experimental conditions, e.g. S:I ratio, will affect the ability to retrieve independent parameter estimates. For Monod-type kinetics, the best independent parameters of maximum specific growth rate (which is directly proportional to maximum specific uptake rate) and half-saturation constant are obtained at ratios 20:1 (Grady et al. (1996)). However, for anaerobic batch experiments this high ratio may very likely lead to acidification, thus lower ratios should be defined but still allowing for substrate in excess. In the other hand, because each cycle of the batch experiment goes through nearly all biological status (e.g. high S:I ratio – low S:I ratio – starvation – decay), it makes it stricter with the mathematical model, i.e. it requires more specific initial conditions and parameter values (Feng et al. (2006)).

4.1 Model applications

The application of mathematical modeling to anaerobic batch experiments has increased recently due to the better understanding of the biochemical processes during anaerobic digestion and the need to assess many different organic waste(water)s without the laborious implementation of continuous reactors. However, due to the equipment limitation, it is difficult to assess the dynamic profiles of the different biomass fractions, and the sampling is relatively more time consuming in relation to continuous reactors. In the other hand, each investigated factor was often represented in a different batch bottle making the experiment too big to monitor and handle when assessing different factors. Finally, the heterogenous nature of organic waste(water)s needs to be representatively assessed in volume thus as more complex and less liquid is the waste, bigger bottles are needed. Careful experimental designs and relevant models are needed for each specific case, depending on the aim of the experiment. At Table 2 a summary is presented of the most recent applications of mathematical models to anaerobic mesophilic batch experiments and the relevant factors influencing the success of these implementations together with the most relevant results. We can summarize that three different types of models have been applied: single-stage models assessing overall conversion to methane, two-stage models for acidogenesis and methanogenesis, and more detailed models assessing also inhibitions and initial substrate concentration. For the overall conversion assessment, it was observed that the pH is often controlled and that the lag-phase was thus diminished, making easier to fit model with experimental data. It was observed too, that there was a general inconsistency of hydrolysis described by first-order or Monod kinetics since models often presented this rate out of time, i.e. under/overestimated. Finally, when pH inhibition was included, unsteady cumulative methane productions were better simulated according to experimental data.

4.2 Application of the Anaerobic Digestion Model No.1 (ADM1)

The ADM1 is defined by 26 state variables which have been extended to 32 in order to simulate the undissociation/dissociation processes of the acid-base system formed by the volatile fatty acids, the carbonate system, and the ammonium/ammonia (Blumensaat and Keller (2005)). The reaction system can be divided into two main types: (1) Biochemical reactions, and, (2) Physico-chemical reactions (Batstone et al. (2002)). The model describes 7 groups of bacteria and archaea, catalyzing the 19 biochemical kinetic processes, coupled to 3 gas-liquid mass transfer equations and 8 algebraic variables (Kleerebezem and van Loosdrecht (2006)). COD balancing is implicit in these equations, and in 5 of the conversion processes the inorganic carbon is the source for product catabolism. Blumensaat and Keller (2005) and Rosen et al. (2006) introduced an inorganic carbon balance term for decay processes since initial carbon balance checks revealed that the amount of inorganic carbon released due to decay of biomass was lost in the system. In this way the decay of biomass closed the system assuming that the disintegration into particulate composites and the subsequent hydrolysis deposited the carbon into the soluble substrate.

Table 2. Mathematical models applied to describe anaerobic digestion under mesophilic batch conditions at different levels of detail and complexity.

Processes modeled	Substrate*/ Inoculum	Experimental set-up	Initial conditions	Parameter assessed**	Variables assessed***	Comments	Reference
Overall methane production	Glucose/ Solids pre-adapted to glucose	**S:I ratio:** 4.8-67 (COD) **pH:** Initial 6.8-7.2 Nutrients & minerals medium, and $NaPO_4$ added at t=0	$C_6H_{12}O_6$, Total VFA, C_2H_5OH, CH_4	B, B_0, k	$C_6H_{12}O_6$, Total VFA, C_2H_5OH, Cumulative CH_4 production	**Lag phase:** 2 days at 7>pH>7.2 and S:I ratio>4.8. Sensitive to pH changes 6.4-6.9. **k:** 2 rates identified, k1>k2 probably due to initial CO_2reduction and later acetate cleavage.	Chen and Hashimoto (1996)
Inactivity and overall methane production	Sludge cake spiked with NH_4Cl	**NH_4Cl :** 10 initial concentrations (0-6 g/L) **pH:** 6 constant values (6.5-9) with HCl & NaOH addition	TS, VS, pH	B, B_0, k, k_m, λ, C_{NH4}, NH_3	Specific CH_4 production	**Lag phase:** Dependent on NH_3 conc. (>0.5 g/L notable shock), not on NH_4. **k:** Sensitive to NH_4 conc.(-10% at 1.7-3.7 g/L; -50% at 4.1-5.5 g/L; nul at 5.9-6 g/L) combined with 6.5>pH>8.5.	Lay et al. (1998)
	Vegetable waste shredded & wood chips / leachate from previous experiment	**2 runs:** 1st w/o inoc. & buffer addition; 2nd w/inoc.	VS, VFA	B, B_0, k_m, λ, $t(B_{0,95\%})$	Cumulative and specific CH_4 production, CH_4 (%), VFA	**Lag phase:** -16% from 1st to 2nd run **k_m:** +18% from 1st to 2nd run **B_0:** +2% from 1st to 2nd run **$t(B_{0,95\%})$:** -37% from 1st to 2nd run	Hegde and Pullamma nappallil (2007)
	MSW / pre-adapted stabilized solid waste leachate	**8 experiments:** 1st w/inoc. 2nd-8th w/o inoc. All fed with increasing substrate load, and nutrients, minerals & buffer in excess	TS, VS, TKN, CODtot, CODsol, pH, VFA	B, B_0, k_m, λ, Specific formate & acetate activities[a]	Cumulative and specific CH_4 production, VFA	**Lag phase:** Longer with no pre-adapted inoculum, which can be done with waste's CODsol **k_m:** Insoluble fraction < soluble fraction. No difference amongst particle size 2 & 50mm	Nopharata na et al. (2007)
Overall methane production & substrate uptake	Food waste shredded / Anaerobic cattle manure	**Init. TS:** 5 conc.(45-135 g/L) **pH:** Constant (6.8-7.4) with $NaHCO_3$ addition **C:N ratio:** Constant (25:1 adjusted with $(NH_2)_2CO$)	TS, VS, TOC, TKN, fat, protein,cellul ose, lignin, pH,Acetate, COD, TAlk	B, B_0, k, D, BCE	Specific CH_4 & CO_2 production, pH, Acetate, COD, TAlk	**Gas:** Corrected to STP, based on ideal gas law and expressed in grams. **k:** Increasing at initial VS≤ 59.7 g/L	Rao and Singh (2004);Ra o et al. (2000)

Processes modeled	Substrate*/ Inoculum	Experimental set-up	Initial conditions	Parameter assessed**	Variables assessed***	Comments	Reference
Readily degradable & slowly degradable	Co-digested filtered food waste & SS / Effluent lab-scale reactors	**Init. VS:** 1-4 g/L **S:I ratio:** 80:20 (ml) Nutrients, minerals, & buffering medium solution added	TS, VS, TSS, VSS,TOC, DOC (sol), $C_nH_aO_bN_c$	B, $B_{0(i)}$, $B_{0(s)}$, k_i, k_s, λ_i, λ_s	DOC, Total VFA, CH_4 & CO_2 production	**Rate-limiting step:** Acidogenesis at low food waste additions; Methanogenesis at high. **$B_{0(i)}$ & λ_i:** increased by increasing food waste **k_i:** increased < 50% food waste < decreased	Kim et al. (2003)
Acidogenesis & methanogenesis	Glucose, starch, cellulose, casein, & various/ unspec.	Unspecified. Assuming ideal environmental conditions and constant biomass concentrations.	Unspecified	k, k_{ac}, k_{meth}, DR	VFA and CH_4 production	**Rate-limiting step:** Acidogenesis **k:** Affected by components distribution ratio (Glucose>Starch>Cellulose).	Shin and Song (1995)
Overall degradation by Contois kinetics	Cattle manure and SS / Unspecified	**Init. VS:** 34.9-87.2 g/L	Substrate and biomass conc (COD)	I_f, K, μ_m	Substrate conc. (COD)	**I_f:** 58.4% in cattle manure & 32.2% in sludge and proportional to influent concentration.	Chen and Hashimoto (1980)
Hydrolysisby Contois kinetics and non-competitive inhibition	Fresh potato cut into cubes / return sludge cake with hydrolytic enzymes and buffer	**pH:** 1st group (5-9); 2nd group (7) **Inhibitor:** 2nd group (Acetate 20g/L) Leaching liquor sampled and replaced by fresh liquor	TS,VS,starch, protein, pH, DOC,N(dissolved),reducing sugar, amino acids.	k_h, K_S, $K_{I,i}$	Substrate VS, DOC, N (dissolved).	**k_h=** Felt rapidly at pH 6.5 in the presence of HAc; fitted R^2=0.99 for carbohydrates hydrolysis but for proteins R^2=0.80 when non-competitive inhibition model was included	He et al. (2006)
Hydrolysis, acidogenesis & methanoge-nesis,inhibitions of HAc & NH_3^-	OFMSW / excess activated sludge	**Init. TS:** 45, 70, 95 g/L **pH:** 1st group uncontrolled, 2nd group (7)	TS, VS, COD, acetate, TKN, pH	μ_i, $\mu_{i,m}$, Y_i, $K_{i,S}$, $k_{i,dec}$, $K_{I,hac}$, K_a, $K_{I,NH3}$, K_{NH3}	Cumulative CH_4 production, pH, acetate, free ammonium	**Hydrolysis:** Predicted degradation rate unvariable (underestimated: beginning, overestimated: end). This deviation increased at higher initial TS. **Overall:** Cumulative trends predicted well even at unsteady periods owing to pH fluctuation.	Liu et al. (2008)

* MSW=Municipal solid waste, OFMSW=Organic fraction of municipal solid waste, SS=Sewage sludge.

** Parameters: B=cumulative methane yield, B_0=ultimate methane yield, k=overall methane production rate, k_m=maximum specific methane production rate, λ=lag phase, C_{FAN}=Critical NH_4/NH_3 concentration at which the reaction can't proceed, D=biodegradable substrate, BCE= D(t) / D, $t(B_{0,95\%})$=time to produce 95% of methane potential, k_{ac}=acidogenenesis rate, k_{meth}=methanogenesis rate, DR=rate-limiting factor, $B_{0(i)}$=initial methane potential, $B_{0(s)}$=secondary methane potential, k_i=initial methane production rate, k_s=secondary methane production rate, λ_i=initial lag phase, λ_s=secondary lag phase, I_f=substrate refractory fraction (COD), K=biomass kinetic parameter relative to growth yield and half saturation coefficient, μ_m=maximum specific growth rate, k_h=hydrolysis rate, K_S=half saturation constant, $K_{I,i}$=inhibition constant, Y=yield coefficient.

*** VFA=acetate, propionate, and butyrate assessed separately, Total VFA=lumped.

The ADM1 employs a large number of constants and coefficients and requires a detailed waste(water) characterization of particulate/soluble carbohydrates and proteins, and lipids in order to achieve accurate model predictions (**II**; Parker (2005); Kleerebezem and van Loosdrecht (2005)). Theoretical COD contributions for each of these fractions should be calculated based on model compounds for proteins and lipids, whilst for the carbohydrates it can be calculated as the remainder COD measured in the organic input (**II**; Batstone et al. (2000b)). The distribution ratio amongst these three fractions is regarded as the critical and difficult issue and the calculation of the biodegradable fraction, i.e. composites, and of the inerts both particulate and soluble introduces a big uncertainty (Feng et al. (2006);Huete et al. (2006)). Furthermore, in almost all cases it is not independently measured (Parker (2005)). At our study (Paper **II**), we calculated it based on the same experimental data from the COD mass balance into the system and as the remainder after anaerobic oxidation, however, we could observe discrepancies amongst this biodegradable fraction and VFA profiles. The calculation of this fraction also influenced the determination of carbohydrates as the remainder COD input, which in some cases was nul. However, it was not possible to find relevant studies for comparison thus it was decided to keep this calculation method. What was particularly observed from some studies was that when previous information regarding the biodegradability of the waste was available, this fraction was more accurately determined. Examples are for sludge and human faeces studies (Siegrist et al. (2002);Ekama et al. (2007);Parker (2005);Feng et al. (2006)). Huete et al. (2006) fitted these fractions based on their elemental composition considering TKN, COD, and VS ratios, until approximating simulations with experimental profiles. Two other inputs introducing uncertainty to the model are the fractionation of input inerts into soluble and particulates and the input bicarbonate alkalinity. Huete et al. (2006) calculated soluble inerts based on influent COD soluble measured and bicarbonate alkalinity according to what was measured by titration, which we also did in our study (Paper **II**). A more accurate method on calculating inert fractions would be by measuring effluent COD as total and soluble, however we lacked of these analytical measurements during our experiments. This inconsistency was evident since in some of our experiments simulated the concentration levels of inerts accumulated to levels which were unrealistically high (data not shown). Finally, in regards to the input fractions, Parker (2005) observed that the concentrations of free ammonium/ammonia nitrogen in the inlet had a substantial impact upon the pH during acidogenesis. We observed this from our study too (Paper **II**), since in all the experiments simulated the model predicted very low initial pH levels, although, these levels were quickly recovered and fitted experimental values in most of the cases (except at the three experiments when experimental pH levels dropped down to inhibitory levels).

The disintegration process showed nearly absent or little influence in kitchen refuse and black water simulations respectively (Feng et al. (2006)). At our study (Paper **II**), we decided to leave this initial process out and keep it only for the disintegration of decay products since we assumed no active biomass contained in the wastewaters assessed (Gosset and Belser (1982);Pavlostathis and Gosset (1986);Huete et al. (2006)). At our study we observed a constant underprediction of propionate, butyrate, and valerate, regardless the different influent composition. This was also observed by Parker (2005), and he attributed this to an overestimation of the rates of oxidation of these substrates. We also noticed at our study that these particular uptake processes resulted not-influencing in six out of nine experiments simulated at our sensitivity analysis. In the other hand, we also noticed a slight overprediction of acetate levels combined with an overprediction of its uptake rate, when comparing the profiles to our experimental data. These simulation results were also observed by Parker (2005). We suggested that the hydrolysis rates of carbohydrates and/or proteins were somehow overpredicted, increasing the simulated levels of acetate but degrading it quicker than what was observed at our experiments. Indeed we also observed hydrolysis of carbohydrates and proteins as influencing parameters in four of our simulated experiments during the sensitivity analysis, showing the hydrolysis parameter as an important value to calibrate in order to get more accurate predictions of VFA accumulation. Concerning the acetate degradation, Feng et al. (2006) also observed a faster acetate degradation and a subsequent faster methane production, which we also observed (Paper **II**). They referred to Batstone et al. (2003) to indicate that this delay may be simulated more accurately by increasing acetogens decay rate, but they did not achieve the same effect. At our study we also tried to emulate this delay by increasing decay and acetate uptake rates proportionally but we observed no effect either. Feng et al. (2006) attributed it to Monod kinetics, since they cannot represent delays. Finally, they also observed that the model was extremely sensitive to significant fractions of acetate in the input (i.e. 30%). We also observed at our study, since one of the experiments having relatively higher input acetate levels presented the most influencing parameters on sensitive output variables. Furthermore, if higher input acetate levels are combined with higher input carbohydrates this makes the model very sensitive too. This was attributed to a high and prompt accumulation of acetate, propionate, and butyrate (Paper **II**).

Finally, the characterization of initial biomass fractions still remains as an unknown but very relevant topic for the application of the ADM1, since these fractions are the catalyzers of all reactions.

5 Food-processing industry wastewaters: Characteristics and factors affecting their composition

Food production wastewater is composed from fractions coming mainly from processing, condensation and transport activities, from cleaning the process machinery, and from washing the installations (Rosenwinkel et al. (2005)). Relevant processing activities for wastewater discharge are soaking, fluming, blanching, scalding, heating, pasteurizing, chilling, and steaming (Casani et al. (2005)).

Food production and cleaning wastewaters contain high concentration of organic matter which is determined by three commonly used analyses: Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), and Biological Oxygen Demand (BOD). The COD has developed into a major parameter, because its results are available more quickly than those of the BOD analysis (Rosenwinkel et al. (2005)). Even though is believed that food industry wastes should be treated easily since their constituents are fundamentally organic, there are a number of elements making the treatment difficult, e.g. high concentrations, organic fractions distribution, temperature, cleaning acids and sanitizers, deficiency in nutrients and/or alkalinity (Wheatley (1995), Papers **I** and **II**). All these factors alter the streams' composition, varying depending on the water consumption, product diversification, industry and/or facility practices, cleaning procedures, by-products recovery, and pre-treatment implementation. Thus, analysis and a complete characterization of the effluents are essential prior to any decisions on a process design for a waste treatment. Furthermore, by knowing the detailed characteristics of the effluent, modeling can be applied assisting on the design (Bernet (2006);Orhon (1998);Wheatley (1995)). Characteristization of food-processing industries should take into account not only the parameters targeted by effluent discharge regulations but also parameters relevant for the (pre)treatment design and operation (Bernet (2006)).

Currently food companies are putting effort on reducing water consumption during the processing activities to remain cost-effective and significant savings have been made by water reuse/recycle and layout design improvements. Whilst the quantity of water required for the different production processes is medium to high, it is often that its quality level required is high to potable (Rosenwinkel et al. (2005); Kirby et al. (2003)). Thus, internal recycling processes affect the composition of the wastewater generated since concentration of pollutants in re-circulated water is higher and halogenated organic compounds are introduced (Rosenwinkel et al. (2005)).

5.1 Product diversification

Food-processing industry has often to manage seasonal production with important consequences on the variability of the wastewater (flows, concentrations, characteristics) (Bernet (2006)). Flows fluctuate according to the water needed for processing the product in-place, which may not be available all-year round, thus, organic constituents in the wastewater may fluctuate too. The production also changes according to how the product is to be processed, and the amount of water required for it. Reuse and recycling of the water and the corresponding addition of disinfectants may also vary accordingly. The specific pollution load per ton of processed product is shown at Figure 8 for two of the six industries which present seasonal production changes. Wastewater discharged was similar in both cases, although their production figures varied amongst seasons, this may have been due to adapted water consumption and/or recycling practices in order to maintain similar wastewater discharge characteristics. Specific pollution loads for the vegetables case was similar too, having the main differences for their COD, lipids, and VFA. Specific loads for the fish meals case study were different. First of all they showed lower numbers and this agrees with Bernet (2006) who defined peas' wastewater as a more concentrated wastewater compared to other food processing's streams. Also solids and lipids were different amongst winter and summer periods. This may have been due to the different composition of the fish during cold and warm periods, decomposing faster at warm temperatures. The higher amounts of solids during the winter may be due simply to the higher amount of raw material processed.

5.2 Reuse and recycling of wastewater streams

Reuse of process water has been primarily limited to non-food and cleaning uses, such as general facility cleaning and performance of cooling functions and fire extinguishing purposes (Casani et al. (2005)). In any extent, process water has to be pre-treated in a certain way before being re-used, altering its qualitative and quantitative characteristics. The suitability of recovered water for use in any food operation is dictated by the quality of the water required in that operation, the quality of the used water, the recovery and distribution method, and the ability to recondition the water to the level required, specially since it comes into contact with food and beverage products or is used to clean surfaces that come into contact with the products (Casani et al. (2005); Kirby et al. (2003)). Nowadays, accepted reusing applications include initial washing of vegetables, fluming of unprepared products, scalding water of meat, and in continuous systems it is reused from the least contaminated water of the final wash to the next-to-last wash and so on (Casani et al. (2005)). A common recycling process utilizes disinfectants to remain protected towards further contamination depending on the remaining concentration of disinfectant (Casani et al. (2005))

.

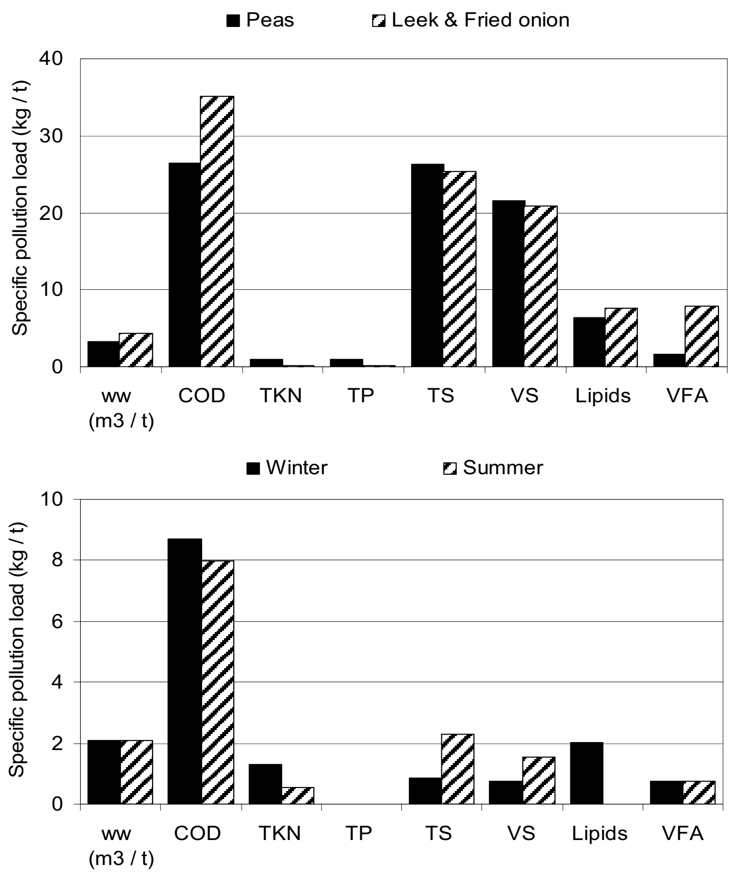


Figure 8. Specific wastewater discharged and pollution loads for the main analytical indicators for Vegetables (top) and Fish meals (bottom) case studies.

5.3 Cleaning production line

Some food-processing wastewaters, e.g. meat processing, frequently include high concentrations of biocides and disinfectants such as hypochlorite (Wheatley (1995)). Hypochlorite, amongst other biocides, are used for extensive cleaning of the production line, particularly for facilities processing high volumes of raw material containing high concentrations of grease, blood, and faeces (Lassen et al. (2001);Wheatley (1995)). This is the case for the slaughterhouses, specially when they are processing high volumes of food. At Figure 9 it is shown the specific wastewater discharged and pollution loads for this case (slaughterhouse). This facility has two production and one cleaning shifts happening at different times of the day, allowing for separate sampling and collection. The specific wastewater discharged was the same since flow is not quantified separately, however it was noticed that the organic constituents varied, showing lower organic matter concentration for the cleaning wastewater. Other particular organic/inorganic compounds are not presented in this chapter, but their source tracking and identification are presented later in this chapter.

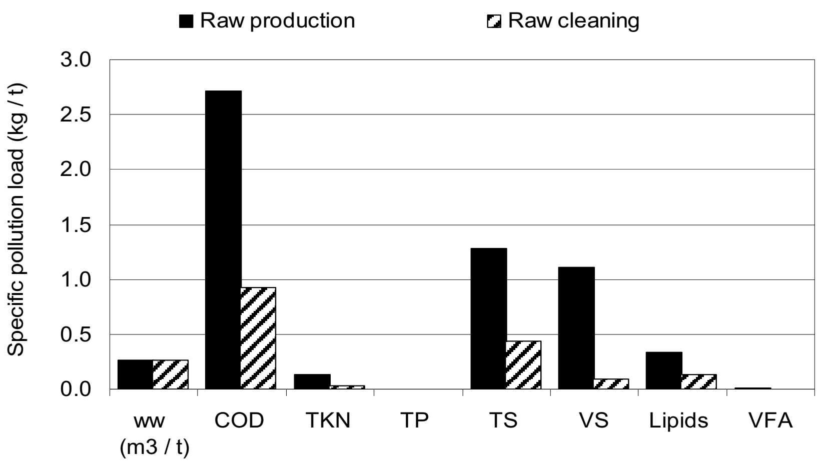


Figure 9. Specific wastewater discharged and pollution loads for the main analytical indicators for slaughterhouse case study (only raw wastewater shown).

5.4 In-site mixture/separation of wastewater streams

One of the strategies commonly suggested in order to make food industries and industrial wastewater treatment more sustainable is treating the wastewater at the place of origin, particularly for anaerobic digestion which is suitable for highly concentrated streams such as food-processing industries. However, during the processing there are streams which are relatively much lower in organic matter concentrations than others and depending on their quality they can be either reused or sent to the sewage (Sekoulov (2002)). More concentrated streams can be treated by anaerobic digestion with the advantage of renewable energy production. At Figure 10 the specific pollution loads for two different streams at the Slaughterhouse case study are shown. It can be seen that wastewater from truck washing and animal sheds is different from the production wastewater. Rosenwinkel et al. (2005) described it as a stream consisting of bedding material, faeces, urine, and hair.

As it is shown at Figure 3 the COD load is much higher and the solids constituents are also relatively higher than the raw production's. There is also a presence of VFA, which may come from the faeces of the animals. It is important to notice that solid particles were screened before the sampling point since the industry sends the screened solid particles to a biogas plant as a regular basis, so the wastewater analyzed did not show very high relative solids contents.

5.5 Pre-treatment

The complex mixture of floating, setteable, suspended, and dissolved materials, and the potential presence of toxic substances in food-processing wastewaters can be prevented from causing health and environmental effects by adequate pre-treatment or a separate management from municipal and excreta (Kirby et al. (2003); Wheatley (1995)).

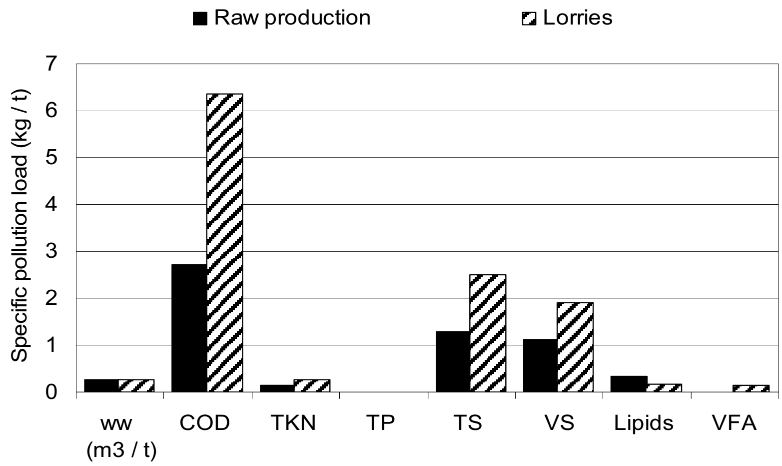


Figure 10. Specific wastewater discharged and pollution loads for the main analytical indicators for Slaughterhouse case study (raw production and lorries' wastewater shown).

According to Rosenwinkel et al. (2005), up to know, the number of processing plants for which physico-chemical or biological operational steps have been added is comparatively small. However, since wastewater companies frequently specify a maximum flow rate as well as the total amount of effluent to be discharged to the sewer, flow balancing may be required to conform to these consent conditions and to even out the chemical composition of the wastewater (Wheatley (1995)). It may be cheaper to remove easily settleable or floatable material phisically or chemically than biologically, but on the other hand the costs of disposal of separate solids may overcome the capital and running costs associated with oxidizing them biologically (Wheatley (1995)). This particularly depends on the regulatory framework of each region in Europe, and dictates the decision of the industries about pre-treating the wastewater or not. The question whether pre-treatment improves the biological treatability of the specific wastewater assessed was evaluated at Paper **I** particularly by anaerobic digestion with the slaughterhouse case study, finding that flocculation improved it but equalization did not. At Figure 11 it is observed that the organic matter load and solids are significantly reduced by pre-treatment, i.e. physico-chemical dissolved air flotation, flocculation, and equalization. Furthermore, the lipids are almost entirely converted to VFA, this may be due to long retention times and that in some areas of the pre-treatment tanks the conditions turn anoxic.

At Figure 12 the specific wastewater discharged and pollution loads for vegetable fats & oils and fish processing for human consumption case studies are shown. It is noticeable the higher numbers in several orders of magnitude for the fish processing case study. This indicates the water practices in the processing facility vary very much from the rest of the case studies presenting higher specific loads. These two industries also do pre-treatment to their wastewaters but fish processing only does it by equalization, whilst vegetable fats & oils does it by flotation and equalization.

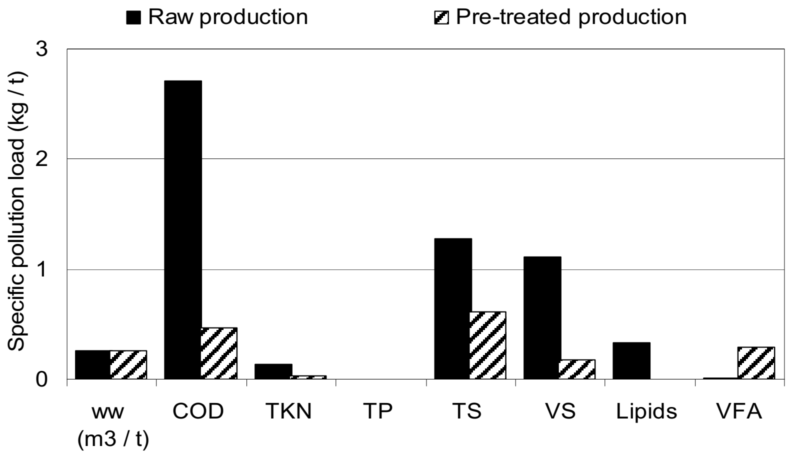


Figure 11. Specific wastewater discharged and pollution loads for the main analytical indicators for Slaughterhouse case study (raw and pre-treated production wastewater shown).

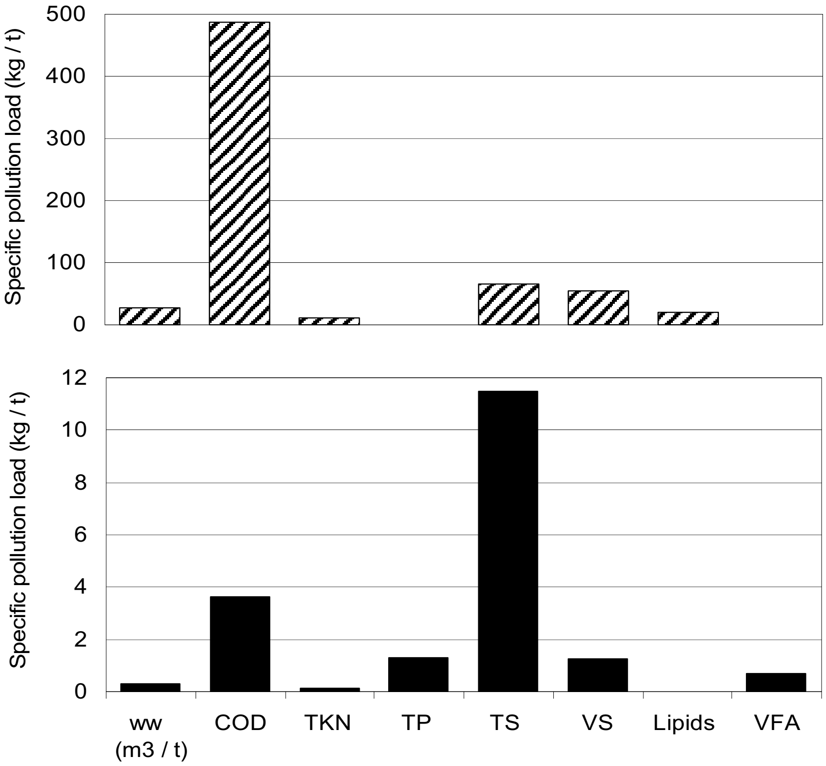


Figure 12. Specific wastewater discharged and pollution loads for the main analytical indicators for fish processing (top) and vegetable fats & oils (bottom) case studies.

Unfortunately, it was not possible to do sampling before the pre-treatment units. The organic constituents showed different, but it was noticed in particular that the vegetable oils & fats wastewater showed relatively high total solids loads, that in this only case phosphorus was present, and finally that again the VFA were present, which may be attributed to the same pre-treatment reasons as for the slaughterhouse case study.

5.6 Biodegradability

It is a well known fact that total organic content measured in a wastewater sample, e.g. as COD, does not adequately reflect its biodegradability. Some organic compounds are readily digestable whereas others are hardly biodegradable (Lyberatos (1997)). Difficulties can be predicted by laboratory treatability studies, and they become essential if there are any doubts about the characteristics of the waste (Paper **I**). Tests in common use are the BOD and the biological methane potential assay (BMP) (Wheatley (1995)).

Often in literature it is found the COD/BOD_5 ratio as a degradability indicator, however this only gives an idea of the readily degradable material in the wastewater under abundance of oxygen, nutrients, minerals, buffering capacity, and preconditioned activated sludge. It is important to remember that these conditions are not always present in a treatment plant, particularly when the sludge is not acclimatized. However, it is a useful indicator and the practicability, easiness, and standardization of the procedure makes the BOD_5 a widely applied biodegradability test. Rosenwinkel et al. (2005) suggested that a food-processing wastewater sample is easily degradable if the ratio<2 which corresponds to Henze et al. (2002), although the later refers only to domestic wastewater. Depending on the equipment and method applied a number of dilutions are applied to the wastewater prior to the test, in order to create oxygen saturation conditions.

Another suggested indicator by Rosenwinkel et al. (2005) is the BMP/COD ratio. However, the outcome of the BMP is the practical methane potential which takes into account not only the wastewater's COD but also other of its physico-chemical components (Paper **I**). A more suitable biodegradability indicator based on BMP outcomes is the methane based biodegradability (MBD) which is calculated from the theoretical and practical methane potentials (Paper **I**). At Table 3 the COD/BOD_5 ratio and the MBD for all the case studies are shown. Six out of eleven showed ratios<2, indicating these wastewaters were readily biodegradable without limitations of oxygen, nutrients and minerals, the other five wastewaters showed to contain organic matter more difficult to degrade under these conditions. On the other hand, only three out of eleven showed to have slowly degradable organic matter under anaerobic conditions at optimal dilutions. Optimal dilution for each wastewater was found by finding the wastewater concentration that showed the highest ultimate practical methane potential (Paper **I**). In this way both indicators were made comparable.

Table 3. Wastewaters' identification with their biodegradability indicators for all case studies. For more details about sampling see Papers **I, II** and **III** and for estimation method for MBD see Paper **I**.

Industry	Sampling point	Sample ID	COD/BOD_5 (unitless)	MBD[a]
Vegetable fats & oils	Discharge to sewage	VFO	1.3	66%
Fish processing for human consumption	Discharge to sewage	FBH	15.7	77%
Vegetable processing	Discharge to sewage during peas processing	VPE	1.1	100%
	Discharge to sewage during leek & onion processing	VLO	1.1	100%
Fish meals for aquaculture	Discharge to sewage during winter production	FMW	1.3	100%
	Discharge to sewage during summer production	FMS	1.5	100%
Slaughterhouse	Discharge to pre-treatment plant during production (raw)	SPR	7.5	100%
	Discharge to pre-treatment plant during lorries' cleaning	SLC	4.2	100%
	At flocculation tank (solid phase)	SPF	4.0	100%
	At flocculation tank (liquid phase)	SPQ	4.7	100%
	Discharge to sewage during production (pre-treated)	SPE	2.4	91%
	Discharge to pre-treatment plant during cleaning (raw)	SCR	1.0	100%
	Discharge to sewage during cleaning (pre-treated)	SCE	4.7	100%

a) From BMP assays at optimal dilutions (see Paper **I** for more details about estimation method).

5.7 Xenobiotic organic compounds (XOC)

Amongst the most frequently mentioned reasons for inhibition of biological wastewater treatment is the presence of ammonia and fatty acids under anaerobic conditions, and the presence of XOC under aerobic and anaerobic conditions, e.g. (Angelidaki and Ahring (1994);Fernandez et al. (2005);Madsen and Rasmussen (1996)). Extensive research has been done concerning strategies to increase the biodegradation of XOC in urban wastewater by activated sludge treatment plants (Khanal et al. (2006);Byrns (2001);Janssens et al. (1997)), and recently attention has been drawn to the anaerobic biodegradation of some of these groups of compounds found in sewage sludge (Fava et al. (2007);Shimada et al. (2007);Dionisi et al. (2006)). The particular concern of XOC found in wastewaters and sludge relies on their persistence and bioaccumulation in the aquatic environment and in the soil, and their toxicity to humans and aquatic life. The occurrence of XOC in discharges from food processing industries has received very little attention in the scientific literature and has typically not been included in environmental hazard identification of wastewater streams (Paper **III**). Raw materials and cleaning products used during food-processing contribute to the discharge of XOC in these wastewaters, although the concentrations are several orders of magnitude smaller than the organic discharge, making their analytical identification very difficult (Paper **III**). Another challenge is the adequate sampling since, as it was realized from our study, more than several composite samples are needed to achieve a higher number of identified compounds by chemical analyses, i.e. sampling and measuring campaigns. In the other hand, it is important to have a clear idea what compounds could be present in a specific stream and what factors are creating their occurrence, before an extensive campaign is planned. For that purpose, a literature review can be carried in order to identify which compounds have been found in previous studies with related wastewaters (Press-Kristensen et al. (2007);Eriksson et al. (2005);Ledin et al. (2006)). In our study (Paper **III**), we lacked of this information, with the exception of a couple of studies related slaughterhouse and olive mill wastewaters (Eriksson et al. (2007);Knupp et al. (1996)). From this evident lack of data, we decided to do an extensive source analysis focusing on the potential migration of compounds to the wastewaters discharged, from raw materials, processing, and cleaning and disinfection chemicals. A literature survey was carried out of XOC identified at any of the food-processing steps shown in Figure 1. It was found that 161 compounds could potentially be present (Figure 13), and that the main sources were raw materials and the processing for several different applications, from cooking of vegetables and disinfection of pork parts, until additives for coloring and flavoring. Cleaning by-products contributed too, mainly by disinfection biocides. More studies were available, focused on polyaromatic hydrocarbons (PAH), pesticides, phytoestrogens, and chlorinated phenols, dioxins, and furans, but still it was found that other chemicals such as biocides, steroids, trihalomethanes, amongst others, had been identified from cooking, frying, animal handling, packaging, and disinfection activities in related food industries. The source analysis was complemented with an analytical screening where 13 compounds/groups of compounds were identified. Lower detection limits had to be increased due to the complexity of the samples. All compounds had been tracked from the source analysis and related to disinfection by-products, processing of peas, migration from raw materials and the environment, and packaging.

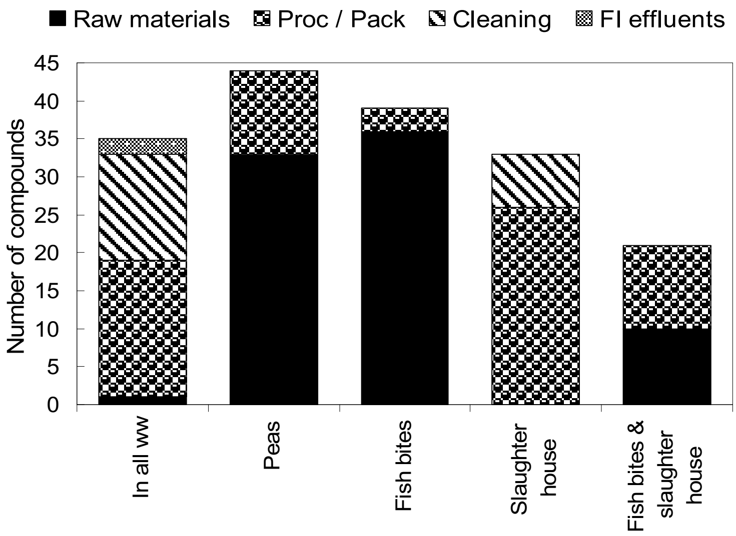


Figure 13. Number of compounds identified per industry according to source analysis. A merged category called 'Fish bites and slaughterhouse' was added since many overlapped compounds were identified (Paper **III**).

The relevance of the information provided from this study can be applied either to the assessment of biological treatability, or to the long term effect that the XOC found can exert on the environment if they persist through the treatment plant, or if they are metabolized to a rather more hazardous compound. It is important to emphasize that a more comprehensive sampling plan should be applied to achieve a higher representativity of the wastewater discharged. Also, a targeted preparation of the sample could favor lower detection limits thus complementing the results provided by the analytical screening carried before. Finally, anaerobic toxicity assays (Speece (1996)) can be carried with the wastewaters sampled in order to also complement the biodegradability information provided by the biological methane production (BMP) assays.

6 Application of life cycle assessment (LCA) and hazard assessment to identify indicators for environmental impact assessment

6.1 The role of Anaerobic Digestion in a sustainable society

Attention to the anaerobic digestion technologies for improving the sustainability of wastewater treatment has been paid mainly after the energy crisis in the 1970s, however it is still not regarded as a top priority in environmental science and industrial development at present (Foresti et al. (2006);Verstraete et al. (2005)). In spite of its advantage of producing methane as a valuable by-product, it also produces other end-products which contain residual organics that require the adoption of post-treatment systems (mainly aerobic) to remove the undesirable constituents (Foresti et al. (2006)). However, there are three clear advantages of the anaerobic treatment of highly concentrated organic wastewaters over their aerobic degradation: (i) the high product and low biomass yield resulting in a limited generation of waste sludge as an unwanted side product; (ii) the *in situ* separation of the product as biogas containing methane; (iii) the use of simple technology, as mixing by the biogas produced circumvents the need for great mixing requirements (Kleerebezem and van Loosdrecht (2007)). In this case, sewage sludge has gained focus from the international arena since it cannot be used on agricultural land due to its high potential as final depository of organic residues such as xenobiotic organic compounds. Furthermore, the biogas generated can overcome the energy spent on an aerobic post-treatment of the effluent's residues. This leaves this alternative with the biggest disadvantage in relation to aerobic treatment, the organic/inorganic residues in the anaerobic effluent. Energy supply for aerobic treatment is extensive, i.e. in the EU the costs related to conventional sewerage treatment such as activated sludge, amount to the order of 100 euro per inhabitant per year (Verstraete et al. (2005)). Even in the case where natural gas has a very low cost, i.e. US$0.5 per kg, and that a network is available, biogas can be distributed through the existing infrastructure. Although, in this case, carbon dioxide and hydrogen sulfide need to be removed from it (Kleerebezem and van Loosdrecht (2007)).

In order to curb the criticism that anaerobic digestion only converts part of the organic matter leading to further handling problems for the left organic residues, anaerobic digestion should be linked up very tightly with processes that can lead with the residuals (Verstraete et al. (2005)). Furthermore, household wastewater should be collected and treated separately from industrial wastewater to take better use of their highly concentrated organics for their conversion to biogas (Hammes et al. (2000)). Finally, the selective pressure required to oxidize the organic matter in the absence of an external electron acceptor, e.g. oxygen, nitrate, or sulfate, can be used by some anaerobes which have the unique capability to use rapidly and efficiently chlorinated organics as electron acceptors (Kleerebezem and van Loosdrecht (2007);Verstraete et al. (2005)). This is an interesting feature for the application of anaerobic digestion on the reduction of some hazardous xenobiotic organic compounds.

6.2 The application of LCA to wastewater treatment systems

In order to evaluate the long-term effects on the environment from any systematic economical activity, life cycle assessment has proven to be a useful tool when considering different alternatives in early stages of the planning, or for optimizing an existing system. Other approaches to combine ecological and economical goals, apart from effluent control, have been applied to allow for trade-offs between ecology and economics. These were reviewed by Starkl et al. (2005), and identified them as environmental risk assessment, cost-benefit analysis, single-objective optimization tools, multi-attributive methods, and outranking methods. They concluded that costs should be evaluated separately from ecological assessment, and that all ecological impacts should be translated into costs so it is possible to take them into consideration. As their opinion, it is found in literature that most of the evaluations are focused on costs rather than on environmental impacts. Another example is the benchmark simulation model which has been developed and implemented in different computer platforms in order to test different operational strategies (Jeppsson and Pons (2004)). The effects of each strategy applied in this standardized activated sludge treatment plant, are evaluated based on a set of performance criteria indicators which have been defined from the most important factors contributing to operational costs (Jeppsson et al. (2006);Vrecko et al. (2006)). These factors are electricity consumption for mixing and pumping, sludge production, and effluent quality. This criteria has further included the evaluation of an anaerobic digestion tank for sludge stabilization before disposal (Rosen et al. (2006);Vrecko et al. (2006)). Anaerobic digestion criteria includes electricity consumption for heating and mixing and electricity production from methane generation (Jeppsson et al. (2006)).

Notably, this criteria coincides with what LCA studies applied to wastewater treatment systems have shown, that energy consumption, sludge production and handling, and effluent quality and handling, are the factors of concern from a long-term ecological perspective (Palme et al. (2005);Beavis and Lundie (2003);Lundin et al. (2000);Houillon and Jolliet (2005);Odegaard et al. (2002);Wenzel et al. (2008)). Much environmental impact is associated with energy-resource utilization. In addition to the manageable impacts of mining and drilling for fossil fuels and discharging wastes from processing and refining operations, the greenhouse gases created by burning these fuels is regarded as a major contributor to a global warming threat. Energy processes lead to many environmental problems, including global climate change, acid precipitation, stratospheric ozone depletion, emissions of a wide range of pollutants including radioactive and toxic substances, and loss of forests and arable and (Dincer and Rosen (2005)). Efficiency is an important measure when assessing the environmental impacts of energy related processes. LCA studies have shown that when the processes under study improve their efficiency, a reduction of environmental impacts during most stages of the life cycle occurs. This means that, for the same services or products, increasing process efficiency leads to less resource utilization and pollution (Dincer and Rosen (2005)). Sludge stabilization and handling, as well as the quality of the wastewater effluent have been focused on their alternative utilization as fertilizers, avoiding the production of industrialized ones. However, since the EU Water Framework Directive (WFD) is inducing to an overall policy to further improve the quality of water bodies with respect to hazardous compounds, in several countries this way of sludge and wastewater effluent disposal has been forbidden. In Denmark as well as in other European countries, the concern is particularly about the migration of nitrates and XOC to groundwater, since that is the main water supply.

The integration of LCA with other tools to promote more sustainable urban wastewater treatment has not yet been applied, with the exception of three studies where specific process models are integrated with data aggregation which are further used for life cycle environmental impacts assessment (Tidaker et al. (2006);Balkema et al. (2001);Jeppsson and Hellstrom (2002)). At one of our studies, we emphasized the relevance of integrating environmental risk assessment with LCA (Paper **IV**) to identify priority substances in the food-processing effluents. We also adapted an evaluation criteria based on Jeppsson et al. (2006) and Vrecko et al. (2006) together with relevant indicators screened from the literature, to integrate process specific models from a life cycle perspective (Abstracts **V**, **VI**). The relevance of including proper technical parameter values and the influence of feeding different wastewaters were the main focus of our studies, particularly to identify main contributors of environmental impacts for anaerobic digestion in comparison with activated sludge treatment.

6.2.1 Goal definition and system boundaries

LCA has demonstrated by several authors that can be an useful tool when integrated to process design and optimization (Azapagic (1999)). In LCA, a model of the technical systems under study is constructed, i.e. the foreground system, and the flows of environmentally relevant substances between the technical systems and the environment are calculated. This technical system delivers the functional unit and comprises the set of processes that directly affect the assessment (Lundin et al. (2000); Azapagic (1999)). The background system is the one supplying energy and materials to the foreground system via operations that may not be identified individually (Azapagic (1999)). Such a systems approach makes it possible to assess changes in wastewater treatment practices and to compare different technical solutions in terms of the estimated environmental loads (Lundin et al. (2000)). The way the foreground system has been modeled in wastewater treatment systems is as a black box from data provided by technical staff designing the plant or by experts (**IV**, Lundin et al. (2000);Wenzel et al. (2008)), or by a process specific model where mass balances are carried according to the wastewater characteristics (**V, VI**, Tidaker et al. (2006);Jeppsson and Hellstrom (2002); Balkema et al. (2001)). The advantages of a black box foreground model is that the model is easier to implement and data is easier to gather, and the advantage of a process specific model is that once implemented it becomes a flexible tool possible to feed with different wastewater influents. Since the main purpose of wastewater treatment system is to collect sewage and reduce emissions of nutrients, BOD, and suspended solids to acceptable levels, but also to produce either biogas or nutrients as electricity/heating and fertilizers replacements, the relevant technical indicators to quantify these removals and productions are defined to control the mass balance flows (**IV**, Wenzel et al. (2008);Lundin et al. (2000)). Furthermore, to cover this double function, the LCA model should include upstream and downstream processes apart from the treatment plant itself (see Figure 14). What shown inside the square is the foreground system which was modeled according to Jeppsson and Pons (2004). Outside the square is the background system which relevant data was gathered and quantified from literature available as black box models.

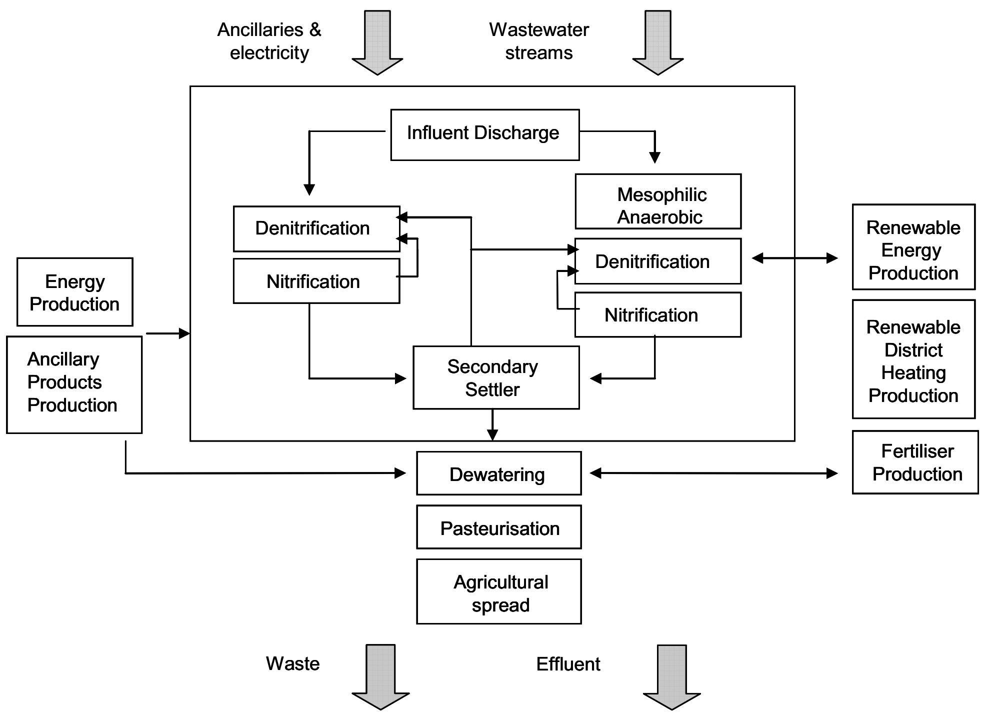


Figure 14. System boundaries of two biological treatment configurations. At the left is Denitrification/Nitrification plant, and at the right is Anaerobic pre-treatment followed by a Denitrification/Nitrification plant. The latter represents an alternative anaerobic pre-treatment unit in the production plant followed by the conventional treatment (**V**, **VI**).

6.2.2 Indicators

According to Palme et al. (2005), a set of preliminary indicators should be defined at the goal and scope definition stage of an LCA, which will be further submitted to the assessment and a set of indicators can be selected from it. The aim at our studies (**V**, **VI**) was to develop a set of indicators which we could use to compare the biological nutrients removal technology with and without an anaerobic pre-treatment step. In addition, we wanted to observe any effect on the assessment when feeding four different wastewater streams from two food-processing industries, which presented different compositions. Thereby, our preliminary indicators were related to carbon and nutrients removal efficiencies, electricity consumption for mixing, pumping, and heating, energy production and utilization in the case of anaerobic inclusion, ancillaries addition to enhance nutrients and buffering capacities, sludge production and quality, effluent quality, and electricity consumption for dewatering and pasteurization, all normalized per person equivalent (0.2 m^3/d).

6.3 Resources consumption and impact assessment

The evaluation of impact assessment is an optional step in LCA. It is usually carried when the results of the aggregated data from the foreground and background systems are not clear enough to show the alternative which performs best. In our studies (**IV**, **V**, **VI**), we performed this evaluation until the normalization step according to Wenzel et al. (1997). The output of the normalization comes in person equivalents according to the amount of resources that an average person in a specific region of the world consumes per year, and to the environmental impacts an average person causes per year also in a specific region. There are a number of considerations and assumptions that this normalization applies which are different for resources consumption than for environmental impacts. To know more about them please refer to Wenzel et al. (1997).

We observed in all three studies that the activated sludge treatment, either as COD or nutrients removal and without the anaerobic digestion plant presented normalized environmental impacts (Paper **IV**) and resources consumption (Abstracts **V**, **VI**) several orders of magnitude higher than when the anaerobic pre-treatment unit was assessed included (**IV**, Figure 15). Resources indicated in Figure 15 resulted with the highest values, and are all related to electricity supply, specially for aeration of the biological tanks and for pasteurization of the sludge. When the anaerobic pre-treatment was included, the energy generated from methane contributed to the supply of electricity for the subsequent denitrification/nitrification step (Abstracts **V**, **VI**). The environmental impacts presented almost the same trend (results not shown). The only difference was that the pet food wastewater, which was very low in nitrogen concentrations, required the addition of industrialized ammonia for achieving the required COD removal by the biological process. This caused a slight increase for the impact assessment of the treatment of this wastewater for all scenarios considered. When taking a closer look at the scenario where anaerobic pre-treatment was included, the pre-treated pet food wastewater presented significantly higher impacts than the other three wastewaters (Figure 16). The reason was that the carbon removal efficiency at the anaerobic tank was only 50% thus methane potential was not fully achieved, furthermore, the nitrogen removal at the denitrification/nitrification treatment was only 24% which together with

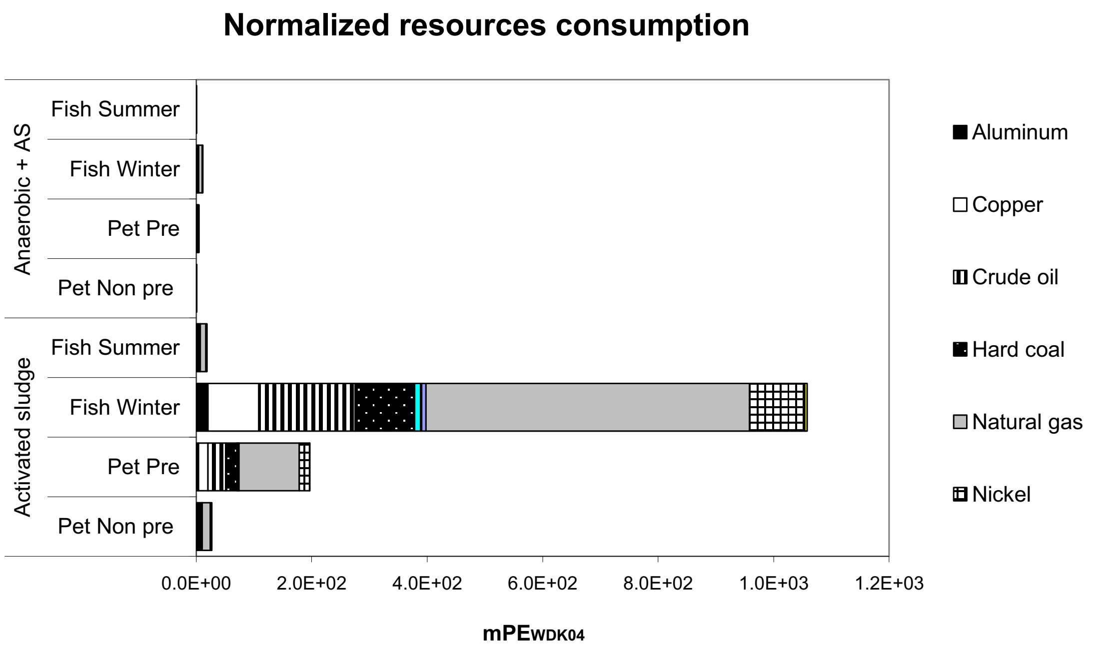


Figure 15. Normalized resources equivalent to one person's consumption during 2004 and one person's wastewater discharge for the two treatment configurations with and without anaerobic pre-treatment (Anaerobic + AS and Activated sludge respectively). Pet food wastewaters included samples with and without physico-chemical pre-treatment (Pet Pre and Pet Non pre respectively). Fish meals wastewaters included samples during summer and during winter time (Fish Summer and Fish Winter respectively). All wastewaters showed different compositions (**VI**).

a higher input COD caused increased oxygen and electricity consumption and sludge production thereby electricity consumption for its pasteurization. This increased the environmental impacts. Moreover, the reason why this was observed only for the environmental impacts evaluation was that the marginal power production applied to the LCA model affected more seriously to nutrient enrichment, global toxicity, and acidification than to resources consumption.

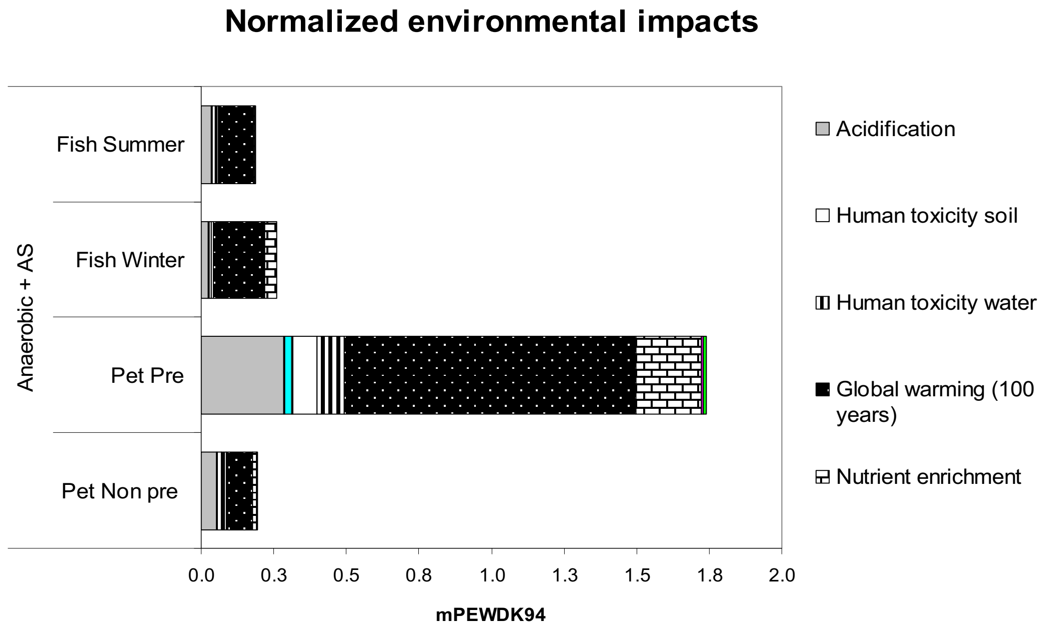


Figure 16. Normalized environmental impacts equivalent to one person's impacts during 1994 and one person's wastewater discharge for the anaerobic plus denitrification/nitrification scenario (Anaerobic + AS) (**VI**).

6.4 Hazard assessment of XOC

Very few LCA studies applied to wastewater treatment have considered hazardous compounds in trace amounts (**IV**; Farre et al. (2007);Wenzel et al. (2008)), and only one has evaluated their toxicity impacts versus the usual indicators found from electricity consumption and sludge handling and disposition (Wenzel et al. (2008)). In spite of the growing interest on evaluating their fate and toxicity effects on the environment, there is a lack of knowledge on the inclusion of these compounds in LCA studies, particularly of XOC and heavy metals. This is due to the difficulties on identifying analytically trace compounds in complex samples such as wastewater, on the uncertainties about their fate in the environment, on the lack of knowledge about their biodegradation by aerobic and anaerobic bacteria, and finally on the uncertainties about the final exposure the aquatic and soil life, as well as the humans have, which determine their toxicity together with the specific chemical properties of the compounds. Particular interest is found in heavy metals and XOC for wastewater treatment studies since they tend to end-up mainly in the sludge, and as mentioned before there is a strong political interest about finding adequate strategies for its handling and disposition.

Even though XOC and heavy metals may constitute as much as 1% of the total wastewater mass (Paper **III**), the long-term fate and effects of XOC have been documented to influence handling options of equally complex compositions (Hospido et al. (2008);Giger et al. (2003);Lundin et al. (2000);Beavis and Lundie (2003);Lundin and Morrison (2002)). At our study (Paper **III**) we found that applying a hazard screening procedure (Baun et al. (2006)), 29 potentially present hazardous XOC in wastewater and 102 in sludge should be aimed for chemical analysis to estimate their concentration and further carry out a risk assessment for determining their exposure to the environment. This systematic approach of source analysis and hazard assessment proved to be a valuable tool on targeting chemical analysis of potentially present XOC in wastewater composite samples, which could reduce the big uncertainty of what compounds to target for analytical identification. From identified hazardous XOC, 12% could not be assessed due to lack of data for aerobic biodegradability, toxicity, and bioaccumulation, whilst as much as 91% could not be assessed for anaerobic biodegradability. This lack of data exemplifies the difficulty to assess these compounds by LCA studies when anaerobic technologies are included in the system (**IV, V**,**VI**).

Urban wastewater has been subject of many studies for the analytical identification of XOC, and the toxicity these effluents exert on the environment. Well identified compounds such as PAH, DEHP, LAS, and estrogens, have been targeted for analytical screenings and complemented with toxicity studies in order to determine their risk to the local environment. Wenzel et al. (2008) developed a methodology for including the effects of endocrine disrupters and an impact category on eco-toxicity in salt water was developed to supplement the life cycle impact assessment. They found that one from three technologies evaluated, i.e. sand filtration, presented higher avoided environmental impacts from the removal of PAH, DEHP, nonylphenol, LAS, and estrogens, than the actual impacts from electricity consumption. Membrane bioreactors and ozonation showed significantly higher impacts from their electricity consumptions than those avoided by removal. However, since many scientists and experts in the area of wastewater argue, separation of the wastewaters at the source should be promoted in order to take advantage of their different chemical characteristics for resource recovery and enhanced biodegradation. Reflecting in this commonly mentioned statement, attention should also be drawn to industrial wastewater streams and their characteristics for treatment, since they may also contribute to the discharge of XOC which could be avoided in the factory rather than at the end-of-pipe wastewater treatment plant (Kohler et al. (2006);Tilche and Orhon (2002)). Food-processing industries also contribute to this discharge (Table 4) due to the high volumes of food they handle in relatively short amounts of time, extensive cleaning processes require the use of disinfectants and biocides. Moreover, the bioaccumulation of these compounds through the food chain can bring them to the wastewater discharge again by their presence in raw materials utilized. At our study (Paper **III**) we identified analytically the presence of 13 compounds in three wastewater composite samples which varied in levels of hazard. From these, ten were hazardous and nine of them predicted to be in the sludge, and from

Table 4. Categorization of the number of xenobiotic organic compounds potentially present in selected food processing industry wastewaters. Categorizations are according to Baun et al. (2006). ‘White’: Compounds which can be excluded from further assessments; ‘Grey’: Compounds that might be included in hazard assessments; ‘Black’: Compounds which must be included in hazard assessments, i.e., the ‘potential priority pollutants’ (‘black’); ‘No data’: Compounds which may be present, but cannot be evaluated due to lack of data.

Phase	Category	In all ww	Peas production (VPE)	Fish bites (FBH)	Slaughter house (SPR, SCR)	Fish bites and Slaughter house (SPR, SCR, FBH)
Water	White	14	28	32	24	8
	Grey	1	4	0	0	0
	Black	7	9	4	7	2
Solid residues	White	8	13	2	10	2
	Grey	1	3	0	0	0
	Black	14	28	36	9	15
Water & solids	No data	13	8	7	12	1
Anaerobic	No data	34	30	39	33	11

these nine, two were predicted to be both in the wastewater effluent and in the sludge, and one only in the wastewater effluent.

6.5 Potentials for integration

Even though plant’s construction and transport were left out from our studies (**IV**, **V**, **V**) energy related indicators introduced the highest consumptions and impacts to the evaluation as for Lundin and Morrison (2002), Lundin et al. (2000), Wenzel et al. (2008), and many others. In the other hand, when the toxicological impacts of some XOC were taken into account, they overcame the consumptions and impacts from the electricity consumption in one evaluated end-of-pipe treatment technology (Wenzel et al. (2008)). Furthermore, when a careful analysis of sludge disposal was done, the heavy metals turned also important on the overall LCA (Hospido et al. (2008);Lundin et al. (2000)). This brings the conflict about what is more important, the reduction of energy consumption or toxicity. While the wastewater sector aims for further reduction of toxic micro-pollutants at the expense of increased energy consumption, other sectors in society aim at greenhouse gases reduction which in some cases may happen at the expense of increased emission of toxic compounds (Wenzel et al. (2008)).

From our case studies we observed than when assessing the treatment of food-processing wastewaters for process selection and design in a life cycle context, the integration of different methodological tools is important. Hazard screening was important to focus our studies on the organic trace compounds which could be present in the wastewater effluent or in the sludge and that represented a hazard for the environment (Paper **III**). In addition, the source analysis required to go up-stream in the food-processing activities and materials which was defined according to the life cycle of the potential compounds in contact with water. Treatment efficiencies are important technical parameters to define so it is possible to predict removal of organic matter, nutrients, and solids in the biological system. Balkema et al. (2001), Lundin et al. (2001), Wenzel et al. (2008) emphasized the importance of the accuracy of these data because it can influence the overall energy demands and the toxicity assessment. From our studies (**IV**, **V**, **VI**), we found that removal efficiencies of COD, nitrogen, and suspended solids influenced the electricity consumption and sludge production of the whole systems defined. Furthermore, the characteristics of the wastewater proved to influence the oxygen, nutrients, and buffers extra requirements, as well as the volumes of sludge produced (Abstract **VI**). This agrees with Wenzel et al. (2008) who performed a sensitivity analysis of the inputs to the system, i.e. influent wastewater, and observed a change on the overall assessment. Finally, the hazard screening from Paper **III** can provide valuable information on different processing shifts to cover in a food industry in order to cover a wider range of potential XOC. Furthermore, targeted chemical analysis can be performed so concentrations in the sample are determined which is the basis to carry out an environmental risk assessment, providing the data needed to calculate the characterization factors for the life cycle impact assessment. The drawback of this point is the lack of biodegradability, bioaccumulation, and toxicity data for many of these compounds (Paper **III**).

7 Conclusions

Food-processing industry generates complex wastewaters with different factors affecting their composition such as seasonal production, cleaning practices, and even their pre-treatment before discharge. These fluctuations, their high content of organic matter, and the stricter regulation for effluents discharge, makes their handling a challenge. However and due to their organic nature, biological treatment results suitable, particularly anaerobic digestion with the generation of methane as a renewable energy source. The assessment of their potential for methane generation has somehow been limited to mainly full-scale biological reactors, and it has been realized that their physico-chemical characteristics can affect the anaerobic digestion process greatly. For this reason it becomes important to find simpler strategies to assess their biodegradation potential, as well as the possibilities and advantages of their treatment by anaerobic digestion, in comparison with the most conventional treatment by activated sludge treatment plants where they are diluted with domestic wastewater.

When assessing the methane potential of food-processing industry wastewaters under batch conditions, it was found that the physico-chemical characteristics of the wastewaters had more relevance, particularly acetate and carbohydrates contents. A coupled effect with bicarbonate alkalinity was also observed only at 25% and at 50% diluted wastewaters. The presence of acetate together with a relatively higher carbohydrates fraction, proved to exert a strong inhibitory effect by decreasing dilution. Furthermore, the inclusion of dilutions in biological methane potential assays provided valuable information on this assessment.

From the literature investigations and from our studies, it was found that a negative effect of the substrate:inoculum ratio on the methane potential assessment of complex organic waste(water)s, was only evident in the presence of acetate or readily degradable carbohydrates in the substrate. In the other hand, for the assessment of waste(water)'s anaerobic biodegradability, this negative effect was only evident in the presence of higher concentrations of organic particulates.

When high fractions of organic particulates were contained in the wastewaters, hydrolysis was the rate-limiting process in their anaerobic digestion. However, the presence of readily degradable carbohydrates together with acetate in the wastewaters not only inhibited methanogenic activity, but also hydrolytic activity when acidification of the liquid media happened, inhibiting the secretion of hydrolytic enzymes, to finally slower down the overall degradation rate. Furthermore, by co-digesting this carbohydrate-rich wastewater with a fatty wastewater, the hydrolysis process was enhanced, switching the dynamics of VFA accumulation from propionate and butyrate

to only acetate accumulation. This promoted the acclimatization of the inoculum re-establishing methanogenic activity, which was not observed when only the carbohydrate-rich wastewater was digested. In general, it was observed that the presence of lipids enhanced the specific methane production in all wastewaters assessed, being more evident at wastewaters with lower organic particulates fractions.
According to our studies and literature findings, it was noticed that a lag phase for methanogenic activity was more likely to happen at pH levels under 7, whilst for hydrolysis was at pH levels under 6.5. The latter happened frequently when assessing carbohydrate-rich food waste(water)s.

The application of mechanistic mathematical models for anaerobic digestion of organic waste(water)s under dynamic conditions has provided valuable information on the identification of rate-limiting processes and variations in substrates degradation occurring by temporal changes in the groups of bacteria. This is of primordial importance when assessing waste(water)s biodegradability under batch conditions, since it is the intention to observe these changes. For this reason it is important that, when conducting batch experiments with this purpose, the inoculum is not pre-acclimatized but still shows initial methanogenic activity. A great disadvantage of applying mechanistic models is that it requires a full characterization of the influent, and of the kinetic and inhibition factors for the specific waste(water). This characterization includes the degradable fraction which indeed requires of previous information about the waste(water) to be assessed. It was constantly noticed that these initial conditions, together with the initial biomass fractions, are the most influencing parameters when applying mechanistic models to biological methane potential assays. When the Anaerobic Digestion Model No.1 was applied, it was observed that more accurate predictions of VFA were needed in order to fit to experimental data. In this case hydrolysis rates were important parameters to calibrate even when assessing wastewaters such as food-processing industries'. Lag phases were very difficult to model, which are indeed relevant when assessing anaerobic batch experiments, and the model was extremely sensitive to input acetate, specially when it was about 15-30% of the total substrate COD. Generally, from literature, it was noticed that as more controlled the experimental conditions are in a batch experiment, as simpler the model definition is since there is no need for inclusion of a lag phase constant or pH inhibition factors. However, when for example pH is controlled or the inoculum is pre-acclimatized, results will not reflect the ultimate capability of both, the waste(water) to be degraded and the inoculum to degrade it.

On identifying trace organic compounds such as xenobiotics in these complex matrixes, it is suggested to plan a more comprehensive campaign. Source analysis as used in this study can support this planning by targeting potential compounds identified in these wastewaters. In addition, an analytical screening revealed that indeed xenobiotics can be discharged by these industries. Together with the common migrating compounds found in other urban streams, others were also found, particularly coming from cooking activities and disinfection practices. This information would not have been possible to gather without carrying the source analysis.

To investigate the possibilities of biological treatment of source separated wastewaters such as food-processing industries', life cycle assessment can provide the systems perspective needed to predict the value or the impact of the resources consumed and the by-products generated by this treatment. However, this methodological tool requires data regarding the technical and the environmental systems with enough representativity to reflect the real conditions. When the technical system does not actually exist, i.e. for process selection or process design, process specific models need to be integrated within the assessment. With this particular purpose and for the wastewaters assessed, it was found that the composition of the wastewaters did influence the overall assessment, particularly for anaerobic digestion which is rather sensitive to the waste(water) composition. Furthermore, they affected energy consumption and generation, sludge production and composition, and effluent quality, which are the most important model parameters in the life cycle assessment of wastewater treatment systems. In addition, the identification and environmental risk assessment of xenobiotic organic compounds as a potential area of integration within the life cycle assessment, also depended on the wastewater characteristics. Thus the importance and relevance of an adequate wastewater sampling and characterization.

8 Future outlook and recommendations

Being still the main practical benefit observed from anaerobic digestion of organic waste(water)s such as food processing industries', the evaluation of methane potential is the most important output to monitor. Simpler and cheaper assessments can be done by biological methane potential assays, with a considerable amount of knowledge about the wastewaters' biodegradability and methane potential. Even the application of process modeling can enrich this knowledge, extending it to the environmental valuation of its effluents and by-products. However, the quality of the results depends very much of an appropriate experimental design, which data can be further applied with a certain reliability to further modeling. A comprehensive sampling and characterization of the wastewater is necessary before carrying out the experiments. Particularly for food-processing industries, the sampling should be representative enough for at least one production shift and if possible one production season. More statistical analysis and reporting is needed when carrying wastewater sampling and analytical characterization. Furthermore, information provided by the industries about the production, cleaning, and pre-treatment practices can save some amount of resources involved in this sampling campaign and provide more representative characterization results. By knowing the organic and inorganic components in the wastewaters, it provides valuable information about the potential organic carbon flows and supports on finding the reasons of process failure. Furthermore, it gives an idea of what will be the rate-limiting process. When carrying out methane potential assays, it is important to define the objective of the assessment. If the aim is to determine the maximum achieved methane potential, dilutions can be considered, and only pH and methane should be monitored. When the application of mathematical models are necessary to complement the information about experimental anaerobic biodegradability, COD, soluble and total, could support enormously to get good hydrolysis data. For this it is indispensable to count on adequate vessels where a representative and, as much as possible, homogenous sample is obtained. In addition, the volume of the vessels should be big enough to get periodical samples, but no too big that they cannot be handled and mixed properly. pH, individual VFA, methane, ammonium, and, if possible, bicarbonate alkalinity should be part of this liquid sampling. Methane is indeed the only necessary gas-phase parameter to measure, but in the case the waste(water) is carbohydrates-rich, it is recommended to monitor hydrogen to determine more accurately the hydrogen inhibition constants. Monitoring is indeed the most laborious and time consuming drawback from biological methane potential assays. It needs to be consistent and periodical, and can be relatively planned in advance according to the characteristics of the waste. However, it is important to do it, if possible, every day. For this reason, we are very much looking forward for the availability, practicability, and economy of more process' monitors on-line. In particular pH since it can rise several decimals when measured manually which can have a tremendous effect on the predictions of substrate's degradation in anaerobic digestion, and VFA because of its laborious sampling and analysis. Inoculum and wastewater should be kept as closer to the original state, when assessing wastewater's biodegradability.

Concerning the modeling area, the adequate determination of the 'biodegradable' fraction of the waste, so-called "substrate", should be defined. Studies showed that previous knowledge of the waste can provide valuable information, however, since food-processing industries' discharges fluctuate very much, this would not be possible. The only way to asses this is by biological methane potential assays, which supports all my recommendations before. There is the need for more studies about the adequate definition of hydrolysis kinetics, separately for the particulate and dissolved polymers. The definition of initial biomass concentrations is quite important for modeling batch experiments, in particular for the VFA and methane degraders. This can be done by carrying control experiments, and estimating their initial fractions by experimental/modeling data with a simpler 2-stages model. Alternatively, it can be done by modeling the methanogenic reactor where the inoculum has been taken from. It is, however, important to remember, that the inoculum will be submitted to physiological changes once it is passed from a continuous to a batch culture. Thus, results from these estimations should be used as reference values, which may be subjected to change.

Regarding the handling of food-processing industry wastewaters, it was noticed that the pre-treatment equalization tanks may have enhanced the fermentation of readily degradable components in some of the wastewaters assessed. It is recommended that the wastewaters do not stay for long retention times in these tanks, specially if they are sent for further anaerobic digestion treatment, since the presence of acetate in the waste(water) can inhibit seriously its anaerobic digestion, particularly if a part of the readily degradable fraction still remains.

Finally, the integration of environmental risk assessment with life cycle assessment is a promising area. However, there is a general lack of knowledge about some emerging trace compounds, particularly xenobiotic organic compounds. This means that attention should be drawn first, to studies about the fate, biodegradation, bioaccumulation, and toxicity of compounds that are actually found in the waste(water)s.

References

Ahring,B.K., Sandberg,M., and Angelidaki,I. (1995) Volatile Fatty-Acids As Indicators of Process Imbalance Is Anaerobic Digesters. Applied Microbiology and Biotechnology 43 (3), 559-565.

Akram, A. and Stuckey, D. C. Batch assay to assess the microbial population in submerged anaerobic membrane bioreactors (SAMBRs). 11th World Congress on Anaerobic Digestion. 2008. 20-9-2007.

Angelidaki,I. and Ahring,B.K. (1994) Anaerobic Thermophilic Digestion of Manure at Different Ammonia Loads - Effect of Temperature. Water Research 28 (3), 727-731.

Angelidaki,I. and Ahring,B.K. (1997) Codigestion of olive oil mill wastewaters with manure, household waste or sewage sludge. Biodegradation 8 (4), 221-226.

Angelidaki,I. and Sanders,W.T.M. (2004) Assessment of the anaerobic biodegradability of macro-pollutants. Reviews in Environmental Science and Bio/Technology 3 141-158.

Alatriste-Mondragon,F., Samar,P., Cox,H.H.J., Ahring,B.K., and Iranpour,R. (2006) Anaerobic codigestion of municipal, farm, and industrial organic wastes: A survey of recent literature. Water Environment Research 78 (6), 607-636.

Austermann-Haun, Ute, Seyfried, Carl Franz, and Rosenwinkel, Karl Heinz. (1997) Full scale experiences with anaerobic pre-treatment of wastewater in the food and beverage industry in Germany. Water Science and Technology 36[2-3], 321-328.

Azapagic,A. (1999) Life cycle assessment and its application to process selection, design and optimisation. Chemical Engineering Journal 73 (1), 1-21.

Babuna,F.G., Ince,O., Orhon,D., and Simsek,A. (1998) Assessment of inert cod in pulp and paper mill wastewater under anaerobic conditions. Water Research 32 (11), 3490-3494.

Balkema,A.J., Preisig,H.A., Otterpohl,R., Lambert,A.J.D., and Weijers,S.R. (2001) Developing a model based decision support tool for the identification of sustainable treatment options for domestic wastewater. Water Science and Technology 43 (7), 265-270.

Batstone,D.J. (2006) Mathematical Modelling of Anaerobic Reactors Treating Domestic Wastewater: Rational Criteria for Model Use. Reviews in Environmental Science and Bio/Technology 5 (1), 57-71.

Batstone, D. J., Balthes, C., and Barr, K. (2007) Model assisted startup of anaerobic digestion fed with thermally hydrolysed sludge. 11th World Congress on Anaerobic Digestion.

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) The IWA Anaerobic Digestion Model No 1 (ADM1). Water Science and Technology 45 (10), 65-73.

Batstone,D.J., Keller,J., Newell,R.B., and Newland,M. (2000a) Modelling anaerobic degradation of complex wastewater. I: model development. Bioresource technology 75 (1), 67-74.

Batstone,D.J., Keller,J., Newell,R.B., and Newland,M. (2000b) Modelling anaerobic degradation of complex wastewater. II: parameter estimation and validation using slaughterhouse effluent. Bioresource technology 75 (1), 75-85.

Batstone,D.J., Pind,P.F., and Angelidaki,I. (2003) Kinetics of thermophilic, anaerobic oxidation of straight and branched chain butyrate and valerate. Biotechnology and Bioengineering 84 (2), 195-204.

Baun,A., Eriksson,E., Ledin,A., and Mikkelsen,P.S. (2006) A methodology for ranking and hazard identification of xenobiotic organic compounds in urban stormwater. Science of the total environment 370 (1), 29-38.

Beavis,P. and Lundie,S. (2003) Integrated environmental assessment of tertiary and residuals treatment - LCA in the wastewater industry. Water Science and Technology 47 (7-8), 109-116.

Bernet,N.P.E. (2006) Application of biological treatment systems for food-processing wastewaters. In: Cervantes,F.J.P.S.G.v.H.A.C. (ed), Advanced biological treatment processes for industrial wastewaters. Principles and applications. 237-266, IWA Publishing.

Biswas,J., Chowdhury,R., and Bhattacharya,P. (2006) Kinetic studies of biogas generation using municipal waste as feed stock. Enzyme and Microbial Technology 38 (3-4), 493-503.

Blumensaat,F. and Keller,J. (2005) Modelling of two-stage anaerobic digestion using the IWA Anaerobic Digestion Model No. 1 (ADM1). Water Research 39 (1), 171-183.

Bozinis,N.A., Alexiou,I.E., and Pistikopoulos,E.N. (1996) A mathematical model for the optimal design and operation of an anaerobic co-digestion plant. Water Science and Technology 34 (5-6), 383-391.

Buffiere,P., Loisel,D., Bernet,N., and Delgenes,J.P. (2006) Towards new indicators for the prediction of solid waste anaerobic digestion properties. Water Science and Technology 53 (8), 233-241.

Buswell, E. G. and Neave, S. L. (1930) Laboratory studies of sludge digestion. Illinois Div.of State Wat.Survey. 30.

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

Casani,S., Rouhany,M., and Knochel,S. (2005) A discussion paper on challenges and limitations to water reuse and hygiene in the food industry. Water Research 39 (6), 1134-1146.

Chen,T.H. and Hashimoto,A.G. (1996) Effects of pH and substrate:inoculum ratio on batch methane fermentation. Bioresource technology 56 179-186.

Chen,Y.R. and Hashimoto,A.G. (1980) Substrate Utilization Kinetic-Model for Biological Treatment Processes. Biotechnology and Bioengineering 22 (10), 2081-2095.

Confer,D.R. and Logan,B.E. (1998) Location of protein and polysaccharide hydrolytic activity in suspended and biofilm wastewater cultures. Water Research 32 (1), 31-38.

Davidsson,Å. (2007) Increase of biogas production at wastewater treatment plants: Addition of urban organic waste and pre-treatment of sludge. PhD Water and Environmental Engineering, Department of Chemical Engineering, Lund University, Lund, Sweden.

Davidsson,Å., Gruvberger,C., Christensen,T.H., Hansen,T.L., and Jansen,J.L.C. (2007) Methane yield in source-sorted organic fraction of municipal solid waste. Waste Management.

Dincer,I. and Rosen,M.A. (2005) Thermodynamic aspects of renewables and sustainable development. Renewable & Sustainable Energy Reviews 9 (2), 169-189.

Dionisi,D., Bertin,L., Bornoroni,L., Capodicasa,S., Papini,M.P., 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 1496-1505.

Ekama,G.A., Sotemann,S.W., and Wentzel,M.C. (2007) Biodegradability of activated sludge organics under anaerobic conditions. Water Research 41 (1), 244-252.

Eriksson,E., Baun,A., Mikkelsen,P.S., and Ledin,A. (2005) Chemical hazard identification and assessment tool for evaluation of stormwater priority pollutants. Water Science and Technology 51 (2), 47-55.

Eriksson, E., Ledin, A., and Hansson, K. Identifiering och prioritering av miljöfarliga ämnen från verksamheter i Eslöv – deletapp i ReVAQ-samarbetet. 2007. Eslöv, Sweden, in preparation.

Farre,J., Garcia-Montano,J., Ruiz,N., Munoz,I., Domenech,X., and Peral,J. (2007) Life cycle assessment of the removal of Diuron and Linuron herbicides from water using three environmentally friendly technologies. Environmental Technology 28 (7), 819-830.

Fava,F., Bertin,L., Capodicasa,S., Occulti,F., Girotti,S., and Marchetti,L. (2007) Microbial processes associated to the decontamination and detoxification of a polluted activated sludge during its anaerobic stabilization. Water Research 41 2407-2416.

Feng,Y., Behrendt,J., Wendland,C., and Otterpohl,R. (2006) Parameter analysis of the IWA Anaerobic Digestion Model No. 1 for the anaerobic digestion of blackwater with kitchen refuse. Water Science and Technology 54 (4), 139-147.

Fernandez, A., Sanchez, A., and Font, X. (2005) Anaerobic co-digestion of a simulated organic fraction of municipal solid wastes and fats of animal and vegetable origin. Biochemical Engineering Journal 26[1], 22-28.

Fernandez,B., Porrier,P., and Chamy,R. (2001) Effect of inoculum-substrate ratio on the start-up of solid waste anaerobic digesters. Water Science and Technology 44 (4), 103-108.

Foresti,E., Zaiat,M., and Vallero,M. (2006) Anaerobic processes as the core technology for sustainable domestic wastewater treatment: Consolidated applications, new trends, perspectives, and challenges. Reviews in Environmental Science and Bio/Technology (5), 3-19.

Frederic, S., Buffiere, P., Marty, B., and Delgenes, J. P. (2007) Importance of waste composition on the kinetics of hydrolysis and methane production in anaerobic digestion: soluble and non-soluble fractions. 11th World Congress on Anaerobic Digestion.

Gavala,H.N. and Lyberatos,G. (2001) Influence of anaerobic culture acclimation on the degradation kinetics of various substrates. Biotechnology and bioengineering 74 (3), 181-195.

Giger,W., Alder,A.C., Golet,E.M., Kohler,H.P.E., McArdell,C.S., Molnar,E., Siegrist,H., and Suter,M.J.F. (2003) Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. Chimia 57 (9), 485-491.

Goel,R., Mino,T., Satoh,H., and Matsuo,T. (1998) Comparison of hydrolytic enzyme systems in pure culture and activated sludge under different electron acceptor conditions. Water Science and Technology 37 (4-5), 335-343.

Gosset, J. M. and Belser, R. L. (1982) Anaerobic Digestion of Waste Activated Sludge. Journal of Environmental Engineering-Asce (Press-Kristensen et al., 2007), 1101-1120.

Grady,C.P.L., Smets,B.F., and Barbeau,D.S. (1996) Variability in kinetic parameter estimates: A review of possible causes and a proposed terminology. Water Research 30 (3), 742-748.

Gungor-Demirci,G. and Demirer,G.N. (2004) Effect of initial COD concentration, nutrient addition, temperature and microbial acclimation on anaerobic treatability of broiler and cattle manure. Bioresource technology 93 (2), 109-117.

Hammes,F., Kalogo,Y., and Verstraete,W. (2000) Anaerobic digestion technologies for closing the domestic water, carbon and nutrient cycles. Water Science and Technology 41 (3), 203-211.

Hansen,T.L., Jansen,J.L., Davidsson,A., and Christensen,T.H. (2007) Effects of pre-treatment technologies on quantity and quality of source-sorted municipal organic waste for biogas recovery. Waste Management 27 (3), 398-405.

Hashimoto,A.G. (1989) Effect of Inoculum/Substrate Ratio on Methane Yield and Production Rate from Straw. Biological Wastes 28 247-255.

Hashimoto,A.G., Varel,V.H., and Chen,Y.R. (1981) Ultimate methane yield from beef cattle manure: effect of temperature, ration constituents, antibiotics and manure age. Agricultural Wastes 3 241-256.

He,P.J., Lu,F., Shao,L.M., Pan,X.J., and Lee,D.J. (2006) Enzymatic hydrolysis of polysaccharide-rich particulate organic waste. Biotechnology and Bioengineering 93 (6), 1145-1151.

Hegde,G. and Pullammanappallil,P. (2007) Comparison of thermophilic and mesophilic one-stage, batch, high-solids anaerobic digestion. Environmental Technology 28 (4), 361-369.

Henze,M., Harremoes,P., la Cour Jansen,J., and Arvin,E. (2002) Wastewater treatment: Biological and chemical processes., Springer.

Hospido,A., Moreira,M.T., and Feijoo,G. (2008) A comparison of municipal wastewater treatment plants for big centres of population in Galicia (Spain). International Journal of Life Cycle Assessment 13 (1), 57-64.

Houillon,G. and Jolliet,O. (2005) Life cycle assessment of processes for the treatment of wastewater urban sludge: energy and global warming analysis. Journal of Cleaner Production 13 (3), 287-299.

Huete,E., de Gracia,M., Ayesa,E., and Garcia-Heras,J.L. (2006) ADM1-based methodology for the characterisation of the influent sludge in anaerobic reactors. Water Science and Technology 54 (4), 157-166.

IWA Task Group. (2006) Harmonization of Anaerobic Biodegradation, Activity and Inhibition Assays Report. 2006. Report.

Janssens,I., Tanghe,T., and Verstraete,W. (1997) Micropollutants: A bottleneck in sustainable wastewater treatment. Water Science and Technology 35 (10), 13-26.

Jeppsson,U. and Hellstrom,D. (2002) Systems analysis for environmental assessment of urban water and wastewater systems. Water Science and Technology 46 (6-7), 121-129.

Jeppsson,U. and Pons,M.N. (2004) The COST benchmark simulation model - current state and future perspective. Control Engineering Practice 12 (3), 299-304.

Jeppsson,U., Rosen,C., Alex,J., Copp,J., Gernaey,K., Pons,M.N., and Vanrolleghem,P.A. (2006) Towards a benchmark simulation model for plant-wide control strategy performance evaluation of WWTPs. Water Science and Technology 53 (1), 287-295.

Khanal,S.K., Xie,B., Thompson,M.L., Sung,S.W., Ong,S.K., and Van Leeuwen,J. (2006) Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems. Environmental Science & Technology 40 (21), 6537-6546.

Kim,H.W., Han,S.K., and Shin,H.S. (2003) The optimisation of food waste addition as a co-substrate in anaerobic digestion of sewage sludge. Waste Management & Research 21 (6), 515-526.

Kirby,R.M., Bartram,J., and Carr,R. (2003) Water in food production and processing: quantity and quality concerns. Food Control 14 (5), 283-299.

Kleerebezem, R. and van Loosdrecht, M. C. M. (2005) Waste characterization for implementation in ADM1. The First International Workshop on the IWA Anaerobic Digestion Model No.1 (ADM1). 193-200.

Kleerebezem,R. and van Loosdrecht,M.C.M. (2007) Mixed culture biotechnology for bionenergy production. Current opinion in Biotechnology (18), 207-212.

Kleerebezem,R. and van Loosdrecht,M.C.M. (2006) Critical analysis of some concepts proposed in ADM1. Water Science and Technology 54 (4), 51-57.

Knupp,G., Rucker,G., Ramos-Cormenzana,A., Garrido Hoyos,S., Neugebauer,M., and Ossenkop,T. (1996) Problems on identifying phenolic compounds during the microbial degradation of olive mill wastewater. International biodeterioration and biodegradation 62 (5), 277-282.

Kohler,A., Hellweg,S., Escher,B.I., and Hungerbuhler,K. (2006) Organic pollutant removal versus toxicity reduction in industrial wastewater treatment: The example of wastewater from fluorescent whitening agent production. Environmental Science & Technology 40 (10), 3395-3401.

Kyazze,G. (2007) Hydrogen production via dark fermentation of carbohydrate-rich substrates. PhD thesis. University of Flamorgan/Prifysgol Morgannwg.

Lassen, C., Skaarup, S., Hagen, S., Kjølholt, J., Nielsen, P. J., and Samsoe-Petersen, L. (2001) Inventory of biocides used in Denmark. Danish EPA. 13-108. Denmark. EU Directive 98/8/EC.

Lay,J.J., Li,Y.Y., and Noike,T. (1998) The influence of pH and ammonia concentration on the methane production in high-solids digestion processes. Water Environment Research 70 (5), 1075-1082.

Ledin,A., Eriksson,E., Mikkelsen,P.S., and Baun,A. (2006) CHIAT: Chemical hazard identification and assessment tool for selection of priority pollutants. In: Malmqvist,P.A., Heinicke,G., Karrman,E., Stenstrom,T.A., and Svensson,G. (eds), Strategic Planning of Sustainable Urban Water Management. 55-67, IWA Publishing, London.

Liu,C.F., Yuan,X.Z., Zeng,G.M., Li,W.W., and Li,J. (2008) Prediction of methane yield at optimum pH for anaerobic digestion of organic fraction of municipal solid waste. Bioresource technology 99 (4), 882-888.

Lundin,M., Bengtsson,M., and Molander,S. (2000) Life cycle assessment of wastewater systems: Influence of system boundaries and scale on calculated environmental loads. Environmental Science & Technology 34 (1), 180-186.

Lundin,M. and Morrison,G.M. (2002) A life cycle assessment based procedure for development of environmental sustainability indicators urban water systems. Urban Water (4), 145-152.

Lyberatos,G., Gavala,H.N., Stamatelatou A. (1997) An integrated approach for management of agricultural industries wastewaters. Nonlinear Analysis, Theory, Methods & Applications 30 (4), 2341-2351.

Madsen,T. and Rasmussen,H.B. (1996) A method for screening the potential toxicity of organic chemicals to methanogenic gas production. Water Science and Technology 33 (6), 213-220.

Maya-Altamira,L., Baun,A., Angelidaki,I., and Schmidt,J.E. (2008) Influence of wastewater characteristics on methane potential in food processing industry wastewaters. Water Research. *In press*.

McCarty,P.L. and Mosey,F.E. (1991) Modeling of Anaerobic-Digestion Processes (A Discussion of Concepts). Water Science and Technology 24 (8), 17-33.

Mosche,M. and Jordening,H.J. (1999) Comparison of different models of substrate and product inhibition in anaerobic digestion. Water Research 33 (11), 2545-2554.

Mosey,F.E. (1981) Anaerobic biological treatment of Food Industry Waste Waters. Water Pollution Control 273-291.

Muller,W.R., Frommert,I., and Jorg,R. (2004) Standardized methods for anaerobic biodegradability testing. Reviews in Environmental Science and Bio/Technology 3 141-158.

Nopharatana,A., Pullammanappalli,P.C., and Clarke,W.P. (2007) Kinetics and dynamic modelling of batch anaerobic digestion of municipal solid waste in a stirred reactor. Waste Management 27 (5), 595-603.

Odegaard,H., Paulsrud,B., and Karlsson,I. (2002) Wastewater sludge as a resource: sludge disposal strategies and corresponding treatment technologies aimed at sustainable handling of wastewater sludge. Water Science and Technology 46 (10), 295-303.

Orhon,D. (1998) Evaluation of industrial biological treatment design on the basis of process modelling. Water Science and Technology 38 (4-5), 1-8.

Palme,U., Lundin,M., Tillman,A.M., and Molander,S. (2005) Sustainable development indicators for wastewater systems - researchers and indicator users in a co-operative case study. Resources Conservation and Recycling 43 (3), 293-311.

Parawira,W., Murto,M., Zvauya,R., and Mattiasson,B. (2004) Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet leaves. Renewable Energy 29 (11), 1811-1823.

Parker,W.J. (2005) Application of the ADM1 model to advanced anaerobic digestion. Bioresource technology 96 (16), 1832-1842.

Pavlostathis,S.G. and Giraldogomez,E. (1991) Kinetics of Anaerobic Treatment - A Critical-Review. Critical Reviews in Environmental Control 21 (5-6), 411-490.

Pavlostathis, S. G. and Gosset, J. M. (1986) A Kinetic Model for Anaerobic Digestion of Biological Sludge. Biotechnology and Bioengineering 28, 1519-1530.

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), 122-130.

Rao,M.S. and Singh,S.P. (2004) Bioenergy conversion studies of organic fraction of MSW: kinetic studies and gas yield-organic loading relationships for process optimisation. Bioresource technology 95 (2), 173-185.

Rao,M.S., Singh,S.P., Singh,A.K., and Sodha,M.S. (2000) Bioenergy conversion studies of the organic fraction of MSW: assessment of ultimate bioenergy production potential of municipal garbage. Applied Energy 66 (1), 75-87.

Raposo,F., Banks,C.J., Siegert,I., Heaven,S., and Borja,R. (2006) Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests. Process Biochemistry 41 1444-1450.

Rosen, C. and Jeppsson, U. (2006) Aspects of the ADM1 implementation within the BSM2 framework. Department of Industrial Electrical Engineering and Automation, Lund University, Lund, Sweden.

Rosen,C., Vrecko,D., Gernaey,K.V., Pons,M.N., and Jeppsson,U. (2006) Implementing ADM1 for plant-wide benchmark simulations in Matiab/Simulink. Water Science and Technology 54 (4), 11-19.

Rosenwinkel,K.H., Austermann-Haun,U., and Meyer,H. (2005) Industrial wastewater sources and treatment strategies. In: Jordening,H.J. and Winter,J. (eds), Environmental biotechnology: Concepts and applications. 49-77, Wiley-VCH, Weinheim.

Rozzi,A. and Remigi,E. (2004) Methods of assessing microbial activity and inhibition under anaerobic conditions: a literature review. Reviews in Environmental Science and Bio/Technology 3 93-115.

Sanders,W.T.M., Zeeman,G., and Lettinga,G. (2002) Hydrolysis kinetics of dissolved polymer substrates. Water Science and Technology 45 (10), 99-104.

Schink,B. (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiology and Molecular Biology Reviews 61 (2), 262-280.

Schmidt,J.E. and Ahring,B.K. (1993) Effects of Hydrogen and Formate on the Degradation of Propionate and Butyrate in Thermophilic Granules from An Upflow Anaerobic Sludge Blanket Reactor. Applied and Environmental Microbiology 59 (8), 2546-2551.

Sekoulov,I. (2002) Sustainable development of wastewater treatment strategies for the food industries. Water Science and Technology 45 (12), 315-320.

Shimada, T., Zilles, J. L., Morgenroth, E., and Raskin, L. (2007) Effects of the antimicrobial tylosin on the performance of anaerobic sequencing batch reactors. 11th World Congress on Anaerobic Digestion. Brisbane, Australia.

Shin,H.S. and Song,Y.C. (1995) A Model for Evaluation of Anaerobic Degradation Characteristics of Organic Waste - Focusing on Kinetics, Rate-Limiting Step. Environmental Technology 16 (8), 775-784.

Siegrist,H., Vogt,D., Garcia-Heras,J.L., and Gujer,W. (2002) Mathematical model for meso- and thermophilic anaerobic sewage sludge digestion. Environmental Science & Technology 36 (5), 1113-1123.

Speece,R.E. (1996) Anaerobic biotechnology for industrial wastewaters., Archae Press, Tennessee.

Starkl,M., Brunner,N., Grasser,U., Moog,O., Stagl,S., Karrman,E., Wimmer,J., Szewieczek,R., and Haberl,R. (2005) Analysis and evaluation of methodologies to assess technical urban water systems. Water Science and Technology 52 (9), 43-51.

Tidaker,P., Karrman,E., Baky,A., and Jonsson,H. (2006) Wastewater management integrated with farming - an environmental systems analysis of a Swedish country town. Resources Conservation and Recycling 47 (4), 295-315.

Tilche,A. and Orhon,D. (2002) Appropriate basis of effluent standards for industrial wastewaters. Water Science and Technology 45 (12), 1-11.

Turick,C.E., Peck,M.W., Chynoweth,D.P., and Jerger,D.E. (1991) Methane fermentation of woody biomass. Bioresource technology 37 141-147.

Vavilin,V.A., Rytov,S.V., and Lokshina,L.Y. (1996) A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. Bioresource technology 56 (2-3), 229-237.

Verstraete,W., Morgan-Sagastume,F., Aiyuk,S., Waweru,M., Rabaey,K., and Lissens,G. (2005) Anaerobic digestion as a core technology in sustainable management of organic matter. Water Science and Technology 52 (1-2), 59-66.

Vrecko,D., Gernaey,K.V., Rosen,C., and Jeppsson,U. (2006) Benchmark Simulation Model No 2 in Matlab-Simulink: Towards plant-wide WWTP control strategy evaluation. Water Science and Technology 54 (8), 65-72.

Wenzel,H., Hauschild,M., and Alting,L. (1997) Environmental assessment of products: Methodology, tools and case studies in product development., Kluwer Publishers.

Wenzel,H., Larsen,H.F., Clauson-Kaas,J., Hoibye,L., and Jacobsen,B.N. (2008) Weighing environmental advantages and disadvantages of advanced wastewater treatment of micro-pollutants using environmental life cycle assessment. Water Science and Technology 57 (1), 27-32.

Wheatley, A. D. (1995) Food Industry and the Environment in the European Union. 111-228.

Zeeman,G. and Sanders,W. (2001) Potential of anaerobic digestion of complex waste(water). Water Science and Technology 44 (8), 115-122.

Zhang,R., El-Mashad,H.M., Hartman,K., Wang,F., Liu,G., Choate,C., and Gamble,P. (2007) Characterization of food waste as feedstock for anaerobic digestion. Bioresource technology (98), 929-935.

Appendices

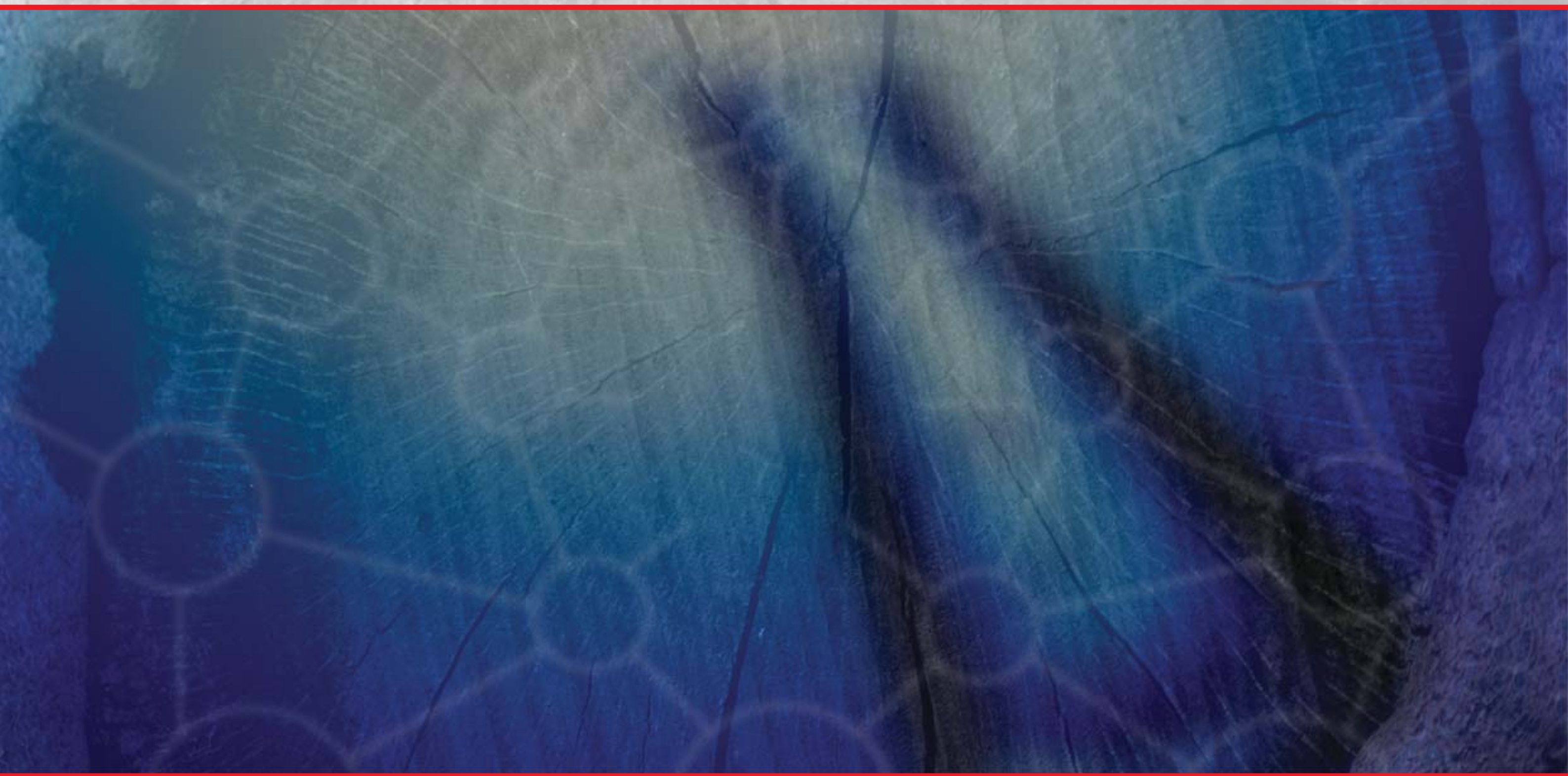
I. Maya-Altamira, L., Baun, A., Angelidaki, I. & Schmidt, J.E. (2008) Influence of wastewater characteristics on methane potential in food-processing industry wastewaters. *Water Research 42(8-9), 2195-2203.*

II. Maya-Altamira, L., Baun, A., Ramirez, I., Steyer, J.P., Gernaey, K. & Schmidt, J.E. Anaerobic biodegradability on the digestion and co-digestion of six food-processing industry wastewaters. *Manuscript.*

III. Maya-Altamira, L., Eriksson, E. & Baun, A. Source analysis and hazard screening of xenobiotic organic compounds in wastewater from food-processing industries. *Accepted in Water, Air & Soil Pollution: Focus.*

IV. Maya-Altamira, L., Batstone, D.J., Baun, A. Hauschild, M. & Schmidt, J.E. (2005). Use of life cycle assessment and environmental risk assessment as tools for design and optimization of a wastewater treatment system for effluents from the food-processing industry. 2nd International Conference on Life Cycle Management, September 5-7, Barcelona, Spain, *volume II, p.402-405*.

The papers are not included in this www-version but can be obtained from the library at the Department of Environmental Engineering, Miljoevej, Building 113, Technical University of Denmark, DK-2800 Kgs. Lyngby (library@env.dtu.dk).



Department of Environmental Engineering
Technical University of Denmark
Miljoevej, Building 113
DK-2800 Kgs. Lyngby
Denmark

Phone: +45 4525 1600
Fax: +45 4593 2850
e-mail: reception@env.dtu.dk

Please visit our website www.env.dtu.dk

ISBN 978-87-91855-50-4