DISSERTATIONS IN

FORESTRY AND NATURAL SCIENCES

SAMI LUSTE

Anaerobic Digestion of Organic By-products from Meat-processing Industry

The Effect of Pre-Treatments and Co-digestion

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Academic Dissertation
To be presented by permission of the Faculty of Sciences and Forestry for public examination in the Auditorium L1, Canthia building, University of Eastern Finland, Kuopio, on December, 16, 2011, at 13 o`clock p.m.

Department of Environmental Science

Kopijyvä
Kuopio, 2011
Editors: Prof. Pertti Pasanen
Distribution:
Eastern Finland University Library / Sales of publications
P.O. Box 107, FI-80101 Joensuu, Finland
tel. +358-50-3058396
http://www.uef.fi/kirjasto

ISBN 978-952-61-0522-2 (Paperback); ISSNL 1798-5668; ISSN 1798-5668
ISBN 978-952-61-0523-9 (PDF); ISSNL 1798-5668; ISSN 1798-5676

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ABSTRACT

Anaerobic digestion is a multi-beneficial biological treatment during which micro-organisms degrade organic material producing biogas (i.e. methane) and stabilised end-product (i.e. digestate). Methane is a versatile renewable energy source and digestate can be used as an organic fertiliser and/or soil improver. Because of the increasing consumption and tightening environment and health legislation, production of organic wastes suitable for anaerobic digestion increases.

Animal by-products (ABP) from the meat-processing industry are often rendered (contaminated material), used as feedstock (in fur breeding), or composted. However, ABPs studied could not be utilised in fodder or in animal food production and have currently been rendered or directed to composting, despite being mostly considered unsuitable for composting. Many ABPs are energy-rich, wet and pasty materials and suitable for the anaerobic digestion process. Moreover, suitable pre-treatment to hydrolyse solid materials and/or co-digestion of two or several materials may improve the anaerobic digestion with ultimate goal to increase the methane production, stabilisation and reusability of digestate.

The case chosen for more detailed research was that of a middle-sized Finnish meat–processing industry. The aim of the thesis was to evaluate the feasibility of different ABPs presently available for treatment as raw material for anaerobic digestion. Another objective was to enhance the anaerobic digestion process via specific pre-treatments and co-digestion cases with the ultimate aim to increase the methane production and the quality of the digestate. The general goal was to observe the overall process from the perspective of real-circumstances in Finland to rise to needs in practice and to produce exploitable information for adopting sustainable development locally and case-specifically into practice via versatile anaerobic digestion technology.

The ABPs studied were highly bio-degradable and especially suitable for anaerobic co-digestion. The co-digestion of the ABPs with sewage sludge and cattle slurry resulted improved methane production and reusability of the digestate. These enhancements were further improved by the pre-treatments studied. The most suitable (ultrasound and bacterial product addition) and synergistically beneficial (pre-hygienisation) pre-treatments were found to enhance the complex degradation of materials. Pre-treatments effects on the whole process and on the end-products were depended on the hydrolysis values, but especially on the content of the materials and qualities of the solubilised compounds. Economical feasibility of ultrasound and hygienisation pre-treatments is attainable.

Materials and process methods studied in this thesis offer required new information and aspects about the case- and material-specific factors of process requirements, process optimisation according to the requirements in practice, degradability of the ABP materials, hygienic matters and mechanisms involved in pre-treatments and co-digestion of ABPs. The information produced could be directly utilised in the practical implementations of the anaerobic digestion of studied or corresponding materials and feed mixtures.

Universal Decimal Classification: 534-8, 628.336.5, 628.385, 628.4.034, 628.4.042, 637.513.12, 637.514.9

CAB Thesaurus: waste treatment; anaerobic treatment; anaerobic digestion; meat and livestock industry; abattoirs; slaughterhouse waste; pretreatment; ultrasonic treatment; meat byproducts; gas production; biogas; methane; methane production; hygiene

Yleinen suomalainen asiasanasto: jätteet - - käsittely; anaerobiset menetelmät; mädätys; lihateollisuus; teurastamot; esikäsittely; ultraääni; kaasuntuotanto; biokaasu; metaani; hygienia

Acknowledgements

I am grateful for Maj & Torr Nessling Foundation (2007-2009), Maa- ja vesitekniikan tuki association (2010), Foundation of Emil Aaltonen (2010), the European Union and city of Mikkeli for financing the study. Järvi-Suomen Portti Ltd., wastewater treatment plant of Kenkävero, NCH Finland Ltd. and farmer Timo Lyytikäinen are thanked for providing the studied materials and MTT for the management of finances.

The research work for the thesis was carried out at the University of Eastern Finland (UEF, Previously University of Kuopio) in Laboratory of Applied Environmental Chemistry (LAEC) in Mikkeli during January 2007 – December 2008. I am grateful for Professor Mika Sillanpää for giving me the opportunity to work in LAEC. I am also highly grateful for my supervisor and the most important UEF contact Professor Juhani Ruuskanen for the guidance and encouragement during the entire era. I also want to thank Dr. Helvi Heinonen-Tanski from UEF for inspirational co-study and all her wise advises. Thanks also to all the co-workers in LAEC (especially M.Sc. Heikki Särkkä and laboratorian Taija Holm).

Grants and stipends for the research did not cover the whole period, so part of the study was done on side of the other works. I especially want to thank for my current employer, Mikkeli University of Applied Sciences (MUAS), especially Hanne, Kari and all the ladies of the lab. Thank you for your support and toleration of my occasional tiredness at work.

I am truly grateful for my amazing head supervisor Dr. Sari Luostarinen, who not only made this study possible but supported, encouraged and advised me all this time despite the huge workload on her own. Sari, your effectiveness, proficiency, solid confidence and commitment to your work and promises to your friends are incredible and truly admirable. You opened the scientific world for me which changed the course of my life. You are my idol and my dear friend, without you this would not have happened. I also want to say my thanks to Sari´s family, Tuomas and Elias, for their patience. I sincerely hope that this project has not taken too much of your family time.

My sincere gratitude goes to my confidants, my dear parents Marja-Liisa and Juhani and my dear sister Eeva and her husband Jarmo for always supporting me. I especially want to thank Eeva for your understanding, empathy and unselfish support and help has always been amazing and indispensable. I feel deep gratitude to you.

The most grateful I am for my Sari, my beloved wife and colleague, who also had a contribution to the analysis and articles of the present study. However, most of all I want to thank you beautiful for your love, your cool clarity and your unfailing support in our everyday life. You have showed me another world with more light and beauty, than I could have ever realised. I love you!

I also have to mention our little goblin Kaapo Joonatan, who did not really help with this project, but has similarly proven much more complicated thesis: how 3.34 kg can overbalance everything else in this world.

Lahti, Finland, September 2011

Sami Luste

ABBREVIATIONS

ABP	Animal by-products
APS	Average particle size
BMP	Biological methane potential (from batches)
CFU	Colon forming units
CH_4	Methane
CHP	Combined heat and power
COD	Chemical oxygen demand
COD_{sol}	Soluble chemical oxygen demand
DAF	Dissolved air flotation
E. coli	*Escherichia coli*
Es	Specific energy (input)
Eo	Energy output
FID	Flame ionisation detector
GC	Gas chromatography
HRT	Hydraulic retention time
LRC_{sol}	Soluble lignin related compounds
NH_4^+-N	Ammonium nitrogen
NH_3-N	Ammonia nitrogen
N_{sol}	Soluble nitrogen
N_{tot}	Total nitrogen
OLR	Organic loading rate
PSD	Particle size distribution
SMA	Specific methanogenic activity
SMP	Specific methane production (Reactor studies)
TS	Total solids
VS	Volatile solids
VSS	Volatile suspended solids
VFA	Volatile fatty acids
LCFA	Long chain fatty acids

LIST OF ORIGINAL PUBLICATIONS

The thesis consists of a summary and the five original papers which are referred to by Roman numbers (Papers **I-V**) in the text.

I Luste S., Luostarinen S., Sillanpää M. (2009): Effect of pre-treatments on hydrolysis and methane production potentials of by-products from meat-processing industry. J Haz Mat. 164: 247–255.

II Luste S., Vilhunen S., Luostarinen S. (2011): Effect of ultrasound and bacterial product on hydrolysation of by-products from meat-processing industry. Int Biodet Biodeg. 65: 318-325.

III Luste S., Luostarinen S. (2010): Anaerobic co-digestion of meat-processing by-products and sewage sludge– Effect of hygienisation and organic loading rate. Biores Tech. 101: 2657-2664.

IV Luste S., Heinonen-Tanski H., Luostarinen S (2011): Enhanced co-digestion of ultrasound and hygienisation pre-treated dairy cattle slurry and animal by-products from meat processing industry. Biores Tech. In Press.

V Luste S., Luostarinen S. (2011): Enhanced methane production from ultrasound pre-treated and hygienised dairy cattle slurry. Waste Manag. 31: 2174-2179.

Author's contribution:
Sami Luste planned the experiments with the supervisors and performed all the experimental work and at least 90% of the writing for the Papers **I-V**.

DESCRIPTION OF ORIGINAL PUBLICATIONS

I Screening the suitability of chosen by-products from Finnish meat-processing industry for anaerobic digestion and the effect of different pre-treatments (hygienisation, ultrasound, addition of acid, base and bacterial product) on their hydrolysis and methane production potentials.

II Hydrolysis of the chosen by-products and their mixture (ABP mixture) using different durations of ultrasound and addition of bacterial product as pre-treatments (the highest hydrolysis achieved in study I) with special attention on the reduction of particle sizes and the highest increase in VS-based hydrolysis parameters.

III A case-study on anaerobic co-digestion of ABP mixture + sewage sludge in a ratio produced by middle-sized companies/municipalities in Finland (1:7, w.w.) and in an optimal ratio described in the literature (1:3, w.w.). The effect of different operational parameters and pre-hygienisation. Evaluation of the possibility to co-digest ABPs in existing digesters at wastewater treatment plants.

IV A case-study on anaerobic co-digestion of ABP mixture + dairy cattle slurry (1:3, w.w.) and on the effect of their pre-hygienisation and ultrasound pre-treatment with special attention on hydrolysis, methane production and the quality of the digestates (incl. VS removal, pathogen reduction).

V The effect of hygienisation and ultrasound pre-treatment on the methane production potential of dairy cattle slurry in order to enhance its anaerobic digestion e.g. at farm-scale plants.

Contents

1 Introduction

Anaerobic degradation is a biological process occurring in anoxic natural ecosystems (e.g. rumen, sediments, swamps), in which organic matter is degraded and converted into a gaseous mixture of methane and carbon dioxide through the concerted action of a close-knit community of bacteria. Anaerobic digestion has also become an established environmental technology as a means of treating and stabilising organic wastes (De Baere, 1999). As such, it has a several advantages when compared to the other common treatment processes (i.e. incineration and aerobic degradation, i.e. composting). The produced biogas is a versatile renewable energy source which can be used as electricity, heat, vehicle fuel and/or through injection to natural gas network to replace fossil fuels. The digestion process destroys pathogens and the stabilised digestate produced enables recycling of materials (organic material, nutrients) when reused e.g. as organic fertiliser or soil improver (Mata-Alvarez et al., 2000; Mata-Alvarez, 2003). Moreover, the process increases the solubility of nutrients, thus making them more available for plants. Simultaneously dispersion of unpleasant odours is diminished.

Anaerobic digestion offers a response to the demands of several environmental programmes for sustainable development (e.g. EU; Environment 2010; Our Future, Our Choice, 2001-2010) and to the tightening health and environmental legislation (e.g. landfilling of organic waste 31/1999/EC; health rules for treatment and disposal of animal by-products (ABP; 1774/2002/EC).

Also, global commitments to decrease the greenhouse gas emissions according the Kyoto protocol supports the utilisation of anaerobic digestion technology preventing greenhouse gas emissions from uncontrolled degradation of the biodegradable materials in several ways. Utilisation of the technology enables (at least nearly) closed cycles (e.g. agriculture, industry) and controlled releases to water, air and soil (Salminen et al., 2002). Moreover, the utilisation of the biogas and digestate produced decreases the direct and indirect greenhouse gas emissions from energy production and consumption as well as from fertiliser industries. Also, possible benefits from the increasing regulations (i.e. feed-in tariffs, requirements to increase production and consumption of renewable energy, more complete utilisation and reuse of wastes) enable the active presence of anaerobic digestion in the changing energy sector.

ABPs are from the meat-processing are challenging materials to be treated in anaerobic digestion process, but similarly those have high potential to improve the methane production, quality of digestate, such as the digestion process itself. There are only few digestion studies of ABPs from meat-processing and thus more extensive and comprehensive studies are needed.

The summarised main motive for the present study is: Anaerobic digestion is an effective and current way to adopt sustainable development locally and case-specifically into practice and rise to the challenges to which environmental technology is expected to answer. It is noteworthy that the benefits mentioned above are achieved with the digestion of significant amounts of regularly produced waste materials which should be treated and stabilised in any case.

2 Literature overview

2.1 ANAEROBIC DIGESTION PROCESS

Anaerobic digestion is a multi-step microbiological process which converts organic materials (i.e. protein, cellulose and grease) to biogas (mixture of methane and carbon dioxide) and stabilised digestion residue, i.e. digestate. It can be divided into four main degradation steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis, which are performed by several different bacterial consortiums (Fig. 1).

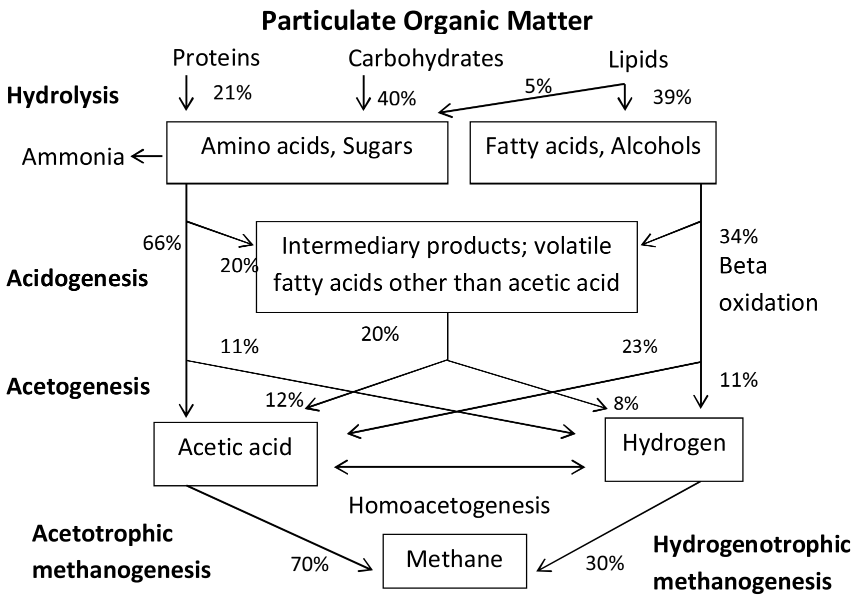

Fig.1. *Summary of the anaerobic digestion chain of organic material previously reviewed by Luostarinen, 2005; Pavlostathis and Giraldo-Gomez, 1991.*

2.1.1 Hydrolysis

In hydrolysis, acidogenic bacteria excrete hydrolytic enzymes which enable solubilisation of particulate and of insoluble colloidal matter. In more detail, hydrolysis is biological decomposition of organic polymers to monomers or dimers via degradation of cell walls and disintegration of flocs. Without hydrolysis the polymeric molecules, i.e. lipids, proteins and carbohydrates are too large to pass through the bacterial cell membrane and thus are not directly available for micro-organisms (Batstone et al., 2000). During hydrolysis, carbohydrates are converted to sugars, lipids (triglycerides) to glycerol and long-chain fatty acids (LCFA; > 10 carbon atoms chains) and proteins to amino acids. Lipases (triacylglycerol ester hydrolases) are enzymes which catalyse the hydrolysis of triacylglycerol to glycerol and free fatty acids, while other enzymes such as different proteases and amylases catalyse the hydrolysis of proteins and cellulose (Mendes et al., 2006). When digesting complex particulate substrates, hydrolysis can be rate-limiting (Miron et al., 2000; Massé et al., 2001).

2.1.2 Acidogenesis

The monomers and dimers produced during hydrolysis are further degraded inside acidogenic bacteria to fermentation intermediates, namely volatile fatty acids (VFA), alcohols, carbon dioxide and hydrogen. As acidogenesis occurs without external electron acceptor, low amount of reduced intermediates such as lactate, VFAs and alcohols are formed by the degradation of lipids and amino acids (Schink, 1997). LCFA are degraded to shorter chain VFAs and hydrogen via β-oxidation.

2.1.3 Acetogenesis

The VFAs and alcohols are further converted (oxidised) to acetate, carbon dioxide and hydrogen by proton-reducing acetogenic bacteria. However, at this point, hydrogen partial pressure has to be low for the acetogens to activate. This is achieved by syntrophic association with hydrogenotrophic methanogenesis maintaining the low hydrogen partial pressure and thus allowing syntrophic acetogenesis to proceed. If hydrogen is not consumed, acetogenesis is inhibited, causing accumulation of degradation intermediates (VFA), followed by decreasing pH and inhibited methanogenesis.

2.1.4 Methanogenesis

During methanogenesis, methane producing bacteria, i.e. methanogens consume acetic acid or carbon dioxide and hydrogen to produce methane and carbon dioxide. Major amount of methane (70 %) is produced via more sensitive and slower acetotrophic pathway. Methanogens are the most sensitive group of micro-organisms in the digestion chain toward changes in the digestion conditions. This is mainly due to their slow growth-rate. Accordingly, conditions of anaerobic digestion processes are usually optimised for methanogens.

2.2 FACTORS INFLUENCING ANAEROBIC DIGESTION

For the anaerobic digestion to proceed from hydrolysis to methane production, the micro-organisms have to survive and grow, and possible inhibitions have to be prevented. Thus, environmental and process factors such as the suitability of raw materials have to be favourable for the full occasion of the digestion pathway. The main factors affecting to the anaerobic digestion process are introduced below.

***Hydraulic retention time* (*HRT*)** describes the relative duration the raw material stays in a digestion process. In practice, a typical HRT for digestion of sewage sludge is approximately 20 days, during which usually a VS removal (biodegradation) of 25-60% is achieved.

(HRT = volume of the digester divided by the volume of the daily feed).

Organic loading rate (OLR) describes the amount of organic materials to be treated in a specific digestion process at a given time. OLR changes with the change in HRT, if the volume of the material in the digester remains constant. OLR cannot be risen to a higher level than the case-spesific bacterial consortium can efficiently degrade. E.g. a reported highest achievable OLR for

reactors co-digesting meat-processing wastes is 1.3-2.9 kgVS/m^3 d for the non-pre-treated (Alvarez and Liden, 2008; Rosenwinkel and Meyer, 1999) and 3.9-4.2 kgVS/m^3 d for the mechanically pre-treated material (Murto et al., 2004) With higher OLR, the biogas production starts to decrease.
(OLR = the amount of volatile solids (VS) in daily feed divided by the volume of digester).

Temperature. Anaerobic digestion can be divided into three different temperature ranges: 0-20 °C for psychrophilic, 20-40 °C for mesophilic and 50-60 °C for thermophilic micro-organisms. The higher the temperature, the more active micro-organisms are. Usually, optimal mesophilic (35-37 °C) or thermophilic temperature for methanogens (55 °C) is used where as psychrophilic temperature (< 20 °C) is not relatively effective. Thermophilic digestion process is usually characterised by higher growth rate of micro-organisms and accelerated interspecies hydrogen transfer resulting in an increased methanogenic potential at lower HRTs. However, it is also more energy-intensive and sensitive to changes in operational conditions (e.g. varying quality and quantity of raw materials, temperature, pH, amount of intermediates) than mesophilic processes. Thus thermophilic process is more easily disturbed and/or inhibited (Bitton, 1999; Zábranská et al., 2000) and subsequently it may result in lower methane content in the biogas produced (Ecke and Lagerkvist, 2000). Still, thermophilic digestion is more effective in destroying pathogens due to the higher process temperature (Watanabe et al., 1997; Huyard et al., 2000; Lu et al., 2008), while mesophilic process alone may not be adequate (Iranpour et al., 2004) depending on the feed materials.

Mixing. Adequate mixing is very important while it improves the distribution and contact between raw materials, enzymes and micro-organisms throughout the digester (Lema et al., 1991; Mata-Alvarez, 2003). It also ensures the desired temperature throughout the digester contents (see also below).

Total solids (TS). Too high or too low TS content may have a detrimental effect on the contact between the raw material(s), enzymes and micro-organisms in anaerobic reactors (Lema et al., 1991; Mata-Alvarez, 2003). It may also affect the HRT negatively resulting in decreased degradation and specific methane production (SMP; Lema et al., 1991; Mata-Alvarez, 2003). Accordingly, shorter HRT requires low TS content to improve the methane production rate. E.g. thicker cattle slurry (TS 10%) has reported to achieve a lower SMP (HRT: 16 days) than the slurry with a half lower solid contents (TS 5%; Karim et al., 2005). Too high TS may also deteriorate the quality of mixing resulting in less contact between the raw materials and the bacteria and thus longer treatment time or less stabilised sludge, when compared to the more diluted contents. The appropriate TS level inside the reactor is on the range 10-50 gTS/l (Chamy et al., 1998; Angelidaki et al., 2006; Chamy et al., 2010). However, it should be noted that these TS examples mentioned consider of wet anaerobic digestion technology, when TS contents in semi-dry and dry anaerobic digestion processes is > 15 %, usually 20-50% (Nallathambi Gunaseelan, 1997).

Organic content of raw materials. The relative proportions of carbohydrates, proteins and lipids affects the quality and amount of degradation intermediates (i.e. VFA, LCFA, NH_4^+-N, NH_3) during anaerobic digestion. Ideal C:N ratio for the growth of micro-organisms is reported to be 25–30:1, but in practice the C:N ratios are often considerably lower or higher than this (Kizilkaya and Bayrakli, 2005). Optimal ratio of chemical oxygen demand (COD), nitrogen and phosphorus for the anaerobic micro-organisms is reported to be 600:7:1 (Hobson and Wheatley 1993; Mata-Alvarez 2003).

pH and alkalinity. Though all micro-organisms have their optimal pH, in anaerobic digestion the methanogens are the most sensitive with a working range of 6.5-7.5 and optimal range of 7.0-7.2 (Bitton, 1999). Usually anaerobic processes are thus operated in the optimal pH range for methanogens. While the formation of degradation intermediates (VFA) tends to lower the process pH, ammonia (NH_3), formed during degradation of proteins, may increase process pH and affect the non-adapted micro-organisms. A balanced and adequate content of proteins and organic acids in the raw materials enhances the ion content and buffering capacity of the anaerobic process and thus increases its resistance toward organic overloads and enhances the treatment "equilibrium" (Alvarez and Liden, 2008). Possible unwanted changes in process pH can be anticipated through analysis of alkalinity (g $CaCO_3$/l) which indicates the buffering capacity of the process. Desired alkalinity in digesters is usually in the range of 2000-4000 mg $CaCO_3$/l and VFA/alkalinity ratio should be < 0.3 (Cecchi et al., 2003).

Volatile fatty acids (VFA). Accumulating intermediates are usually a sign of an overloaded digestion process which is shortly also noticed in lowered biogas and/or methane production. Different anaerobic processes are adapted to different concentrations of VFAs. E.g. previously reported inhibiting levels for total VFAs are 2.2-4.9 g /l (Kalle and Menon, 1984; Siegert and Banks, 2005; Climet et al., 2007), while the most inhibitive VFAs are excess amounts of propionate and butyrate (Mata-Alvarez 2003). Accumulating VFA, especially acetate and excess amount of butyrate (precursor of acetate) and/or branched VFA (isovalerate, isobutyrate), indicates slow growth or inhibition of the acetate-utilising methanogenic micro-organisms (Kalle and Menon, 1984; Wang et al., 1999).

Long chain fatty acids (LCFA) are formed during lipid degradation and in too high amounts they may accumulate and decimate the degradation of propionate thus preventing further hydrolysis (Salminen et al., 2000). LCFA interacts with hydrogen produced by acetogenic bacteria, which are responsible for the β-oxidation of LCFA, the limiting step of anaerobic digestion of lipid-rich materials (Hanaki et al., 1981; Rinzema, 1988). Thus high amount of LCFA slows down the degradation rate of lipids (Cirne et al., 2007). The most inhibiting LCFAs are reportedly saturated fatty acids with 12-14 carbon atom chains (Lauric acid, Myristoleic acid) and unsaturated acid with 18 carbon atoms (Oleic acid). Oleic acid may be inhibitive already in the concentration of 0.03-0.3 g/l (Broughton et al., 1998; Alves et al., 2001; Lalman and Bagley, 2001). LCFA inhibition was long believed to be irreversible (Rinzema et al., 1994), but new studies have shown it reversible, though recovery takes a long time (Pereira et al., 2004). Moreover, already the lipids may cause physical inhibition of the process and form a floating sludge layer depending on the reactor type. Also, the hydrophobic nature of lipids may lead to the adsorption on the surface of sludge flocs and/or onto the cell walls of bacteria disturbing the transportation functions and consequently causes the conversion rate in substrates to decrease (Sayed et al., 1988; Rinzema et al, 1993).

Ammonium- and ammonia nitrogen (NH_4^+-N, NH_3). High concentration of ammonium nitrogen and especially ammonia may be inhibitive and pose problems when digesting protein-rich materials (Hansen et al., 1998). A part of ammonium nitrogen always exists as unionised ammonia depending on the pH and temperature of the anaerobic digestion process. As ammonia is unionised, bacterial cell membranes cannot prevent it from entering the cells and disrupting their normal functions. This makes it more toxic than its ionised counterpart ammonium nitrogen (Angelidaki and Ahring, 1993; Kadam and Boone, 1996). Different concentrations of ammonia and ammonium nitrogen are reported toxic or inhibitive in different anaerobic processes (e.g. 1.5-2.5 g NH_4^+-N/l in non-adapted process: Van Velsen, 1979; Koster and Lettinga, 1984; Hashimoto, 1986; Buendia et al., 2009; 1.13 g NH_4^+-N/l causing 50% inhibition in methane production: Buendia et al., 2009; 3-7 g NH_4^+-N/l in adapted processes: Van Velsen, 1979; Pechan et al., 1987; 0.15–2.0 g NH_3-N /l: Braun et al., 1981; Angelidaki and Ahring, 1993; Hansen et al., 1998) and "safe" concentrations are nearly impossible to determine. Any process can, however, be adapted to higher ammonium/ammonia concentrations by gradually increasing its content in the process.

Cellulose and lignin. Too high content of recalcitrant cellulose and lignin compounds (Rosenwinkel and Meyer, 1999; Buendía et al., 2008) may also lower biodegradation and specific methane production. Lignin compounds act as glue between polysaccharide filaments and fibres thus slowing down their degradation, while 12% of cellulose is estimated to remain in the flotation layer of a biogas reactor (Rosenwinkel and Meyer, 1999). Moreover, lignin related fractions with their various functional groups may re-flocculate easily (Lehtomäki et al., 2007a) which not only slows down the digestion process, but makes the treatment difficult to control.

Other factors. Anaerobic digestion may also be inhibited by excess amount of various compounds, such as excess salinity, detergents, toxic compounds, foreign matter (i.e. pesticides) and hydrogen sulfide (Mata-Alvarez 2003).

2.3 ANAEROBIC CO-DIGESTION

Anaerobic co-digestion means the digestion of two or more raw materials together in one process, which may improve the rate of the process, biodegradation, stabilisation of the raw materials, digestate and methane production. E.g. methane production of farm-scale digesters has been reported to increase by 80–400% when manure and sewage sludge are co-digested with other organic wastes and by-products (Braun et al., 2003; Table 1). Co-digestion may also improve the different factors influencing the digestion process (see 2.2), such as dilute toxic materials/inhibitors and achieve an improved TS content, nutrient balance, C:N -ratio and alkalinity (Mata-Alvarez et al., 2000; Mata-Alvarez, 2003). Co-digestion may also increase the nutrient content (ammonium nitrogen, potassium, phosphorous, calcium, magnesium) and thus reuse-potential of the digestate, when compared to digesting the materials alone (Field et al., 1985). It should, however, be noted that the additional raw materials may affect the technical requirements of the biogas plant and/or the end-use possibilities of the digestate (possible requirements from legislation). Moreover, transportation costs and various policies of waste producers may limit the use of additional materials in digestion processes (Mata-Alvarez et al., 2000). Optimal relation of the mixture of the available co-substrates is substantial.

In practise, e.g. co-digestion of sewage sludge with other organic materials of higher energy content (e.g. food industrial waste, municipal waste) has been widely studied (Table 1) and also performed in practice. This is because anaerobic digestion of sewage sludge is a common process in many wastewater treatment plants, where mass reduction and improved dewatering properties are the main features expected from the process (Mata-Alvarez et al., 2000). However, slow degradation (> 20 days) and the relatively low VS removal (30–40%) are often the disadvantages of the process as the digesters are rarely optimised for biogas production and are operated with too low C:N ratios and OLRs (Murto et al., 2004; Climent et al., 2007).

Another potential co-digestion raw material, which is steadily produced, available throughout the year and which energy content is scarcely utilised (although widely studied) is animal manure. Both sewage sludge and animal manure are rather dilute (low TS content) wherefore their own biological methane potential (BMP) is rather low (120-260 m^3 CH_4/tVS_{added}; Ahring et al., 2001; Møller et al., 2004; Amon et al., 2006; Bougrier et al., 2006a; Lehtomäki et al., 2007a; Luostarinen et al., 2009), but offer a good dilution matrix for other more concentrated organic raw materials (Table 1). Animal manures alone may also have high nitrogen content and thus too low C:N -ratio for anaerobic digestion (especially pig and poultry manure), which can then be enhanced with carbohydrate-rich materials (Hobson and Wheatley 1993).

Table 1. *Examples of previous co-digestions with different organic by-products and wastes.*

Co-digestion materials	Temp.	HRT	OLR	CH4	VS-removal	Author
Pig and cow digestive tract content/flotation tailings and sewage sludge (1:3)	37 °C	a)17, b)15 d	a) 2.9, b) 1.5 kg TS m^3d	a) 230, b) 320 m^3/t TS	a) 61%, b) 55%	Rosenwinkel & Mayer, 1999
Food industrial waste (grease trap sludge, bakery-, confectionary-,dairy product- and mill waste) and sewage sludge (1:2)	35 °C	20-13 d	1.6-1.9 kg TS m^3d	390 m^3/t VS	Not analysed	Murto et al., 2004
Slaughterhouse and food industrial waste with big manure(1:3)	35 °C	30-36 d	2.6 kg VS/m^3d	560-680 m^3/t VS	Not analysed	Murto et al., 2004
Organic municipal wastes and sewage sludge (1:3)	a)36, b)56	a)17, b)38	a) 3.1, b) 1.5	a) 230, b) 190 m^3/t	Not analysed	Sosnowski et al., 2008
ABP (digestive tract content, blood), manure and vegetable wastes (1:3)	35 °C	30 d	1.8 kg VS/m^3d	270–350 m^3/t VS	51-67%	Alvarez & Liden, 2007
Municipal bio-waste and cow manure (1:4)	a)35, b)55 °C	a, b) 20 d	2.2-2.7 kg VS/m^3d	a)210-250, b)220-290 m^3/t VS	a, b) 31- 48%	Paavola et al., 2006
Poultry ABPs (bones, trainings, blood, offal, feather; 3:1:2; TS: 3.1-9.4%) and water	31 °C	a)13, b)25, c)50, d)100	a, b) 2.1, c, d) 0.8 kg VS/m^3d	a) 90, b) 310, c) 550,	a)31%, b)63%,	Salminen & Rintala, 2002
Grease trap- (Feed VS: 5-46%) and sewage	35 °C	16-18 d	1.14-3.46 kg	350-460 m^3/t VS	54-63%	Luostarinen et al., 2009
Chopped energy crops and cattle manure (3:7)	30 °C	20 d	2.0 g VS/ld	210-270 m^3/t VS	33-43%	Lehtomäki et al., 2007a
a) Ruminal waste + wastewater sludge (1:3); b) Sludge and cattle manure (1:3)	35 °C	Batch study	Batch study	a) 170, b) 180 m^3/t VS	a)75%, b)57 %	Buendìa et al., 2009
Cattle blood, rumen contents (1:3) and water	35 °C	20 d	1.5-3.6 kg TS/m^3d	100-270 m^3/t TS	49-63%	Banks &Wang, 1999
Poultry entrails, digestivetract content and organic fraction of municipalwaste (1:5) and water (TS	35 °C	a)36, b)25 d	a) 2.56, b) 3.7 kg TS/m^3d	300-500 m^3/t TS	80-83%	Cuetos et al., 2008
ABPs (digestive tract content, blood, food waste, manure slurry), diluted with water (TS 19-38%)	37°C	22-40 d	2.5 kg VS/m^3d	310 m^3 biogas/t VS	Not analysed	Edström et al., 2003
By-products (slaughterhouses, pharmaceutical, food, beverage, distillery industry), biowastes and sewage sludge or cattle manure. (1:6)	30 °C	12-60 d	1.1-4.5 kg TS/m^3d	300-1400 m^3/t VS	Not analysed	Braun et al., 2003
ABPs and iquid from municipal bio-waste (1:3)	38 °C	21 d	10 kgCOD/m^3d	140 Nm^3/ kg COD	Not analysed	Resch et al., 2006

2.4 PRE-TREATMENTS

Pre-treating organic materials prior to anaerobic digestion aims at enhanced hydrolysis (i.e. separate liquid organic material from solid organic material) and thus more complete utilisation of the raw material by micro-organisms. Pre-treatments may also loosen particulate structures and disrupt flocs or cell walls for further hydrolysis by anaerobic micro-organisms (Chu et al., 2002). Enhanced hydrolysis aims at intensified digestion process resulting in increased biogas production and more complete degradation of the raw material (Fernandes et al., 2009). This, in turn, leads to improved biodegradation and more stabilised digestate (Bougrier et al., 2006b). Suitable pre-treatments may also accelerate the digestion process or microbial activity and avoid and/or overcome process inhibition (Vidal et al., 2000; Alves et al., 2001; Massé et al., 2001; Cammarota and Freire, 2006; Bormann et al., 2007). Pre-treatments may also concentrate the material and destroy pathogens and unwanted micro-organisms responsible for sludge bulking (Bougrier et al., 2005; Dewil et al., 2006; Paavola et al., 2006). Effective pre-treatments enable process intensification: higher OLR, shorter HRT and/or smaller digester volume (Alvarez and Liden, 2008; Rosenwinkel and Meyer, 1999).

There are several different process technologies which can be used as pre-treatment for anaerobic digestions. The technologies include physical, chemical and biological processes which are discussed in more detail in the following sections. Higher OLR could be also treated with the technical application, where hydrolysis and acidogenesis steps are separated (i.e. two-phase anaerobic digestion; Wang and Banks, 2003; Demirer and Chen, 2005; Lu et al., 2008).

2.4.1 Physical pre-treatments

Many physical pre-treatment methods are studied and used to concentrate, to homogenise, to degenerate the particle sizes and loosen the solid structures with similar solubilisation of solid material.

There are many mechanical applications already in use to homogenise, concentrate, dewaters and to cause mechanical disruption to cells and flocs. Lysis centrifuge hydrolysis via re-suspension of dewatered material (waste activated sludge, Zábranská et al., 2006), liquid shear solubilised via high liquid flows due to a high pressure changes (collision plate, high pressure homogeniser, activated, sewage sludge and mixed sludge; Barjenbruch and Kopplow, 2003; Onyeche, 2007) and grinding/chopping (stirred ball mills, activated sludge; Kopp et al., 1997; Agricultural chaff-cutter, plant biomass, Lehtomäki et al., 2007a) are very effective methods to cut up filaments and open channels for hydrolysing enzymes of anaerobic digestion. Maceration is a usual method as attempted with cattle manure (Angelidagi and Ahring, 2000; Hartmann et al., 2000).

Thermal pre-treatment (see also 2.3.4) has been used for treating e.g. waste activated sludge, sewage sludge, cattle manure and biowaste (Bougrier et al., 2006a, b; Paavola et al., 2006) with the focus of concentrate the material and degrade or loosen the structures with similar release of the linked water (Bougrier et al., 2006b). Thermal treatment may also intensify the activity of anaerobic micro-organisms (Lu et al., 2008; Carrère et al., 2010). Also, microwaves, γ-irradiation and ultrasound (see also 2.3.4; Table 2) methods aims at physically disrupt the cell and floc structures and it is mainly used with waste activated sludge and sewage sludge. (Tiehm et al., 1997; Chu, et al., 2002; Lafitte-Trouqué and Forster, 2002; Bougrier et al., 2006b; Climent et al., 2007; Eskicioglu et al., 2008; Saifuddin and Fazlili, 2009; Braguglia et al., 2010; Carrère et al., 2010). Microwaves, such as thermal treatments, increases the viscosity of sludge via increased temperature (Eskicioglu et al., 2007), while γ-

irradiation has also the pasteurise effect due to the high energy content of it (Bougrier et al., 2006b; Table 2).

2.4.2 Chemical pre-treatments

Chemical pre-treatments usually with base addition aim at disrupt of molecule structures via high pH change, but that with the neutralisation may also dilute the feed materials. They have been reported to increase the ratio of soluble COD (COD_{sol}) and to reduce VS (Lin et al., 1997; Cárdenas et al., 2010a) and lipid content e.g. in waste activated sludge and solid ABPs (Karlsson, 1990). Moreover, Heo et al. (2003) reported alkali addition (NaOH, 45 meq/l, 4 h, 35 °C) to increase COD_{sol} by 31% and biogas production by 73% when digesting waste activated sludge after the pre-treatment. Massé et al. (2001) noticed NaOH (5-40 meq, pH 13, 4 h) to be more efficient with proteins than with lipids when pre-treating slaughterhouse wastewater. Similarly, acid pre-treatment (60 meq HCl, 30-120 min, 35 °C) has been reported to increase solubilisation and to reduce particle size of organic matter in septic tank sludge (Lin and Lee, 2002).

Oxygenation (H_2O_2, wet air oxidation) and/or ozonation has also been studied as a pre-treatment of sewage and waste activated sludge prior to anaerobic digestion (Weemaes et al., 2000; Grönroos et al., 2005; Bougrier et al., 2006b; Chu et al., 2009; Braguglia et al., 2010; Table 2) and applied in combination with activated sludge process for wastewater treatment (Sakai et al., 1997). The goal of these pre-treatments is that formed oxygen radicals reduce the soluble, particulate, organic or mineral fractions. Moreover, oxygenation modifies viscosity and settlement of sludge (Battimelli et al., 2003; Bougrier et al., 2006b). The optimal ozone dose (0.1-0.15 g O_3/g COD) enhances the biodegradation of organic material (Weemaes et al., 2000; Bougrier et al., 2007), but because it is oxidative, that may decrease the methane production (Carrère et al., 2010).

2.4.3 Biological pre-treatments

During anaerobic degradation, acidogenic bacteria excrete hydrolytic enzymes which enable the degradation of particles into smaller compounds. Thus, biological treatments using pure enzymes have been studied with lipid-rich dairy and slaughterhouse waste waters (pancreatic lipase PL 250 –enzyme; Massé et al., 2003; Mendes et al., 2006) and mixed sewage sludge (Carbohydras –enzyme; Barjenbrush and Kopplow, 2003). PL 250 has reported to increase the lipid hydrolysis by 40% (24 hours) with the reducing particle sizes (Mendes et al., 2006; Table 2). However, in another study at 25 °C, the PL 250 pre-treatment only slightly enhanced lipid digestion and transformation into methane, but the effects were suggested to be more pronounced at higher temperatures (Massé et al, 2003).

Hydrolytic enzymes are not effective in degrading lignin structures under anoxic conditions (Hataka, 2001). However, Zhen-Hu et al. (2004, 2005) have studied pre-treating plant cellulose using rumen micro-organisms and Lehtomäki (2006) also reported pre-treating plant biomass with white-rot fungi, but usually biological pre-treatments have been attempted with pure enzymes and are often limited to lipid–rich wastewaters (Cammarota and Freire, 2006).

Biological pre-treatment aims at intensification by enhancing the hydrolysis process in an additional stage prior to the main digestion process. Thus, separate thermal hydrolysis-step or hyper-thermophilic prehydrolysis step (55-70 °C) can also be considered as a biological pre-treatment (Carrère et al., 2010). This is because it not only increase the particles degradation rate, but is attributed to increased hydrolytic activity (Gavala et al., 2003; Climet et al., 2007; Lu et al., 2008; Ge et al., 2010).

Different pre-treatments, such as thermal and microwave is combined with pressure or chemical treatments (KOH, NaOH, maleic acid) (Valo et al., 2004; Dogan and Sanin, 2009; Eskicioglu et al., 2009; Fernandes et al., 2009), to intensify the solubilisation and further hydrolysis by anaerobic micro-organisms (Table 2). In practise materially could also be mechanically crushed and homogenised to the smaller particle size prior to actual pre-treatment.

Table 2. *Examples of previous pre-treatments with various materials.*

	Instrument	Mode	Materials	Main effects (Compared to the original material)	Author
Physical	Lysing centrifuge	12-200 m^3/h, 2250-3140 rpm	Sewage sludge	Biogas yield increased by 15-26%; organic matter in removal by 48-49%.	Zabranska et al., 2006
	Grinding, Ballmill	db: 0.25 mm, vb: 10 m s^{-1}, 60 ºC,	Activated and secondary sludge	COD_{sol} increased from 1-5% to 47%, BMP by 10 62%; COD degradation by 20-50%	Baier and Schmidheiny, 1997
	Ultrasound	2500 kJ/kg TS	Sewage sludge	Hydrolysis increased by 4%, SMP by 26%; VS removal by 19%	Braguglia et al., 2010
	Ultrasound	6250, 9350 kJ/kg TS	Waste activated sludge	COD_{sol} increased by 15%, BMP by 49 ±2%; biodagradability by 48 ±2 % .	Bougrier et al., 2006
	γ-irradiation	500 krad	Waste activated sludge	Biogas yield increased by 4-8%; notable reduction(3-log) in coliforms	Lafitte-Trouque & Forster, 2002
Thermal	Thermostatic bath	70 °C, 2 d	Primary sludge	BMP increased by 48%; VS removal by 12 %.	Lu et al., 2008
	Heating chamber	70 °C, 60 min	Municipal biowaste and cow manure	Increased hydrolysis, SMP increased by 8-24% with total destruction of *Salmonella*- bacteria.	Paavola et al., 2006
	Thermostatic bath	70 °C, 9 hour	Mixture of tickened sludges	COD_{sol} increased from 5 to 50%, BMP by 20-30%: VS removal by 10%.	Ferrer et al., 2008
	Autoclave	80-121 °C, 60 min	Mixed sewage sludge	BMP increased by 17-22%; VSS removal by 4-7%; foaming ended.	Barjenbrush & Kopplow, 2003
	Oven	100-140 °C, 20-40 min	Solids from cattle and pig manure	No hydrolysis; BMP increased by 8-24%; VS removal by 35%; Increased conversion rate.	Mladenovska et al., 2006
	Heating plate and oven	60 °C/60 min	Solid ABPs (80% of rumen content)	COD_{sol} increased by 29%, BMP by 39%; VS removal by 8.8%.	Cárdenas et al., 2010a
Chemical	NaOH	45 meq/l, 4 h, 35	Waste activated	COD_{sol} increased by 31%; SMP by 73 %.	Heo et al., 2003
	Ozone	0.1-0.16 g O_3/ g TS	Waste activated sludge	COD_{sol} increased by 23 ±2%; BMP by 11-22%; biodagradability by 11-24%.	Bougrier et al., 2006
	NaOH	0.5 g NaOH/ g VSS	Solid ABPs (80% rumen content)	CODsol increased by 66 %, COD removal by 58%; VS removal by 45%	Cárdenas et al., 2010b
Biological	*Pancreas lipase* - enzyme	1770 units/mg	Lipid-rich wastewaters from dairy industry	Hydrolysis of lipids and proteins increased by 28-40%, BMP from 210 to 354-450 ml, COD removal by 90%; APS decreased by 60%	Mendes et al., 2006
	Pancreatic lipase- enzyme	250 mg/l, 5.5 h	Slaughterhouse wastewater	Hydrolysis of lipids increased by 35%, increase in SMP; reduction of particles was < 5%.	Masse et al., 2003
	Carbohydras- enzyme	Not reported	Mixed sewage	BMP increased by 14%; VSS removal by 2%.	Barjenbrush, 2003

2.4.4 Examples of the pre-treatments

Two physical pre-treatment methods, ultrasound and thermal pre-treatments, to hydrolyse liquid organic material from solid organic material and to reduce the particle sizes are introduced more detailed below. Ultrasound and thermal pre-treatments are the main pre-treatment methods applied in the experiments of the present thesis.

Ultrasound pre-treatment is a novel physical pre-treatment method, which has mostly been applied for the treatment of sewage and waste activated sludge (Tiehm et al., 1997; Chiu et al., 1997; Bougrier et al., 2006b; Braguglia et al., 2006; Van Leeuwen et al., 2006; Nickel, U. Neis, 2007), municipal wastewaters (Antoniadis et al., 2007) and industrial wastewaters (Gonze et al., 2003; Silva et al., 2007). Ultrasound evokes cavitation by bubble formation in the liquid phase (Tiehm et al., 1997). Cavitational collapse of bubbles produces local heating (~4700 °C) and pressure (~50 MPa) at liquid/gas interface, turbulence, formation of radicals ($OH^{\bullet}$, $HO_2^{\bullet}$, $H^{\bullet}$) and high-rate shearing phenomena in the liquid phase (Gonze et al., 1999). Disintegration of cellular structures is most significant at low frequencies (e.g. 20-40 kHz), because the bubble radius is inversely proportional to the frequency and large bubbles indicate large shear forces (Tiehm et al., 1997, 2001; Laurent et al., 2009). On the other hand, higher frequencies (e.g. 3200 kHz; Tiehm et al., 2001; Laurent et al., 2009) have higher radical formation ability and disinfection efficiency (Blume and Neis, 2004).

Previous ultrasound experiments with waste activated sludge and sewage sludge report reduction of average floc/particle sizes (APS: 6-70%; Chu et al., 2001, 2002; Bougrier et al., 2006b, 2005), cellular lysis (Tiehm et al., 1997, 2001), increased COD_{sol} (7-1200%; Tiehm et al., 1997, 2001; Chu et al., 2001, 2002, Lafitte-Troquette and Foster, 2002; Grönroos et al., 2005) enhanced biodegradation (i.e. VS reduction of 4-50%: Tiehm et al., 1997, 2001; Neis et al., 2000; Bougrier et al., 2005, 2006b) and improved biodegradation rate (Schläfer et al., 2002). Ultrasound pre-treatment has also increased BMP by 10-100% (batch studies; Chu et al., 2002; Grönroos et al., 2005; Bougrier et al., 2005, 2006b), SMP by 10-40% (semi-continuous reactor studies: Neis et al., 2000; Tiehm et al., 2001; Lafitte-Troquette and Foster, 2002) and methane yields in pilot processes by 5-10% (Clark and Nujjoo, 2000), when compared to untreated raw materials (Table 2). Enhancements depend on the used power (100-350 W) and frequency (9-360 kHz; Es < 20 000 kJ/kg TS) of ultrasound unit, but also on the treatment time (5-60 min), temperatures during ultrasound pre-treatment (25-70 °C) and the following digestion process (HRT: 8-20 days; 35-55 °C; Table 2).

Lower specific energy (Es) inputs (see 4.4; Eq. 2) may break cell and floc structures and release weakly adsorbed molecules between the flocs and on the surface of particles (Laurent et al., 2009). Es of 1000 kJ/kg TS has been reported to be the minimum specific energy requirement needed for the degradation and hydrolysis of waste activated sludge to begin (Bougrier et al., 2005; Dewil et al., 2006). However, even if there is no instant hydrolysis of particulate material, low Es may weaken the structures and thus assist the further hydrolysis and disintegration of material during the following digestion process (Chu et al., 2002). Higher Es inputs degrade solid particles and may release intracellular material (Lehne, 2001; Bougrier et al., 2005). Bougier et al. (2005) reported hydrolysis of waste activated sludge being fast under the Es of 10 000 kJ/kg TS, while with higher Es than this, hydrolysis slows down.

Higher TS content is reported to enhance ultrasound pre-treatment of sludge to be more energy efficient than lower TS content (Wang et al., 2005). With higher TS content, higher amount of solid particles can act as nuclei, which increases the efficiency of the disintegration (Onyeche et al., 2002; Khanal et al, 2006). Moreover, higher TS content of the material to be ultrasound pre-treated may facilitate its disruption due to improved particle-to-particle collision (Khanal et al., 2006). Despite this, pre-treated sludges have usually had a TS content of only 0.7-5.5% (Chiu et al., 1997; Tiehm et al., 1997; Neis et al., 2000; Chu et al., 2002; Bougrier et al., 2005; Grönroos et al., 2005; Braguglia et al., 2006; Van Leeuwen et al., 2006). However, if the solids concentration is too high, increased viscosity hinders cavitation bubble formation. According to Show et al. (2007) the optimal range of solids content of sewage sludge for ultrasound pre-treatment lies between 2.3% and 3.2% TS. With a TS content of 15 g/l ultrasound waves are scattered by the particles and absorbed by the fluid to generate heat rather than creating cavitation bubbles (Khanal et al, 2006).

Thermal pre-treatment (i.e. high temperatures) is another physical method to hydrolyse liquid organic material from solid organic material. It was first applied to enhance the dewater ability of sludge (Haug et al., 1978), because it concentrates material due to evaporation of water, similarly decreasing the viscosity and the filterability of material (90-130 °C; Bougrier et al., 2008). However, thermal treatment also loosens the cell structure of the solid particles via pressure changes (Bougrier et al., 2005). Low temperatures of below 100 °C have been found more effective in increasing biogas production from waste activated sludge, food industry wastewater and sewage sludge than higher temperatures (Gavala et al., 2003; Climent et al., 2007; Ferrer et al., 2008). In temperature screenings, the temperature of 60 °C produced the highest increase in degradation and methane production from slaughterhouse solid wastes (Cárdenas et al., 2010b). Research with thermally pre-treated lipid- and protein-rich materials is scarce, though Mendes et al. (2006) reported thermally pre-treated lipids to be non-susceptible to flotation in digesters.

In order to ensure hygienisation, i.e. reduce the pathogen content of the raw material, a separate hygienisation treatment is recommended or demanded for certain materials to be treated using anaerobic digestion. E.g. most materials of animal origin are required to be thermally pre- or post-treated in European

Union (hygienisation: 70 °C, 60 min, particle size < 12 mm; sterilisation: 133 °C, 20 min, 3 bar, particle size < 50 mm; 1774/2002/EC). Similarly, mesophilically digested sewage sludge has to be pre- or post-hygienised in Finland in order to reuse it e.g. as soil improver (ENV.E.3/LM, 2000). However, if hygienisation is performed before the biogas process, it will also serve as a thermal pre-treatment.

Thermal pre-treatments have been reported to improve the solubilisation (i.e. increase COD_{sol}) of waste activated and sewage sludge linearly by 40-60% up to 200 °C (Haug et al., 1978; Li and Noike, 1992; Bougrier et al., 2008), when compared to the untreated material. Thermal treatments (70-120 °C/ 0.5 hours -7 days) have also been reported to increase the methane potential (batch studies) of waste activated sludge and sewage sludge by 25-50% (Gavala et al., 2003; Kim et al., 2003; Climent et al., 2007; Ferrer et al., 2008), of cattle manure by 8-24% (Mladenovska et al., 2006) and to increase specific methane production (semi-continuous reactor studies) of sewage sludge and of a mixture of biowaste and manure by 14-30% (Barjenbruch and Kopplow, 2003; Paavola et al., 2006; Ferrer et al., 2008; Table 2), depending on the materials and digestion temperatures (30-55 °C). Thermal pre-treatments have also reported to intensify the degradation of cellulose via prevention the floating layer formation (Bochmann et al., 2010).

The most significant drawback of thermal pre-treatment is probably its energy-intensity, wherefore low temperature and short time are preferred. However, when compared to the other physical treatments, a biogas plant including thermal treatment can reuse the “excess” heat in the different stages plant via heat-exchangers. Moreover, in some cases the heat produced in conjunction with electricity production using combined heat and power (CHP) may be the most efficient to use at the biogas plant itself, offering the required energy for thermal pre-treatment and to keep the total energy balance positive. Moreover, higher thermal treatments of fatty residues (> 100 °C) and sludge (> 170-190 °C; Bougrier et al., 2008) may also lead to decreased biodegradability. This may take place e.g. via Maillard reactions, where carbohydrates and amino acids may react and form melanoidins, which are difficult or impossible to degrade (Bougrier et al., 2008).

2.5 BY-PRODUCTS FROM MEAT-PROCESSING INDUSTRY

The composition of ABPs varies considerably depending on the animal and its nutrition, on seasonal timing, on the size and process technology of the slaughterhouse and the legislation/regulations applied. However, ABPs from meat-processing are usually lipid-rich, small particles or pasties and they have little fibrous structure and a water content of higher than 70% (Rosenwinkel and Meyer, 1999), which makes them eligible substrates for anaerobic digestion. Moreover, their diversity offers potential to increase the alkalinity and buffer capacity during the digestion process. E.g. digestive tract content consists of plant-based cellulose with carbohydrates and lignin, while meat, blood, grease and slaughterhouse wastewaters have high content of proteins and fats. Fatty materials have high methane production potential (Martinez et al., 1995; Batstone et al., 2000; Massé et al., 2001, 2003; Luostarinen et al., 2009), while protein-rich fractions increase the nutrient content and fertiliser potential of the stabilised digestate (Table 3).

However, slaughterhouse wastes and meat-processing by-products are also reported as challenging materials for anaerobic digestion specifically due to their high protein and lipid content and subsequent inhibition due to their degradation intermediates (NH_4^+-N, NH_3, VFA, LCFA; see 2.2). These effects depend on the buffering capacity and degree of adaptation of the micro-organisms in the digestion process. Also, recalcitrance of cellulose and lignin compounds in digestive tract content and ruminant manure may decelerate the digestion process and cause re-flocculation in tandem with the hydrolysis of the materials (Rosenwinkel and Meyer, 1999; Buendía et al., 2008). These challenges can be affected by pre-treatments, by co-digestion (Table 1, 2) and by process technology.

Table 3. *Characteristic relations of the materials (%) used in the present study (meat-processing wastes, cattle manure and sewage sludge) from literature (Pavlostathis and Giraldo-Gomez, 1991).*

Content	Meat-processing wastes	Cattle manure	Sewage sludge
VS	92	72	59-75
Lipids	55	3.5	4.5-12
Cellulose	–	17	7
Hemicellulose	–	19	–
Lignin	–	6.8	–
Protein	29	19	32-41
Ash	8	28	25-41

ABPs may also contain pathogens and risk of spreading diseases (e.g. bovine spongiform encephalopathy (BSE), foot-and-mouth-disease, bird and swine influenza). Thus, treatment, disposal and reuse of ABPs are strictly controlled in EU (1774/2002/EC) and the materials are divided into three different categories according to the risk of diseases (Table 4).

***Table 4.** Categorisation of ABPs from meat-processing industry according to EU regulation (1774/2002/EC). Estimated amounts of ABPs and food supplies produced from cows and pigs per year in Finland (Heinänen et al., 2007).*

Category	**1**	**2**	**3**
Material	TSE-risk, unknown or possible risk for public health, hygienic risk	Risk for other illnesses than TSE, Screened-out material from anti- and post-mortem controls	Materials from animals fit for human consumption, but not used for commercial reasons
Treatment and requirements for anaerobic digestion	Not suitable for digestion	Sterilisation: 133 ºC, 3 bar, 20min, <50 mm	Hygienisation: 70 ºC, 60 min, <12 mm
Example from materials and fractions	Ruminant spinal cord, scull, brains and eyes of animals older than 12 months	Manure, digestive tract content, blood, perished animals, animals died in storage	Catering waste, meat-containing wastes from food industry, dirty residues
ABPs produced in Finland each year	Cow: 13 000 t/a; Pig: -	Cow: 50 000 t/a; Pig: 50 000 t/a	Cow: 13 000 t/a; Pig: 5000 t/a
	Food supplies: Cow: 5000 t/a and pig: 8500 t/a		

Only the materials in categories 2 and 3 can be anaerobically digested, though with process requirements. Materials in category 2 must be sterilised (133 °C, 20 min., 3 bar, particle size > 50 mm) and those of category 3 hygienised (70 °C, 60 min, particle size < 12 mm) before or after the biogas process in order to guarantee the hygienic quality of the digestates (no salmonella and the number of *Escherichia coli* < 1000 CFU/g: 208/2006/EC). Though manure, digestive tract content and milk are included in category 2, they can be digested without sterilisation (Table 4). It has, however, been proposed that if any other material of animal-origin is to be co-digested, hygienisation has to be applied. So far, many category 2 (i.e. blood, milk, dead animals) and category 3 by-products (certain meat containing wastes from food processing: Fig. 2) are utilised in fodder production for pet, fur, zoo, circus and wild animals and/or cultivation of fish baits instead of digestion.

According to Finnish national legislation, manure (such as rumen content of bovine animals) can be digested and reused as organic fertiliser without hygienisation, as it can also be reused as such. Moreover, if the additional raw materials to be co-digested with manure in farm-scale biogas plants or cooperatives involving several farms require hygienisation, only those materials with the hygienisation requirement need to be pre-treated and the manure can be fed into the process as such. However, there is one prerequisite: the digestate cannot then be handed over or sold to anyone outside the farm or farms in the cooperative. Co-digestion of materials is widespread digestion technique (see 2.3), but co-digestion of ABPs from meat processing is not studied extensively. Previous few co-digestions with various ABPs are given in table 1, while anaerobic treatment of slaughterhouse wastewater has been proven feasible in several investigations (e.g. Sayed et al., 1984, 1987; Sayed and De Zeeuw, 1988; Harper et al., 1990; Borja and Banks, 1994; Borja et al., 1995a,b,c,d; Borja et al., 1998; Pozo del et al., 2000). Solo-digestion of different ABPs and municipal waste has also studied in the content of slaughterhouses (Edström et al., 2003; Resch et al., 2006, 2010).

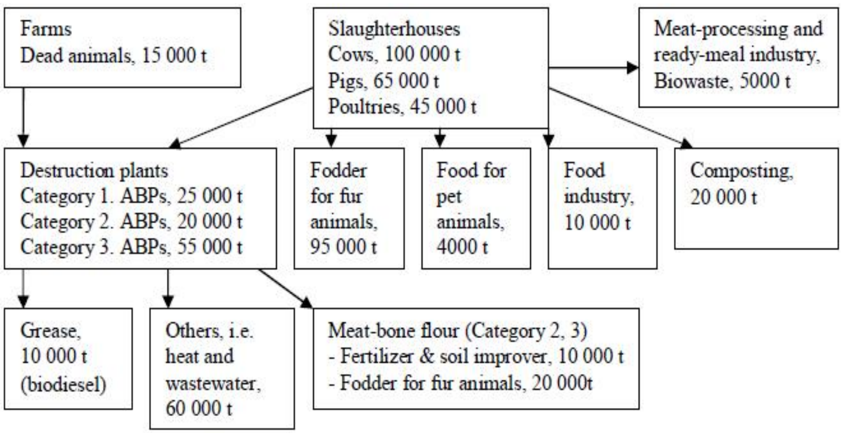

Fig. 2. *ABP streams of the Finnish meat-processing industry (Heinänen et al., 2007).*

The pre-treatments attempted include physical (e.g. particle size reduction and thermal treatment; Dalev, 1994; Wang and Banks., 2003), chemical (e.g. alkali addition: Dalev, 1994; Massé et al., 2001) and biological (e.g. enzymes: Dalev, 1994; Massé et al., 2001, 2003; Mendes et al., 2006; Valladão et al., 2007) methods. To our knowledge, studies on pre-treating presently studied raw materials are few or nonexistent, and the most of pre-treatments applied in this thesis (e.g. ultrasound and bacterial product) have not been previously studied with by-products from meat-processing industry or with cattle slurry. Thermal pre-treatment experiments (inc. hygienisation) with the similar materials have been made previously (Table 2).

The almost total energy self-sufficiency of the slaughterhouse industrial complex is reported to be obtained if the ABPs produced (rumen, blood, grease trap waste, DAF sludge, colon and digestive tract content) is digested and converted to the energy via CHP (Waltenberger et al., 2010).

3 Aims of the study

The motive for this study emerged from the increasing need and requirements for feasible and safe treatment of organic wastes and by-products. Moreover, depletion of un-renewable resources, such as fossil fuels and phosphorus, demands implementation of processes producing renewable energy and reusing materials. ABPs from meat-processing form an increasing group of materials with tightening treatment and disposal requirements. Many ABPs can be reused as energy- and nutrient-rich raw materials for anaerobic digestion as long as the safety regarding the quality (i.e. hygiene) of the end-products is ensured.

This study was conducted to evaluate anaerobic digestion of organic by-products from meat-processing, with special consideration to the effect of pre-treatments and co-digestion. ***The scientific objective*** was to understand the mechanisms involved in pre-treatments and co-digestion of ABPs. The case chosen for more detailed research was that of a middle-sized Finnish meat–processing industry. ***The specific aims*** were*:*

1. To evaluate the feasibility of different ABPs presently available for treatment as raw material for anaerobic digestion (Paper I-V).
2. To evaluate the effect of different pre-treatments on hydrolysis and methane yields of the ABPs studied (Paper I, II, IV, V).
3. To study optimal conditions and techniques for pre-treating ABPs and feed mixtures as well as for co-digesting them in mesophilic digestion processes (Paper II-IV).
4. To enhance the digestion process (increased methane production, quality of digestate) of the ABPs with the use of

pre-treatments (Paper I-V) and/or in co-digestion with sewage sludge and slurry (Paper III, IV).

5. To evaluate the possibility to co-digest ABPs in existing digesters at wastewater treatment plants and in farm reactors in the case presented (Paper III, IV).

The baseline of this research was to observe the overall process from the perspective of real circumstances in Finland (legislation, availability of raw materials, feed ratios, pre-treatment option, possible co-substrates etc.) in order to provide practical information despite laboratory-scale experiments. The aspects of economic profitability and environmental sustainability of the enhanced processes were estimated via indicative energy balances.

The general goal was to produce easily-exploitable information for adopting locally and case-specifically sustainable processing technologies of organic wastes and by-products into practice via anaerobic digestion technology.

4 Materials and methods

All materials, methods, analyses and calculations are described in more detail in the original articles (Paper I–V).

4.1 MATERIALS

Meat-processing industry produces different kinds of organic wastes (210 000 t per year in Finland) with different kinds of treatment requirements (e.g. Animal by-products (ABP) regulation 1774/2002/EC), reuse possibilities and treatment solutions, as discussed in section 2.5.

The ABPs studied presently (digestive tract content, drumsieve waste, dissolved air flotation (DAF) sludge and grease trap sludge) were chosen according to their availability for treatment in Finnish meat-processing industry and were received from a middle-sized slaughterhouse handling cows and pigs (Lappeenranta, Finland) and a meat-processing plant (Mikkeli, Finland). These ABPs could not be utilised in fodder (95 000 t of ABPs per year in Finland) or in animal food production (4000 t of ABPs per year in Finland) and have currently been directed to destruction plants (55 000 t of category 3 ABPs per year in Finland) or to composting (25 000 t of ABPs per year in Finland), despite being mostly considered unsuitable for composting. At the time of sampling, approximately 5300 tons of digestive tract content (~2400 t), drumsieve waste (~490 t) and DAF sludge (1800 t) were produced annually in the slaughterhouse, and 75-100 tons of grease trap sludge (530-700 t with the water content included) was produced in the meat-processing plant.

Only digestive tract content (and cattle slurry) of the materials studied was categorised in the ABP regulation (Category 2; see

2.5), while the other materials, i.e. drumsieve waste, DAF sludge and grease trap sludge, were not included in the regulation due to passing 6 mm sieves.

After the suitability of the ABPs studied on anaerobic digestion was tested (by the biodegradation and BMP studies in the batches) and preliminary screening of different pre-treatments was performed (Paper I), a new portion of raw materials were collected and mixed according to their produced wet weight (w.w.) ratios (44:34:13:9) in order to form the ABP mixture used (Paper II-IV). The mixture was frozen at -18 °C prior to melting for use in the experiments. The quality of the ABPs used in paper I and the rest of the studies (Paper II-IV) varies due to the different dilution of DAF and grease trap sludges (Table 5). Moreover, digestive tract content and drumsieve waste was first collected and studied separately (Paper I), but for papers II-IV they were collected in the end of the process line in which they are mixed together according to produced amounts (82:18; Paper II).

Table 5. *Characteristics of the untreated ABP fractions of the present study. Standard deviation given where applicable.*

	Digestive tract content	**Drum-sieve waste**	**Digestive tract content + drumsieve waste**	**DAF sludge**		**Grease trap sludge**		**ABP mixture**
Paper	I	I	II	I	II	I	II	II-IV
TS %	12 ±0.5	14 ±2.0	17 ±0.5	4.3	7.9	11 ±0.8	16 ±0.9	13 ±0.2
VS %	11 ±0.5	14 ±2.0	15 ±0.4	3.5	6.7	11 ±0.8	16 ±0.9	12 ±0.2
TS/VS	0.9	1.0	0.8	0.8	0.9	1.0	1.0	0.9
COD_{sol} (g/l)	4.0 ±0.1	0.9±0.1	7.8 ±0.5	5.6	4.6	6.6	7.2	11 ±0.1
VFA (g/l)	2.6 ±0.4	0.2±0.1	4.8 ±0.4	1.6	1.2	3.4	3.9	6.4 ±0.2
VFA from COD_{sol} (%)	65	22	62	29	26	52	54	58
LCFA (mg/l)	-	-	5.0	-	30	-	10	30
NH_4^+-N	0.1	0.1	0.3	0.5	-	0.48	-	-
N_{sol} (g/l)	-	-	0.4	-	0.6	-	0.7	0.7
N_{tot} (g/l)	-	-	1.1 ±0.1	-	2.1		2.1	1.6
LRC_{sol} (g/l)	-	-	0.06	-	-	-	-	0.03
pH	7.2	6.6	7.1	6.9	6.8	5.6	5.5	6.0-6.2

Cattle manure, categorised in the ABP regulation (category 2; Table 4), was not included in the ABP mixture studied, though it is also produced in slaughterhouses and during the transportation of animals. As this manure usually does not resemble the manure produced on farms, the cattle slurry used in the experiments was collected from a dairy farm housing 40 dairy cows (Mikkeli, Finland; TS 5.9 ±0.1, VS 4.5 ±0.2, pH 7.2-7.5; Paper IV, V).

The other co-digestion matrix, sewage sludge (TS 4.5 ±0.8%; VS: 3.0 ±0.6%; pH: 7.1; Paper III), was collected from a municipal wastewater treatment plant (Mikkeli, Finland). The plant treats wastewaters not only from residential areas, but also from small- and medium-sized industries and produces approximately 36 400 m^3 of sewage sludge per year. New sewage sludge and cattle slurry were collected once a month and kept at 4 °C prior to use in the experiments.

The inocula used in the digestion experiments were digested sewage sludge from a municipal wastewater treatment plant (Mikkeli, Finland; TS 3.3, VS 2.0, pH 7.5; Paper I, III) and digestate from a farm-scale biogas plant digesting cattle slurry, plant biomass and confectionery waste (Laukaa, Finland; TS 4.4, VS 3.5, Ph 7.5; Paper IV, V).

4.2 EXPERIMENTAL SET-UP

4.2.1 Pre-treatments

The effect of hygienisation (Paper I-V), ultrasound (Paper I, II, IV, V), chemical (acid, base; Paper I) and biological pre-treatments (bacterial product; Paper I, II) on hydrolysis of the ABPs (Paper I, II, V) and feed mixtures (Paper III, IV) was studied. Of these, the pre-treatment of ABPs with ultrasound and addition of bacterial product were chosen for a more detailed study (Paper II). The optimal duration for bacterial product treatment (3, 6, 12, 24 and 48 hours; except for DAF sludge 3, 6, 12 and 24 hours; Paper II) and the optimal Es input for ultrasound pre-treatment (1000, 3500, 6000, 8500 and 14 000 kJ/kg TS) of by-products (Paper II, V) and ABP mixture + cattle slurry (1:3; Paper IV) were determined according to the highest increase in VS-based hydrolysis parameters (Tables 8, 9 and Fig. 3). The pre-treatments chosen for semi-continuous co-digestion case-studies (ABP mixture + sewage sludge or cattle slurry; Paper III-IV) and for experiments of cattle slurry alone (Paper V) were ultrasound pre-treatment and hygienisation. These treatments were chosen due to their efficiency (Paper I, II), lack of previous studies in literature and potential synergy benefits for the process. hygienisation, required for such by-products of category 2, is not only offering pathogen removal, but simultaneously possible enhanced degradation and capability to use the heat produced during the process chain.

Thermal pre-treatment was the hygienisation required by ABP regulation (70 °C, 60 min, particle size < 12 mm; Paper I, III-V). Materials were hygienised by heating them to 70 °C using the heater in a magnetic stirrer (Heidolph MR 3001, Germany) and then keeping them in an incubator (Termaks TS 8056, Norway) at 70 °C for one hour. Before use in the experiments, the materials were cooled to 35 °C. Feeds for the co-digestion experiments were either hygienised separately (Paper IV) or mixed together (Paper III, IV).

Ultrasound treatment was achieved with Hielscher UP200H (Germany; 24 kHz, pulse range of 60%; Paper I) and Hielscher UP100H ultrasound processors (Germany; 30 kHz, pulse range of 80%; Paper II, IV, V) at 25 ±5 °C.

Bacterial product pre-treatment was performed with Liquid Certizyme 5™ (Certified Laboratories, NCH Finland Ltd.), designed to prevent grease from solidifying in sewers and removal tanks (Paper I, II). The product consists of three different bacteria: *Bacillicus subtilis*, *Bacillicus licheniformis* and *Bacillus thuringiensis* (108 CFU/ml), which proliferate and produce protease, amylase and lipase enzymes when exposed to viable conditions. The manufacturer's dose recommendation, 300 CFU/500 ml, was followed using nitrogen-flushed vessels at 23 ±2 °C with agitation (HS 501 digital, IKA Labortechnik, Germany). Grease trap sludge was already treated with a bacterial product at the meat-processing plants for preventing formation of solid grease in removal tanks. It have apparently not been studied for pre-treatment purposes

Base (2 M NaOH; 6-14%; pH 12-12.2; 4 hours) and acid (6 M HCl; 2-8% pH 2-2.5, 4 hours) pre-treatments were carried out in nitrogen flushed, mixed (HS 501 digital, IKA Labortechnik, Germany) vessels and neutralised with NaOH (2 and 0.1 M) or HCl (6 and 0.1 M) to pH 7.0 prior to batch experiments (Paper I).

4.2.2 Batch experiments

Methane production potentials of digestive tract content, drumsieve waste, DAF sludge, grease trap sludge, cattle slurry (Paper I, V) and co-digestion feed of ABPs and cattle slurry (1:3; Paper IV; Table 6) were determined in batch experiments in duplicate 2 liter glass bottles incubated statically at 35 ±1 °C. The potentials were determined with and without pre-treatments (hygienisation, ultrasound: Paper I, IV, V; bacterial product, acid, base: Paper I) and a set of bottles were prepared with inoculum alone with its methane production subtracted from the materials studied. Inoculum (750 g/batch) and the materials studied were added into the bottles in a $VS_{substrate}/VS_{inoculum}$ ratio of 1. Distilled water was added to produce a liquid volume of 1.5 liter. pH of each batch was adjusted to 7.0 with 2 M NaOH or 6 M HCl, and sodium bicarbonate ($NaHCO_3$, 3 g/l) was added as buffer. Headspaces of the bottles were flushed with nitrogen gas for five minutes, after which the bottles were sealed with rubber septa. Biogas was collected into aluminium gas bags (Tesseraux Spezialverpackungen GmbH, Germany). The more divided information of the batch experiments are given in table 12 (see 5.3).

Table 6. Characteristics of the raw materials studied in co-digestion studies.

	ABP mixture + sewage sludge (1/7)	ABP mixture + sewage sludge (1/3)	ABP mixture + cattle slurry (1/3)	Cattle slurry
Paper	III	III	IV	IV, V
TS %	6.3 ±0.7	7.2 ±0.6	7.6 ±0.3	5.9 ±0.1
VS %	4.6 ±0.3	5.6 ±0.3	6.2 ±0.2	4.5 ±0.2
VS/TS	0.7	0.8	0.8	0.8
COD_{sol} (g/l)	6.6 ±2.0	7.8 ±2.0	15 ±1.4	14 ±1.0
VFA (g/l)	4.7 ±1.0	5.3 ±1.0	5.5 ±1.1	6.0 ±0.2
VFA from COD_{sol} (%)	71	68	37	43
LCFA (mg/l)	3.0-42	3.0-22	3-22	-
NH_4^+-N (g/l)	0.3 ±0.1	0.4 ±0.1	1.1 ±0.2	1.3
N_{sol} (g/l)	-	-	1.4 ±0.2	1.6
LRC_{sol} (g/l)	-	-	1.5 ±0.3	1.9
pH	6.2-6.6	6.1-6.5	6.7-7.1	7.2-7.5
Alkalinity ($gCaCO_3$/l)	2.3 ±0.4	2.9 ±0.5	5.7 ±0.3	-

4.2.3 Reactor experiments

The semi-continuous reactor experiments co-digesting ABP mixture + sewage sludge (Paper III) and ABP mixture + cattle slurry (Paper IV) were conducted in three five liter glass reactors (R1, R2, R3) with a liquid volume of 4 liters at 35 ±1 °C. The reactors were constantly mixed using magnetic stirrers (300 rpm; Heidolph MR 3001, Germany). Feeding and withdrawal were performed once a day, five days per week using a 100 ml syringe. OLR were calculated for five days per week (Table 13, 14).

Co-digestion of ABPs and sewage sludge was designed as a case study on Finnish middle-sized meat-processing company and a middle-sized municipal wastewater treatment plant, and it continued for 175 days (Paper III). HRT was reduced from 25 days (days 0–43) to 20 (days 44-126) and finally to 14 days (127 - 175) with the OLR increasing accordingly. The feed for reactors 1 and 2 contained ABP mixture + sewage sludge in a ratio of 1:7 (w.w.), representing the annual production ratio of the materials, while a feed ratio of 1:3 for reactor 3 represented the optimum co-digestion ratio from the literature (sewage sludge with industrial food waste or slaughterhouse waste and/or municipal food waste; Rosenwinkel and Meyer, 1999; Murto et al., 2004, Sosnowski et al., 2008). The feed for reactor 2 was hygienised, while the other feeds were digested as such. Anaerobic digestion of sludge alone is already used widely in the wastewater treatment plants.

Co-digestion of ABPs and cattle slurry was designed as case study of Finnish middle-sized meat-production company and a large farm or a cooperative of several farms, and it continued for 109 days (HRT of 21 days, feed ratio of 1:3 (w.w.); Paper IV). Cattle slurry, also categorised in an ABP regulation (category 2), was not included in such to ABP mixture, but it was studied at the organic waste produced by agriculture, that could also utilised it in farm scale biogas plants (or plant involving several farms) co-digesting ABPs (from meat-processing industry, farm-scale slaughterhouse, fur farming and food production) + slurry (1:3, w.w.; Paper IV) or pre-treated cattle slurry alone (Paper V). Reactor 1 was fed with untreated mixture, while the feed for reactor 2 was ultrasound pre-treated (6000 kJ/kg TS in the batch experiments and days 0-67 of the reactor study; 1000 kJ/kg TS days 68-109 of the reactor study). The feed materials for batch experiment and for reactor 3 were initially hygienised separately (days 0-67), but on days 68-109, the feed materials were first mixed together as then hygienised as a ready-made mixture. The data in the tables is presented from day 22 onwards in order to avoid the variation during the start-up phase. The more specific parameters of the continuous reactor experiments are given in table 13 and 14 (see 5.4.1 and 5.4.2).

4.3 ANALYSIS

Biogas volume was measured with water displacement and methane content with gas chromatography (Agilent 6890N: PerkinElmer Elite-Alumina column 30 m x 0.53 mm, flame ionisation detector 225 °C, oven 100 °C, inlet 225 °C, carrier gas helium 10 ml/min, split ratio 35:1, injection volume 100 µl, Paper I, III-V). Specific methanogenic activity (SMA, m^3 CH_4/tVS d) was calculated from the steepest slope of the cumulative methane production curves (Paper I, IV, V). TS and VS were analysed according to standard methods (APHA, 1998). COD_{sol} was determined after filtration through Whatman GF/A glass microfibre-filters (Scheicher & Schuell, Germany) according to the Finnish standard method SFS 5504. VFA (acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic acid) and LCFA (palmitic, oleic acid; Paper II-IV) were measured using gas chromatography with flame ionisation detector (Agilent 6890N GC-FID, column Agilent HP-FFAP 30 m x 0.32 mm x 0.25 µm). The parameters for VFA were: oven 100-160 °C 25 °C/min, injector 225 °C, detector 230 °C, carrier gas helium 2.6 ml/min, split ratio 2.3:1, injection volume 1 µl, and for LCFA: oven 50-230 °C, 10 °C/min, injector and detector 230 °C, carrier gas helium 3.3 ml/min, split ratio 2.3:1 and injection volume 1 µl. For VFA, the samples were filtered through 0.45 µm syringe filters (VWR International Ltd.). LCFA analysis was made from 2 ml of filtered samples (Whatman GF/A glass microfibre-filters, 1.6 µm), extracted with tert-metylbutylether (TMBE, Merck; ISO 5508). Oleic and palmitic acids were chosen for analysis as they are the most common LCFAs in animal fats (68%) and sewage sludge (65%; Miron et al., 2000; Fernández et al., 2005). pH was measured with WTW 340i pH-meter and electrode (Germany) and alkalinity (Paper III, IV) was measured according to the European standard ISO 9963-1. Total nitrogen (N_{tot}, Paper II) was studied from the four parallel samples (Kjeltec system 1002 – disteller; ISO 20483). Soluble and ammonium nitrogen (N_{sol}, Paper II, IV, V; NH_4^+-N, Paper I, III-V) was analysed photometrically (HACH LANGE DR 2800 VIS photometer, Germany) from filtered samples (Whatman GF/A) using cuvette tests (HACH LANGE LCK302, 47-130 mg/l; LCK338, 20-100 mg/l, Germany). Particle size distribution (PSD; Paper I-III) was analysed using high-end dispersion analyser LUMiSizer® (L.U.M. GmbH, Germany), measuring APS as arithmetic average volume diameters (nm). To give an exact diameter of particles, the density of the particles should be known. However, as the studied materials were heterogeneous composites, particle density was considered constant (1.0) and the results were reported only with percentual comparison of particle sizes (untreated vs. pre-treated). Soluble lignin related compounds ($LRC_{sol,}$ Paper II, IV, V) were estimated from filtered (Whatman GF/A glass microfibre-filters, 1.6 μm) materials assumed to contained in plant-originated cellulose content materials (i.e. digestive tract content + drumsieve waste, cattle slurry and ABP mixture; Paper II, IV, V). Degradation of lignin compounds were analysed using Perkin Elmer Lambda 45 UV/VIS spectrometer at the absorbance of 280 nm (Larrea et al., 1989), with lignin model compound and dissolution product 4-hydroxymethyl-2-methoxyphenol (Merck, purity > 98%) as a standard (Crestini et al., 2005; Lahtinen et al., 2008).

4.4 CALCULATIONS

VS-based hydrolysis parameters (Table 7, 8, 10 and 11) were used in order to avoid the changes in VS occurring during the pre-treatments (evaporation, dilution, varying quality of material). According to the previous studies, ultrasound treatment must be optimised separately in each application (Schläfer et al., 2002; Grönroos et al., 2005), thus screening according to the highest increase of hydrolysis parameters (at least COD_{sol}/VS and VFA/VS ratios) were performed (Paper II, IV, V).

The Es input for ultrasound treatments was calculated with equation (Eq. 1), also enabling economical estimates and further energy balance calculations of the biogas process:

$$Es[kJ/kg\ TS] = Pt/VTS_0, \qquad (Eq. 1)$$

where ultrasound power (P), duration of ultrasound treatment (t), volume of ultrasound treated material (V) and initial TS_0 (Bougrier et al., 2005).

The Es input for hygienisation describes the energy needed to increase the temperature of daily feed and was calculated with equation (Eq. 2):

$$Es\ [kJ\ d^{-1}] = ((\varrho\ Q\ \gamma\ (T_1 - T_2)), \qquad (Eq. 2)$$

where ϱ = specific density of sludge calculated from the specific volume of the weighted daily feed; Q = daily feed to the reactor; γ = specific heat capacity of feed (water: 0.00419 kJ/g °C); T_1 = terminal temperature (70 °C); $t_{70\,°C}$ = duration of treatment (60 min), T_2 = digestion temperature (35 °C). Energy needed to maintain the treatment temperature at 70 °C during the one hour was calculated with the hygienisation of ABP mixture + sewage sludge (1:7, w.w.; Paper III), but since excluded. It was approximately 10 % from the total Es input.

Energy output (Eo) describes the differences in methane production between pre-treated and untreated reactor/batches. It was calculated with equation (Eq. 3):

$$Eo\ [kJd^{-1}] = H_{CH4}\ (V_{R2}\, n_{R2}\, Q_{R2} - V_{R1}\, n_{R1}\, Q_{R1}), \qquad (Eq. 3)$$

where H_{CH4} = calorific value of methane (802 kJ/mol); n = feed VS; Q = daily feed; V = methane potential of digesters was calculated with equation (Eq. 4; Lu et al., 2008).

$$V\ [mol\ CH_4\ g^{-1}VS^{-1}] = SMPp/RT_2, \qquad (Eq. 4)$$

where SMP = specific methane production, p = air pressure (1.013 bar), R = gas constant (0.08314 bar dm^3/mol K).

The proportion of gaseous ammonia (NH_3) from NH_4^+-N was calculated according to the equation (Eq. 5) from the article of Martinelle and Häggström (1997):

$[NH_3] = [NH_x] \times 10^{(pH-pKa)}/(1 + 10^{(pH-pKa)})$, (Eq. 5).

The dissociation constant (pK_a) at 35 °C is 8.95 and $NH_x = [NH_4] + [NH_3]$ is calculated with the NH_4^+-N concentration analysed, this being dependent only on temperature and pH.

The statistical significance of microbial numbers between the feed and the corresponding digestate was calculated with paired t-test after log_{10}-transformation with Microsoft Excel programme which was also used to obtain the geometric means.

5 Results

The included papers (I-V) represent the data from applied experiments more immersed.

The suitability of ABPs (digestive tract content, drumsieve waste, DAF sludge and grease trap sludge) for anaerobic digestion, as determined by their methane production potential and the effect of different pre-treatments was firstly studied in a preliminary screening study presented in paper I, II. The pre-treatments chosen were pre-hygienisation, ultrasound (13 000 ±2500 kJ/kg TS) and the additions of acid, base (both 4 hours) and bacterial product (24 hours).

All the studied pre-treatments hydrolysed part of the organic material, but the most effective pre-treatments, ultrasound and addition of bacterial product, were then studied in more detail to determine the optimal treatment mode (Es and duration) when pre-treating ABPs separately and in mixture (Paper II). The hydrolysis parameters used were: COD_{sol}/VS, VFA/VS, LCFA/VS, N_{sol}/VS, NH_4^+-N/VS, LRC_{sol}/VS and change in APS.

After the preliminary screening of ABPs and pre-treatments (Paper I, II), the ABP mixture was used in case-specific experiments co-digesting it with sewage sludge (Paper III) and with dairy cattle slurry (Paper IV). Both of these co-digestion feeds were pre-treated by hygienisation, which is in any case required when these legislative demanding by-products are digested. ABP mixture + cattle slurry feed was also pre-treated by ultrasound. As dairy cattle slurry alone is also a significant by-product, its digestion alone and the effect of hygienisation and ultrasound pre-treatments were further included into the experiments (Paper V). Hygienisation and ultrasound pre-treatments of case-experiments (Paper III, IV, V) were studied in comparison to co-digestion as such.

5.1 THE SCREENING FOR THE MOST EFFECTIVE PRE-TREATMENTS ON HYDROLYSIS OF ABPS

Five different pre-treatments (hygienisation, ultrasound, base, acid, bacterial product) were used in order to hydrolyse by-products from meat-processing industry (Table 7). Hygienisation concentrated the ABPs increasing the VS content by 30 ±4.8%, whereas both chemical treatments (acid, base) diluted the materials by 15 ±4.8%, when compared to the original feed materials. Ultrasound increased the VS content of DAF- and grease trap sludge (+6% and +30%), but decreased the content of digestive tract content and drumsieve waste (-4% and -20%). Hydrolysis of digestive tract content and drumsieve waste could not be measured due to the evaporation and lack of soluble phase that could be filtered and analysed (Table 7).

The most effective pre-treatments for digestive tract content were bacterial product and base, increasing hydrolysis parameters by 70-160%, when ultrasound and bacterial product modalities increased the hydrolysis of drumsieve waste and DAF sludge by 38-1300%. Ultrasound increased the most COD_{sol}/VS ratio of grease trap sludge (+120%), when only acid and hygienisation treatments achieved a low increase in rest of the parameters (Table 7). BMP (140-1040 m^3 CH_4/tVS), VS-removal (i.e. biodegradation and stabilisation; 62-95%) and SMA of inoculum (13-73 m^3 CH_4/tVS d) from the untreated and the pre-treated ABP fractions were determined in the batch experiments and can be reviewed in the section of 5.3.

Table 7. ABP fractions from the meat-processing industry studied before and after the pre-treatments and the change in hydrolysis parameters, when compared to the untreated ABPs.

Material	Treatment	TS (%)	VS (%)	VS/TS	VFA from COD_{sol} (%)	COD_{sol} /VS (%)	VFA /VS (%)	NH_4^+-N /VS (%)
Digestive tract content	Untreated	12 ±0.5	11 ±0.5	0.9	65			
	Thermal	15 ±0.2	14 ±0.2	0.9	- *	- *	- *	- *
	Ultrasound	11 ±0.1	10± 0.1	0.9	78	32	62	95
	Base	11 ±0.2	9.0 ±0.1	0.8	83	67	120	97
	Acid	11 ±0.1	9.5 ±0.1	0.8	76	26	50	96
	Bact. prod.	12 ±0.1	10 ±0.1	0.9	88	70	130	160
Drumsieve waste	Untreated	14 ±2.0	14 ±2.0	1.0	22			
	Thermal	18 ±0.1	17 ±0.1	0.9	- *	- *	- *	- *
	Ultrasound	12 ±1.0	11 ±0.7	0.9	47	540	1300	250
	Base	12 ±2.0	10 ±1.2	0.9	32	810	1200	-5
	Acid	11 ±0.1	10 ±1.5	0.9	29	520	640	33
	Bact. prod.	15 ±0.1	14 ±0.1	0.9	24	920	1040	-63
DAF sludge	Untreated	4.3 ±0.1	3.5 ±0.1	0.8	29			
	Thermal	5.6 ±0.1	4.5 ±0.1	0.8	31	20	27	-12
	Ultrasound	4.6 ±0.1	3.7 ±0.4	0.8	22	76	35	90
	Base	4.4 ±0.1	3.0 ±0.1	0.7	34	56	83	-33
	Acid	4.2 ±0.1	2.8 ±0.1	0.7	37	22	57	7.5
	Bact. prod.	4.2 ±1.0	3.0 ±1.0	0.7	38	79	130	790
Grease trap sludge	Untreated	11 ±1.0	11 ±1.0	1.0	52			
	Thermal	16 ±0.3	15 ±0.3	1.0	18	98	-33	20
	Ultrasound	15 ±0.2	14 ±0.1	1.0	18	120	-21	-13
	Base	12 ±1.0	10 ±0.9	0.9	52	-5.1	-3.3	-54
	Acid	10 ±0.1	9.3 ±0.1	0.9	46	25	13	3.4

-* lack of soluble phase

5.1.1 Ultrasound screening treatments

Ultrasound pre-treatment was considered efficient and thus its optimal Es input was determined according to the highest hydrolysis parameter ratios received using Es inputs of 1000, 3000, 6000, 9000, 14 000 (±500) kJ/kg TS (Table 8; Paper II).

Table 8. Change in hydrolysis parameters (%) and APS reduction (%) during ultrasound pre-treatment of the ABP fractions studied as compared to the raw materials.

Material	Es input	COD_{sol}/ VS	VFA/ VS	LCFA/ VS	N_{sol}/ VS	LRC_{sol}/ VS	APS reduction
Digestive tract	1000	17	-17	190	49	4.9	28
content +	3500	19	35	240	59	50	-15
drumsieve	6000	57	56	150	110	120	-59
	8500	6.4	-3.5	79	41	60	-53
	14000	-27	-39	67	50	90	-57
DAF sludge	1000	114	68	500	19	-	-61
	3500	164	130	270	47	-	-73
	6000	150	160	160	68	-	-75
	8500	286	260	140	85	-	-70
	14000	650	310	110	100	-	-78
Grease trap	1000	5.6	-22	560	-11	-	-42
sludge	3500	24	-16	21000	23	-	-54
	6000	98	3.5	1900	44	-	-67
	8500	63	4.3	33000	86	-	*
	14000	55	-19	26000	-11	-	*
ABP mixture	1000	27	-31	140	2.9	38	-60
	3500	-1.1	-23	120	-7.9	140	-53
	6000	14	-12	120	25	180	-42
	8500	45	27	160	45	260	-45
	14000	8.6	-12	100	30	160	-17

(-) Not measured

Digestive tract content + drumsieve waste was hydrolysed the most with the Es input of 6000 kJ/kg TS, when the hydrolysis parameters increased by 56-150% and APS reduction was 59 % from the original. DAF sludge was the only studied ABP whose hydrolysis increased linearly with increasing Es input, being finally at 14 000 kJ/kg TS 100-650% higher than in raw sludge (Table 8).

Hydrolysis of grease trap sludge was improved the most by the Es input of 8500 kJ/kg TS, when hydrolysis parameters increased by 63-33 000%. Only VFA decreased or remained at original level. The highest APS reduction of DAF sludge and grease trap sludge was 67-78% from their original sizes. However, during the highest hydrolysis of grease trap sludge, the whole structure of the sludge changed from particulate emulsion to paste, preventing the further APS measurements (Table 8).

ABP mixture increased the hydrolysis by 27-260% and achieved an APS reduction of 45% (8500 kJ/kg TS), when compared to the untreated mixture (Table 8). N_{sol}, like other hydrolysis parameters, varied depending on the increasing Es. However, changing Es did not affect to the N_{tot} content, but it was 1.1 ±0.1 g/l for the digestive tract and drum sieve waste, 2.1 ±0.1 g/l for the DAF sludge, 2.1 ±0.3 g/l for the grease trap sludge and 1.6 ±0.1 g/l for the ABP mixture.

5.1.2 Bacterial product screening treatments

Also the hydrolysis by bacterial product added was studied closer in order to find the optimal treatment time for the ABPs. Screening was achieved with the durations of 3, 6, 12, 24, 48 hours (Except DAF sludge 3-24 hours; Table 9; Paper II).

Table 9. *Change in hydrolysis parameters (%) and APS reduction (%) during different pre-treatment durations with bacterial product as compared to the raw material.*

Material	Duration (Hours)	COD_{sol}/ VS	VFA_{tot}/ VS	LCFA/ VS	N_{sol}/ VS	LRC_{sol}/ VS	APS reduction
Digestive tract content + Drumsieve waste	3	140	130	-	150	15	-15
	6	100	99	-	130	13	-18
	12	56	43	-	68	9.1	6
	24	130	110	-	83	23	-40
	48	85	52	-	130	-4.6	-20
DAF sludge	3	120	63	-	82	-	-35
	6	69	38	-	47	-	-70
	12	46	30	-	18	-	-65
	24	38	4.1	-	13	-	-60
ABP mixture	3	15	-13	32	13	20	6
	6	6.5	-19	110	14	38	-22
	12	31	-3.8	100	68	61	-35
	24	24	-15	110	28	62	-40
	48	25	-7.3	100	73	98	-20

(-) Not measured

The most suitable treatment time for the combined fractions of digestive tract content and drumsieve waste was 3 and 24 hours when the hydrolysis parameter increased by 83-150% from the original and APS was reduced by 18 and 40%, respectively (LRC_{sol}/VS increased by 19 ±3%). Three hours treatment of DAF sludge increased the hydrolysis by 63-120%, while the highest APS reduction (65-70%) was achieved between 6-12 hours.

The most of the hydrolysis parameters of ABP mixture increased the most after the treatment of 12 hours (31-73%). However, the VFA/VS ratio merely decreased (12 ±4.5%) from the original value, but similarly the increase of LCFA/VS ratio was improved by 110 ±5% with all durations higher than 3 hours, when compared to the original. The APS reduction was the highest (40%) after the 24 hours of treatment, while LRC_{sol}/VS increased the most (98%) after the treatment of 48 hours (Table 9). Grease trap sludge was already treated with bacterial product in the meat processing plant.

5.2 PRE-TREATMENTS USED IN THE CASE-EXPERIMENTS

Ultrasound (Paper IV, V) and hygienisation (Paper III-V) were used to pre-treat the case-specific co-digestion mixtures (ABPs + sewage sludge, Paper III; ABPs + cattle slurry, Paper IV) and cattle slurry (Paper V) prior to batch (Paper IV, V) or semi-continuous experiments (Paper III, IV). The ultimate aim was to enhance methane production and stabilisation of ABPs at 35 °C. These pre-treatments were selected to the further studies due to their efficiency (see 5.1), lack of previous studies and potential to utilise heat produced from the biogas in a hygienisation treatment required for the ABPs.

5.2.1 Ultrasound

Before the digestion experiments with the ultrasound pre-treated cattle slurry (Paper V) and ABP mixture + cattle slurry (1:3 w.w.; Paper IV), ultrasound was screened in order to find the most efficient Es input (Fig. 3). The observed hydrolysis parameters (COD_{sol}/VS and VFA/VS) of cattle slurry and ABP mixture + cattle slurry increased the most (25-50% and 27-130%) with the Es input of 6000 kJ/kg TS, when compared to the original ratios (Fig. 3).

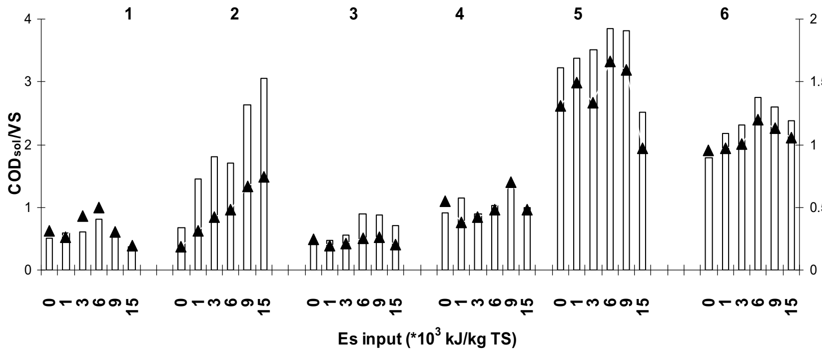

Fig. 3. *COD_{sol}/VS (□) and VFA/VS (▲) ratios in raw materials (0) and after ultrasound pre-treatment with different Es inputs (1000-15000 kJ/kg TS): The materials studied were: 1) digestive tract content + drumsieve waste, 2) DAF sludge, 3) grease trap sludge, 4) ABP mixture, 5) cattle slurry, 6) ABP mixture + cattle slurry (1:3).*

When the same materials were ultrasound pre-treated (6000 kJ/kg TS) during the reactor experiment, the increase in all hydrolysis parameters was only 7.5-31% from the original. Further, when Es was decreased from 6000 to 1000 kJ/kg TS the same hydrolysis parameters were increased to 17-31%, when compared to the ratios of original feed mixture (Table 10).

Table 10. *Characteristics of the ultrasound pre-treated materials used in the methane production studies (Paper I, IV, V) and change in hydrolysis parameters as compared to the untreated materials.*

	Cattle slurry	ABP mixture + cattle slurry (1/3)	
Es input (kJ/kgTS)	6000	6000	1000
Paper / Experimental mode	V, Batch	IV, Batch, reactor	IV, Reactor
TS (%)	6.0 ±0.1	7.2 ±0.1	7.5 ±0.3
VS (%)	4.5 ±0.1	5.9 ±0.2	6.2 ±0.2
VS/TS	0.8	0.8	0.8
COD_{sol} (g/l)	17 ±1.0	18 ±0.8	16 ±1.0
VFA (g/l)	6.7 ±0.2	7.3 ±0.9	6.6 ±0.3
VFA from COD_{sol} (%)	39	41	41
LCFA (mg/l)	-	4.7 ±2.0	4.4 ±1.0
NH_4^+-N (g/l)	1.4	1.4 ±0.1	1.2 ±0.1
N_{sol} (g/l)	1.7	1.7 ±0.3	1.7 ±0.1
LRC_{sol}	2.5	1.9 ±0.1	1.6 ±0.1
pH	7.1	6.8-7.2	6.8-6.9
Alkalinity (g $CaCO_3$/l)	-	5.9	5.6
Enterococci (cfu/g)	-	68 000	5700
Clostridia (cfu/g)	-	14 000	42 000
COD_{sol}/VS (%)	15	17±1.5	19
VFA/VS (%)	10	8-28	17
LCFA/VS (%)	-	23 ±6	28
N_{sol}/VS (%)	10	20 ±1	23
NH_4-N/VS (%)	7.5	10 ±1	31
LRC_{sol}/VS (%)	31	9	30
Alkalinity (%)	-	0	2

(-) Not measured

5.2.2 Hygienisation

Cattle slurry alone (Paper V), ABP mixture + cattle slurry (1:3 w.w.; Paper IV) and ABP mixture + sewage sludge (1:7 w.w.; Paper III) were pre-hygienised before batch (Paper IV, V) or reactor experiments (Paper III, IV). Hygienisation increased the VS content in cattle slurry by 4.5% and hydrolysis parameters studied by 29-96%. Separated hygienisation of ABP mixture + sewage sludge (Paper III) and ABP mixture + cattle slurry (Paper IV) increased the VS content by 13 ±2%, while the hydrolysis parameters increased by 0-23% (Except LCFA/VS of ABPs + sewage sludge decreased by 20 ±6 %) and the APS of ABPs + sewage sludge was reduced by 10% from the original size. The combined hygienisation of ABP mixture + cattle slurry (Paper IV) increased VS content by 5.9% and hydrolysis parameters by 39-100%, when compared to the untreated mixture (Table 11).

Pre-hygienisation process greatly reduced the numbers of enterococci so that results even below the detection limit (10 CFU/g) could be achieved. Also the clostridia were decreased from 36 000 and 14 000 CFU/g to 1300 CFU/g and 35 CFU/g (Table 11).

Table 11. *Characteristics of* hygienised *materials used in the methane production studies (Paper I, III, IV, V) and the change in hydrolysis parameters as compared to the raw materials. A = Separate hygienisation of ABP mixture and cattle slurry; B = Co-hygianisation of ABP mixture and cattle slurry.*

	Cattle slurry	**ABP mixture + sewage sludge (1/7)**	**ABP mixture + cattle slurry (1/3)**	
Paper / experimental mode / pre-treatment mode	V, Batch	III, Reactor	IV, Batch, Reactor, A	IV, Reactor, B
TS (%)	6.1 ±0.1	7.3 ±0.8	8.5 ±0.4	8.1 ±0.3
VS (%)	4.6 ±0.1	5.4 ±0.5	7.0 ±0.2	6.7 ±0.4
VS/TS	0.8	0.7	0.8	0.8
COD_{sol} (g/l)	21 ±1.0	9.2 ±3.0	20 ±1.5	20 ±1.0
VFA (g/l)	8.4 ±0.3	5.8 ±1.0	8.3 ±0.2	7.8 ±0.2
VFA from COD_{sol} (%)	40	63	42	39
LCFA (mg/l)	-	2.7-27	5.0 ±1.0	6.8 ±2.0
NH_4^+-N (g/l)	1.7	1.4 ±0.1	1.5 ±0.2	1.4 ±0.1
N_{sol} (g/l)	2.1	-	1.8 ±0.3	2.1 ±0.1
LRC_{sol}	3.8	-	2.3 ±0.1	1.9 ±0.1
pH	6.9	5.9-6.5	6.8-7.1	6.9-7.1
Alkalinity (g $CaCO_3$/l)	-	2.7 ±0.2	6.6	5.4
Enterococci (CFU)	-	-	<10	10
Clostridia (CFU)	-	-	1300	35
COD_{sol}/VS (%)	44	23 ±6	10 ±1	39
VFA/VS (%)	37	8 ±3	5 - 27	35
LCFA/VS (%)	–	-20 ±6	8 ±1	86
N_{sol}/VS (%)	33	-	9 ±1	40
NH_4-N/VS (%)	29	9.6 ±3.6	0	41
LRC_{sol}/VS (%)	96	-	13 ±1	100
Alkalinity (%)	-	12 ±2	13	1.8

(-) Not measured.

5.3 BATCH EXPERIMENTS

BMPs, potentials in VS removal (indicating the biodegradation and stabilisation of material) and SMA of inocolum of original and pre-treated ABPs (digestive tract content, drumsieve waste, DAF sludge, grease trap sludge; Paper I; see 5.1), cattle slurry (Paper V) and ABP mixture + cattle slurry (1:3; w.w.; Paper IV) were determined in batch experiments at 35 °C.

Methane production of DAF sludge started immediately and the extractable methane was quickly produced during 10-15 days both with untreated and pre-treated fractions. With drumsieve waste and digestive tract content, methane production started also immediately, but continued 3-15 days longer than with DAF sludge. Hygienisation improved the digestion rate (12 days), BMP from drumsieve waste (+48%) and shortened the lag phase of grease trap sludge to approximately two days, when otherwise it varied between 6-16 days. Cattle slurry alone and mixed with the ABPs showed a slight lag phase (approx. 2-3 days) before the methane production started to increase (Fig. 4).

Pre-treated cattle slurry produced relatively more biogas after the intensive period (18 days), than the original slurry. Moreover, the highest methane production from the untreated slurry and the mixture of it ceased 1-2 days earlier than those of the ultrasound pre-treated and hygienised materials (Fig. 4). About 27 ±5% from the total methane yield from untreated and pre-treated ABP mixture + cattle slurry and cattle slurry alone was produced after the intensive periods (10-13 days, total duration of 42 days). Similarly, untreated digestive tract content produced 20% of its methane yield after the intensive period (22 days), when with the other untreated and pre-treated ABPs the corresponding relations was 1-9% (Total duration of 69 days).

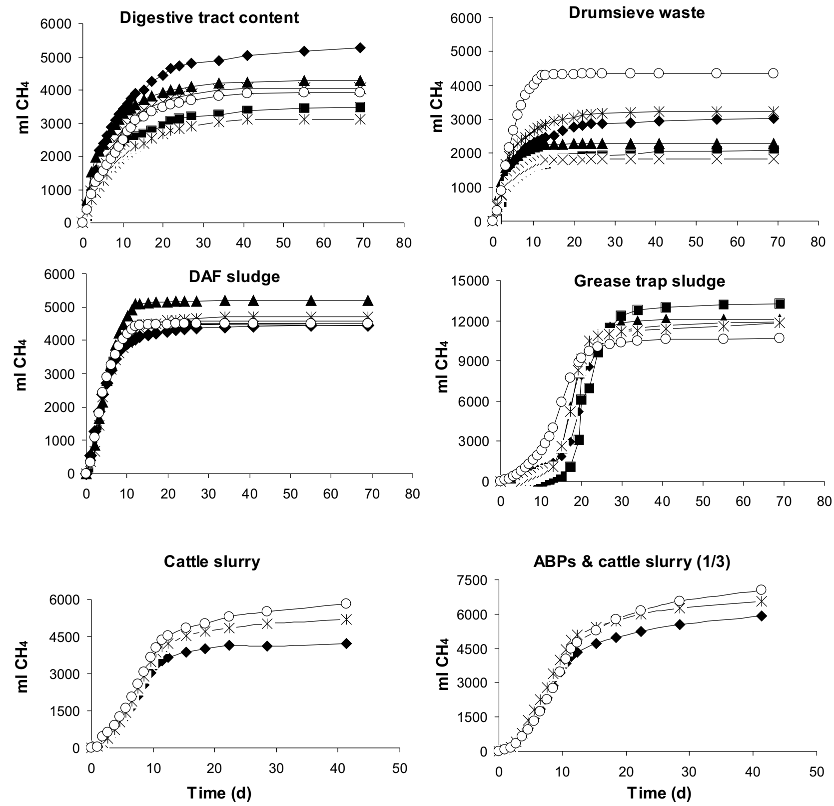

Fig. 4. Cumulative methane production of untreated and pre-treated materials in batch experiments. Raw materials (♦),hygienisation (o), ultrasound (*), bacterial product (×), acid (■) and base (▲).

BMP of cattle slurry was 230 m^3 CH_4/tVS$_{added}$ (Paper V) and that of ABP mixture + cattle slurry 300 m^3 CH_4/tVS$_{added}$ (Paper IV). Ultrasound increased the BMP of cattle slurry and ABP mixture + cattle slurry by 14 ±2% (270, 340 m^3 CH_4/tVS$_{added}$) and hygienisation by 24 ±4% (300, 360 m^3 CH_4/tVS$_{added}$; Table 12). BMPs of the separated ABP fractions were 400 m^3 CH_4/tVS$_{added}$ for digestive tract content, 230 m^3 CH_4/tVS$_{added}$ for drumsieve waste, 340 m^3 CH_4/tVS$_{added}$ for DAF sludge and 900 m^3 CH_4/tVS$_{added}$ for grease trap sludge (Paper I). BMP of ABP mixture calculated from the BMP of each fraction according to its proportion in the mixture was 410 m^3 CH_4/tVS$_{added}$. All the pre-treatments screened increased the BMP from DAF sludge by

3-15%, while only the hygienisation treatment managed to increase the BMP of drumsieve waste (+48%) and acid treatment the BMP of grease trap sludge (+12%). The other pre-treatments, and most notably all the pre-treatments of digestive tract content, decreased the BMP of the ABPs when compared to the raw materials.

SMA of inoculum (measured during the highest methane production rates) of all the untreated materials (Paper I, IV, V) was the highest with ABP mixture + cattle slurry (64 m^3 CH_4/tVS_{added} d; Paper IV) and the lowest with cattle slurry alone (24 m^3 CH_4/tVS_{added} d; Paper V). Hygienisation increased both of the SMAs by 21 ±5%, while ultrasound increased only the SMA of cattle slurry (17%), when compared to the original. However, in the suitability studies (Paper I), all pre-treatments decreased SMA of digestive tract content (25 m^3 CH_4/tVS d) on average by 58 ±9%, except base treatment which increased SMA by 32%. Ultrasound pre-treatment of DAF sludge increased SMA by 19% from original, whereas the other treatments reduced it. With all pre-treated drumsieve waste (18 m^3 CH_4/tVS_{added} d) and grease trap sludge (60 m^3 CH_4/tVS_{added} d), respective average increases in SMA were 15 ±6% and 14 ±3%, compared to the untreated materials. The exceptions were bacterial product treatment of drumsieve waste and hygienised grease trap sludge, the SMA of which decreased by 17 and 22%, respectively (Table 12).

VS removal was 46% for the untreated cattle slurry (Paper V) and 62% for the untreated ABP mixture + cattle slurry (Paper IV), while ultrasound and hygienisation increased the stabilisation to 48% and to 66%, respectively (Table 12). VS removal from separate ABPs (Paper I) was the highest with the original and pre-treated grease trap sludge (93 ±1%) and the lowest after the chemical and bacterial product addition in DAF sludge (62 %). VS removal from original and ultrasound pre-treated DAF sludge was 73 ±1% and 79% for the hygienised DAF-sludge. Pre-treatments did not appreciably affect the VS removal of drumsieve waste, which was 92 ±1% (Table 12).

During all batch experiments, the COD_{sol} content in batches were 2.6-11 g/l), while VFA was not detected (≤ 5 mg/l) from the batches including cattle slurry and ABP mixture + cattle slurry (Paper IV, V). However, batches digesting untreated ABPs contained 0.2-3.4 g VFA/l, as opposed to 1.2-5.1 g VFA/l in the pre-treated batches (Paper I). NH_4^+-N concentrations reduced from the contents before the digestions probably due to the dilution with the inoculum. All-in-all, NH_4^+-N concentrations of all the pre-treated batches remained higher (10-370%), when compared to the untreated digestates, except in the chemically treated DAF- and grease trap sludge (-35 ±6%) and biologically treated DAF sludge (-29%; Table 12).

At the end of the batch experiments of separated ABP fractions (Paper I), digestive tract content treated with the bacterial product had a 58% smaller residual APS, while all the pre-treatments of drumsieve waste resulted in a 11 ±8% smaller APS than the untreated materials. All the pre-treatments increased the APS of digestate of DAF sludge by 250 ±50% while that of grease trap sludge was by 17% smaller after hygienisation and ultrasound pre-treatment, as compared to the untreated materials. APS of all materials was the largest after the chemical treatments (acid, base), except in digestive tract content, where APS was the largest after the physical (ultrasound, hygienisation) treatments, when compared to the untreated fractions (Table 12). The changes in APS of cattle slurry content feeds was not measured.

Table 12. *Methane production potentials, highest achieved methane content of biogas, specific methanogenic activity of the inoculum (SMA), VS removals and the characteristics of the digested ABPs, cattle slurry and ABP mixture + cattle slurry (1:3, w.w.) in the batch experiment at 35 °C.*

Material	Pre-treatment	Paper	CH_4 (m^3/t VS_{added})	CH_4 (m^3/t $w.w_{added}$)	CH_4 (%)	SMA (m^3CH_4/ tVS d)	VS removal (%)	TS (%)	VS (%)	VS /TS	COD_{sol} (g/l)	VFA (g/l)	NH_4^+-N (g/l)	APS (%)	pH
Cattle slurry	Untreated	V	230	11	62	27	27	3.3	2.3	0.7	3.6	-*	1.0	-	7.5
	Ultrasound	V	270	12	63	28	28	3.3	2.3	0.7	3.8	-*	1.2	-	7.5
	Thermal	V	300	14	64	30	30	3.3	2.3	0.7	3.6	-*	1.2	-	7.5
ABP + slurry (1:3)	Untreated	IV	300	10	63	64	62	3.5	2.4	0.7	3.5	-*	0.9	-	7.4
	Ultrasound	IV	340	12	64	74	64	3.6	2.4	0.7	3.0	-*	1.0	-	7.3
	Thermal	IV	360	14	62	64	67	3.6	2.5	0.7	2.6	-*	0.9	-	7.4
Digestive tract content	Untreated	I	400	42	52	25	91	2.0	1.0	0.5	4.0	2.6	0.1	–	7.3
	Ultrasound	I	250	25	42	15	89	2.0	1.0	0.5	5.1	4.2	0.2	30	7.4
	Thermal	I	310	42	48	22	92	2.1	1.1	0.5	- *	-*	- *	30	7.3
	Bact. prod.	I	320	33	48	18	90	1.9	1.0	0.5	6.1	5.1	0.2	-58	7.2
	Base	I	320	30	50	33	89	2.0	1.0	0.5	4.6	3.5	0.2	24	7.4
	Acid	I	270	25	45	18	89	1.9	1.0	0.5	6.5	5.7	0.3	13	7.3
Drumsieve waste	Untreated	I	230	30	46	18	93	1.9	1.0	0.5	0.9	0.2	0.1	-	7.3
	Ultrasound	I	210	23	47	22	89	2.2	1.2	0.6	4.3	2.0	0.5	-12	7.4
	Thermal	I	340	56	60	27	93	2.1	1.1	0.5	- *	-*	- *	-20	7.3
	Bact. prod.	I	140	20	46	15	92	2.0	1.0	0.5	5.9	1.9	0.1	-11	7.5
	Base	I	170	18	47	25	91	1.9	1.1	0.6	4.8	1.2	0.2	-1	7.3
	Acid	I	160	16	42	31	92	2.0	1.1	0.6	9.0	2.2	0.1	-4.6	7.4
DAF sludge	Untreated	I	340	12	58	13	73	1.9	1.0	0.5	5.6	1.6	0.5	–	7.5
	Ultrasound	I	370	14	60	37	72	2.1	1.0	0.5	11	2.4	1.0	260	7.5
	Thermal	I	360	16	68	23	79	2.1	1.1	0.5	8.7	2.7	0.6	240	7.4
	Bact. prod.	I	350	11	60	33	62	2.3	1.1	0.5	7.6	2.6	0.3	130	7.6
	Base	I	390	12	66	31	62	2.4	1.1	0.5	5.7	2.1	0.4	310	7.4
	Acid	I	350	10	54	37	62	2.3	1.1	0.5	8.7	3.3	0.6	310	7.4
Grease trap trap sludge	Untreated	I	900	99	77	60	92	1.7	0.8	0.5	6.6	3.4	0.5	–	7.5
	Ultrasound	I	890	126	71	70	94	1.7	0.8	0.5	19	3.5	0.5	-16	7.4
	Thermal	I	840	129	65	47	95	1.7	0.8	0.5	18	3.2	0.8	-17	7.4
	Base	I	900	92	73	68	92	1.6	0.8	0.5	5.8	3.0	0.2	20	7.4
	Acid	I	1010	93	71	73	91	1.7	0.8	0.5	7.0	3.2	0.4	8	7.5

5.4 REACTOR EXPERIMENTS

Anaerobic co-digestion of ABP mixture + sewage sludge (1:7; 1:3; Paper III) and ABP mixture + cattle slurry (1:3; Paper IV) was studied at semi-continually fed reactors at 35 °C. Both of the feed mixtures were pre-hygienised, but ABP mixture + cattle slurry was also ultrasound pre-treated.

5.4.1 Co-digestion of ABP mixture + sewage sludge

Original (R1) and hygienised feeds (R2) were mixed in the ratio of one part ABPs and seven parts sewage sludge (1:7 w.w.), corresponding the case in which the materials are produced in a middle-sized wastewater treatment and meat-processing plants in Finland. The feed ratio of R3 was 1:3, w.w. corresponding the optimum for similar materials reported in literature (sewage sludge with industrial food waste or slaughterhouse waste and/or municipal food waste; Rosenwinkel and Meyer, 1999; Murto et al., 2004, Sosnowski et al., 2008). The experiment lasted for 175 days and was divided into three different HRTs of 25 (days 0-43), 20 (days 44-126) and 14 days (days 127-175).

Table 13. *Semi-continuous co-digestion of ABP mixture + sewage sludge with HRTs of 25, 20 and 14 days at 35 °C. Feed for R1 and R2 (pre-hygienised) contained ABPs and sewage sludge in the ratio of 1:7 (w.w.) and for R3 in the ratio of 1:3 (w.w.).*

Reactor		**R1**			**R2**			**R3**	
Days	0-43	44-126	127-175	0-43	44-126	127-175	0-43	44-126	127-175
HRT (days)	25	20	14	25	20	14	25	20	14
OLR (kgVS/m^3 d)	1.8 ±0.1	2.4 ±0.1	3.3 ±0.3	2.1 ±0.1	2.8 ±0.2	3.7 ±0.4	2.2 ±0.1	2.9 ±0.2	4.0 ±0.2
Biogas (ml/d)	3600	5200	6400	3600	6300	7400	3800	6200	7600
CH_4 (ml/d)	2000	3300	4200	2200	4100	4800	2400	4000	5100
SMP (m^3CH_4/t VS)	340 ±30	400 ±30	380 ±20	370 ±40	430 ±40	400 ±20	340 ±30	410 ±30	390 ±30
SMP (m^3CH_4/t w.w.)	15 ±1.0	20 ±2.0	18 ±1.0	17 ±2.0	24 ±2.0	20 ±2.0	18 ±2.0	24 ±1.0	22 ±1.0
CH_4 (%)	56 ±3.0	63 ±1.0	65 ±4.0	59 ±4.0	65 ±1.0	64 ±4.0	62 ±2.0	65 ±1.0	67 ±4.0
VS removal (%)	61	38	40	60	37	33	65	36	37
TS (%)	2.9 ±0.6	5.1 ±0.2	4.6 ±0.5	3.9 ±0.9	5.8 ±0.1	5.6 ±0.5	4.0 ±0.9	5.7 ±0.3	5.3 ±0.6
VS (%)	1.7 ±0.3	3.0 ±0.1	2.7 ±0.3	2.4 ±0.6	3.4 ±0.1	3.4 ±0.3	1.9 ±0.4	3.7 ±0.2	3.5 ±0.4
VS/TS	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.7	0.7
COD_{sol} (g/l)	0.7 ±0.2	1.0 ±0.1	1.1 ±0.1	1.7 ±0.6	1.0 ±0.2	1.2 ±0.2	1.2 ±0.8	0.9 ±0.2	1.1 ±0.1
VFA (g/l)	0.2±0.1	0.2 ±0.1	< 0.1	0.9 ±0.4	< 0.1	< 0.1	0.5 ±0.2	< 0.1	< 0.1
LCFA (mg/l)	1.4 ±0.4	0.8 ±0.3	2.0 ±1.0	1.0 ±1.0	0.6 ±0.1	1.6 ±0.7	1.6 ±0.5	1.0 ±0.3	4 .0±4.0
NH_4-N (g/l)	1.0 ±0.1	1.30 ±0.1	1.4 ±0.1	1.1 ±0.2	1.5 ±0.1	1.6 ±0.1	1.1 ±0.1	1.4 ±0.1	1.4 ±0.1
Alkalinity (g	4.5 ±0.6	6.0 ±0.4	5.9 ±0.9	5.3 ±0.6	6.8 ±0.9	6.4 ±0.5	5.0 ±0.1	6.5 ±0.9	6.1 ±0.6
pH	7.4 - 7.5	7.4 - 7.5	7.4 - 7.5	7.4 - 7.5	7.4 - 7.5	7.4 - 7.5	7.4 - 7.5	7.4 - 7.5	7.4 - 7.5

The HRT of 25 days served as a start-up for the reactors, as defined by fluctuations in all reactor parameters, especially increasing SMP and high COD_{sol} and VFA contents especially during the first 23 days. SMP of R1, R2 and R3 was 340, 370 and 340 m^3 CH_4/tVS, respectively (Table 13). In the digestates, VFA varied between 0.2-0.9 g/l in all reactors, and of COD_{sol} it comprised of 21%, 50% and 42% in R1, R2 and R3, respectively. The VFA in the digestates were mostly acetic acid with the content of 90 ±5% of VFA in R2 and R3 and 50% in R1.

The HRT of 20 days was found to be the most suitable of the studied HRTs and the highest SMPs were achieved during days 73-126 (Fig. 5), when it was 400 in R1, 430 in R2 and 410 m^3 CH_4/tVS in R3 (Table 13). The daily methane yield from the R2 and R3 was by 23% higher than that from the R1. VFA increased or remained high during the first 2-3 weeks after lowering the HRT in all reactors (Fig. 5), but on days 73-126 for R2 and R3, total VFA was ≤0.1 g/l (Table 13). Simultaneously, acetic acid content of total VFA decreased from 90 ±5% to 50 ±3%. The VFA from COD_{sol} were 23% in R1, 10% in R2 and 12% in R3 (Table 13). Average LCFA content of the digestates were 0.6-1.0 mg/l and NH_4^+-N increased by 230-270% in all reactors (1.2-1.6 g /l; Table 13).

Decreasing the HRT to 14 d (day 126) decreased SMP of the reactors momentarily by 14% (days 131-140; Fig. 5). Since then, SMP was restored but remained lower than with HRT of 20 d (R1: 380, R2: 400, R3: 390 m^3 CH_4/tVS). VS removal of R1 remained at 40%, when the removals from R2 and R3 decreased to 34 ±1% from the original VS content (Table 13). VFA remained ≤0.09 g/l with acetic acid content of 48 ±2% in all reactors. VFA from COD_{sol} in the R1 was 8.8%, R2: 6.7% and R3: 7.6%. LCFA increased in the feeds by 150 ±20%, when compared to the previous HRTs due to new grease trap sludge collected. LCFA in the digestates increased to 0.8-1.6 mg /l (Table 13).

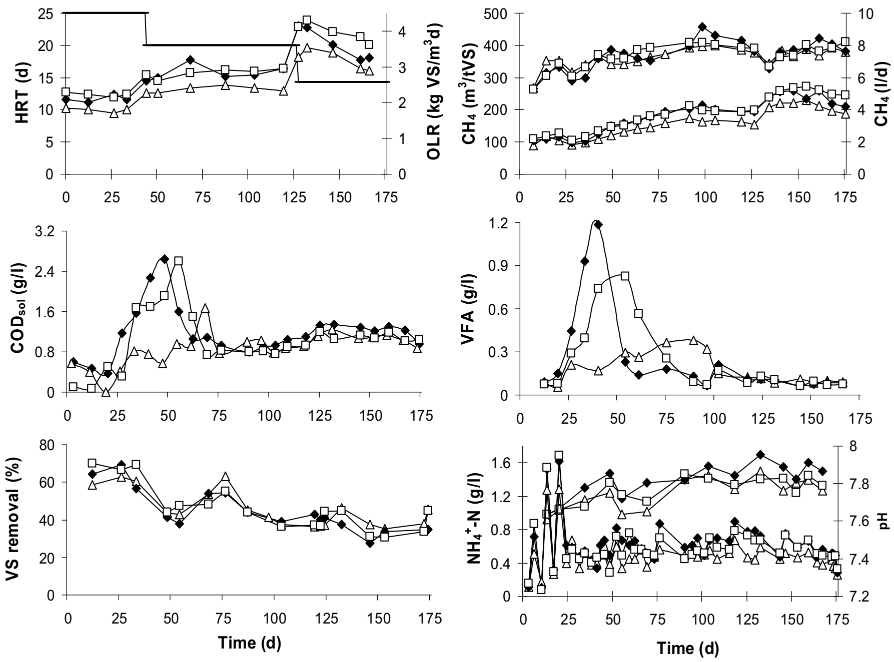

***Fig. 5**. HRT (□), OLR, SMP, daily methane yield, VS removal and the NH_4^+-N, COD_{sol}, VFA and pH in the digestates of R1 (Δ), pre-hygienised R2 (♦) and R3 (□) during the semi-continuous co-digestion of ABP mixture + sewage sludge at 35 °C. Feeds for R1 and R2 contained ABPs and sewage sludge in the ratio of 1:7 (w.w.) and for R3 in the ratio of 1:3 (w.w.).*

5.4.2 Co-digestion of ABP mixture + cattle slurry

ABPs and cattle slurry were mixed in a w.w. based ratio of 1:3 representing the optimal co-digestion ratio of by-products and sewage sludge from the literature (see 5.3.1). Also, the optimal ratio for co-digestion of energy crops and dairy cattle slurry has been reported to be similar (Lehtomäki, 2006). The feed was co-digested in semi-continuous reactors with HRT of 21 d and OLR varied between 2.9-3.3 kgVS/m^3 d (Fig. 6). Results are presented from day 22 onwards to disregard start-up variation.

Table 14. *Semi-continuous co-digestion of ABP mixture + cattle slurry (1:3 w.w.) with HRT of 21 days at 35 °C. On days 22-67 Es value of ultrasound pre-treatment was 6000 kJ/kg TS and* hygienisation *was performed separately to raw materials prior to mixing together and feeding. From day 68 onwards the Es was reduced to 1000 kJ/kg TS and raw materials were firstly mixed together and then pre-treated*

Reactor	**R1**		**R2**		**R3**	
Days	22-67	68-109	22-67	68-109	22-67	68-109
OLR (kgVS/m^3 d)	3.0 ±0.1	3.0 ±0.1	2.9 ±0.1	3.0 ±0.1	3.3 ±0.1	3.0 ±0.2
Biogas (ml/d)	3900	4400	4200	4500	4800	4800
CH_4 (ml/d)	2300	2700	2500	2800	2900	3000
SMP (m^3CH_4/t VS)	260 ±10	270 ±10	290 ±20	300 ±20	280 ±20	290 ±10
SMP (m^3CH_4/t w.w.)	16 ±1.0	17 ±1.0	17 ±1.0	18 ±1.0	20 ±2.0	19 ±1.0
CH_4 (%)	59 ±3.0	62 ±2.0	60 ±2.0	63 ±1.0	60 ±2v	63 ±2.0
VS removal (%)	29	32	31	31	32	31
TS (%)	5.6 ±0.2	5.7 ±0.3	5.4 ±0.1	5.6 ±0.1	6.2 ±0.3	6.2 ±0.2
VS (%)	4.2 ±0.2	4.3 ±0.2	4.1 ±0.1	4.3 ±0.1	4.8 ±0.2	4.8 ±0.2
VS/TS	0.8	0.8	0.8	0.8	0.8	0.8
COD_{sol} (g/l)	5.3 ±0.4	4.8 ±0.7	5.0 ±0.6	4.5 ±0.4	5.2 ±0.4	4.6 ±0.2
VFA (g/l)	0.5 ±0.1	0.1 ±0.1	0.4 ±0.1	0.3 ±0.1	0.6 ±0.2	0.2 ±0.1
LCFA (mg/l)	4.4 ±1.0	4.6 ±1.0	3.1 ±1.0	4.1 ±1.0	3.2 ±1.0	2.5 ±0.5
NH_4-N (g/l)	1.4 ±0.2	1.5 ±0.2	1.4 ±0.2	1.5 ±0.1	1.5 ±0.2	1.5 ±0.1
N_{sol} (g/l)	1.7 ±0.1	1.8 ±0.1	1.6 ±0.2	1.8 ±0.1	1.9 ±0.1	1.7 ±0.1
Alkalinity (g	8.0 ±0.5	8.9 ±0.1	8.0 ±0.4	8.6 ±0.2	8.5 ±0.5	8.9 ±0.2
pH	7.6- 7.7	7.6-7.9	7.5-7.6	7.6-7.8	7.5-7.6	7.7-7.8
Enterococci (CFU/g)	1000	1900	1200	1800	200	<10
Clostridia (CFU/g)	44 000	13 000	27 000	13 000	21 000	1700

R1 treated the feed mixture as such, while the feed for R2 was ultrasound pre-treated with 6000 kJ/kg TS (days 22-67) and the feed for R3 hygienised separately prior to mixing together and feeding (days 22-67). The average SMP of R1 was then 260, of R2 290 and of R3 280 m^3 CH_4/t VS_{added} (Table 14). The remaining VFA content in reactors varied from 0.4 to 0.6 g VFA/l (Table 14) comprising 10% of COD_{sol}, while VFA from COD_{sol} of feeds was 40 ±1.7 during the whole experiments. NH_4^+-N accounted for 77-85% of the N_{sol}, while the proportion of NH_3-N from NH_4^+-N was around 5% (Eq. 5).

On day 68, the Es of ultrasound was reduced to 1000 kJ/kg TS and ABP mixture + cattle slurry were co- hygienised. In addition, the characteristics of the untreated feed changed (Table 14). The average SMP was 270 in R1, 300 in R2 and 290 m^3 CH_4/t VS_{added} in R3. The VFA concentration was low (0.1–0.3 g/l, Table 14) comprising only 2%, 6% and 5% of $COD_{sol,}$ respectively. NH_4^+-N was responsible for 83-87% of N_{sol}, while NH_3-N from NH_4^+-N varied between 5-8% (Eq. 5). During the entire experiment the LRC_{sol} remained at 2.7-2.9 g/l in all reactors and acetic acid was 95% in R1 and 100% in R2 and R3 of VFA.

Pathogen content of feeds and reactors was evaluated. No salmonella were detected in any of the treated or untreated samples of 25 g. AD reduced the number of enterococci; but if omitted, in the cases when the feed had been hygienised, the geometric means of enterococci in feed medium were 15 000 CFU/g but it was only 1 400 CFU/g in the digestate. This difference was statistically significant according to the paired t-test ($p=0.023$). The geometric means of clostridia were 4 900 CFU/g and 14 000 CFU/g in feed and digestate with respect to all treatments, and 23 000 CFU/g and 21 000 CFU/g if the prior hygienising process was omitted but none of these differences were statistically significant (Table 14).

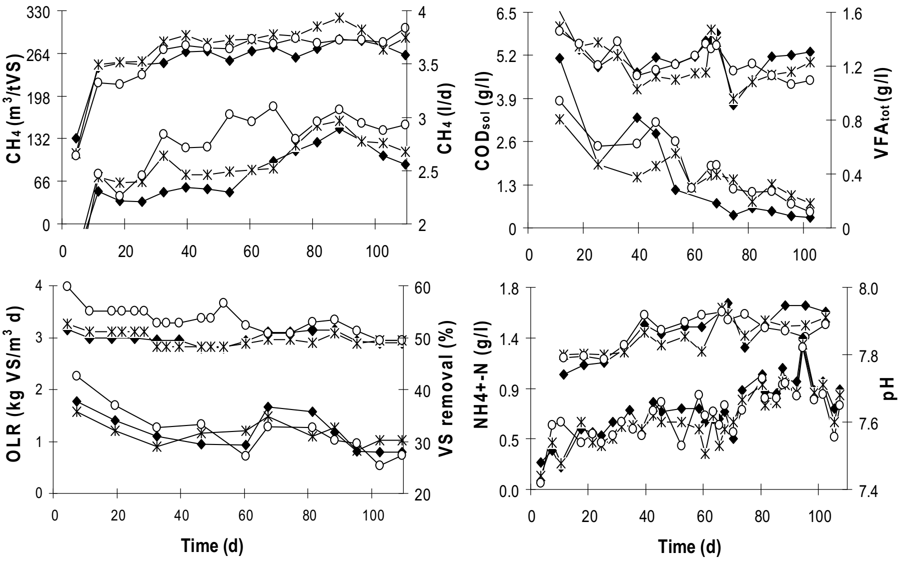

Fig. 6. *SMP, daily methane production, OLR, VS removal and the NH_4^+-N, COD_{sol}, VFA and pH in the digestates of untreated (R1; ◊), ultrasound pre-treated (R2; ■) and hygienised feeds (R3; ▲) during the semi-continuous co-digestion of ABP mixture + cattle slurry (1:3, w.w.). The feed for R2 were ultrasound pre-treated and R3 were hygienised.*

6 Discussion

The case of this research was to study the utilisation of ABP from a middle-sized Finnish meat–processing industry in the anaerobic digestion. The feasibility of different ABPs (Paper I) as raw material for anaerobic digestion and the possibility of improving the overall process and the methane production with the use of pre-treatments (Paper I-V) and/or with co-digestion with sewage sludge (Paper III) and cattle slurry (Paper IV, V) are discussed. More detailed discussion from the subjects can be found from the Papers I-V.

6.1 SUITABILITY OF ABPS FOR ANAEROBIC DIGESTION AND EFFECT OF PRE-TREATMENTS

As the digestion process has to be optimised according to case- and material-specific factors, research on different raw materials has to be performed separately. The ABPs studied (digestive tract content, drumsieve waste, DAF sludge and grease trap sludge) were highly bio-degradable (VS removal 73-93%) and suitable for anaerobic digestion with BMPs between 230-900 m^3 CH_4/t VS_{added}. All the pre-treatments studied (hygienisation, ultrasound, base, acid, bacterial product) hydrolysed the ABPs (defined as increased hydrolysis parameters by 18-1300%, especially COD_{sol}/VS ratios 70-920%), while only few pre-treatments (except with DAF sludge) managed to increase BMP, when compared to untreated material (see 6.3.2).

Bacterial product as a pre-treatment method was the most effective in hydrolysing digestive tract content (COD_{sol}/VS ratio 70%) and drumsieve waste (> 900%), probably due to the facultative bacteria *Bacillius licheniformi*, available in the product and capable of producing protease and amylase which hydrolysed proteins and carbohydrates to VFA (64-160%). Also, base (NaOH) addition increased the hydrolysis parameters of plant-based digestive tract content (COD_{sol}/VS ratio 67%) and drumsieve waste (COD_{sol}/VS ratio 810%) notably and accordingly, it has been reported to hydrolyse carbohydrates efficiently (Karlsson, 1990). With drumsieve waste, the overall hydrolysis was notably high (e.g. COD_{sol} content increased from 0.9 g/l up to 9 g/l).

Bacterial product addition and ultrasound treatment increased hydrolysis parameters of DAF sludge the most, while ultrasound and hygienisation increased COD_{sol}/VS ratio (98-120%) the most with grease trap sludge. Simultaneously, VFA content decreased, indicating that only high amounts of LCFA were released, as observed in subsequent experiments (Paper II). N_{sol} content decreased during ultrasound treatments, probably due to increased reactions between N_{sol} compounds and lipids/LCFA (Sayed et al., 1988; Xu et al., 2005; see 6.2; 6.2.1).

Despite the positive effect of chemical treatments to the hydrolysis of digestive tract content (and to the BMP of DAF and grease trap sludge (see 6.3.2)), these pre-treatments were not general as effective as ultrasound and bacterial product addition and were excluded from further experiments. In practice, chemical treatments are load for the environment, complicated to perform and thus rather expensive. Moreover, they may cause production of molecular nitrogen (N_2) and unwanted loss of valuable NH_4^+-N. In the present studies, base addition decreased the concentration of NH_4^+-N, apparently due to evaporation as NH_3 at the high pH (van Velsen et al., 1979, also noticed with septic tank sludge; Lin and Lee, 2002). Also, acid treatment (HCl) may remove NH_4^+ via salt formation as chloride amines (ammonium chloride; NH_4Cl with nitric acid, ammonium nitrate; Robinson et al., 1996). The base addition is also known to react with glycerol and LCFA and form unboiled soap, which in case of grease trap sludge may explain the present decrease of hydrolysis.

6.2 PRE-TREATMENT OF ABPS AND FEED MIXTURES

The most efficient hydrolysing pre-treatments, ultrasound and bacterial product (Paper I), were screened for optimal treatment modes (Paper II). Ultrasound (Paper IV, V) and hygienisation (Paper III-V) were then chosen for semi-continuous digestion experiments (see 6.3) due to the noted hydrolysing efficiency of ultrasound and the legislative requirements for hygienisation for many cases of ABP digestion (Paper III-V).

The most characterising feature in the screening experiments of ABP (Paper II) and ABP mixture + cattle slurry (Paper IV) pre-treatments was that hydrolysis did not increase linearly with increasing ultrasound Es or treatment time with bacterial product. This may be due to the release of flocculating agents (i.e. lignin related compounds with their various functional groups; Larrea et al., 1989; Stewart, 2008; Renault et al., 2009; Zahedifar et al., 2002) from the cellulose-structures of the plant-cells, present in digestive tract content, drumsieve waste and cow slurry. The noted changes in APS and LRC_{sol} correlated reciprocally with coefficient of 0.98, promoting re-flocculation and shown as increase in APS and reduction in the concentration of LRC_{sol}. Rumen-lignin is reported to bound effectively to the proteins (Zahedifar et al., 2002), while carbohydrates and amino acids may form recalcitrant structures via Maillard reactions (Bougrier et al., 2008). Flocculation with proteins aptitude for further adsorptions with lipids, carbon hydrates and water (Sayed et al., 1988; Rinzema et al., 1994; Xu et al., 2005; Dewil et al., 2006), may also contribute to re-flocculation, but also to low content of soluble compounds in the raw materials, which, in turn, are mostly rather easily hydrolysed (see 6.2.1).

When the flocculation mentioned was assumed to occur (Paper II), the size of the smallest particles in PSD (16-50% of particles) increased by 35 ±5%, while the larger particles in PSD (16% of particles) remained at their original sizes or decreased only a little (7 ±2%), when compared to the same untreated materials. PSD indicates that the volume occupied by the smallest half of the particles (50% of particles) increased the total APS. Thus, it is possible that the smaller particles released by the pre-treatments re-bonded, adsorbed or reacted with each other, while certain larger particles were quite inert to the screened pre-treatments.

Though flocculation is an unexpected reaction which may decrease the subsequent methane production, re-flocculated structures may also be easier for micro-organisms to degrade, than the original structures (Chu et al., 2002). Thus, despite the supposed flocculation (Paper III-V), ultrasound and hygienisation pre-treatments improved methane production in the present batch and semi-continuous experiments and most likely achieved to rupture the usually poorly degraded (Mata-Alvarez. 2003) lignin bound structures of cellulose as also reported by Hartmann et al. (2000) and Myint et al. (2007). This is further supported by the high LRC_{sol} after the pre-treatments, when compared to the original materials. Nonetheless, the pre-treatments which weakened the hydrolysis of separate ABP fractions (Paper I) produced less methane and contained higher APS in digestate, than the untreated fractions. This indicates that the supposed re-flocculation of cellulose-rich ABPs (e.g. digestive tract content) may have also eliminated the further hydrolysis of material by the hydrolysing bacteria.

All-in-all, the pre-treatments used in the screening and digestion experiments (ultrasound, hygienisation and bacterial product) ruptured the original structure of ABPs (Paper I, II) and feed materials (Paper III-V) by decreasing the APS and releasing material to the soluble phase with simultaneous pH decrease as a sign of acid formation. These observations together with the increased methane production are reported to be indicators of a successful pre-treatment (Gavala et al., 2003; Kim et al., 2003; Climet et al., 2007; Fernandes et al., 2009).

6.2.1 Ultrasound optimisation and use for semi-continuous digestion

The optimal Es input for ultrasound treatments was found to be 6000-8500 kJ/kg TS with all the materials studied (ABPs: Paper II, cattle slurry: Paper V, ABP mixture + cattle slurry: Paper IV). The higher Es inputs applied decreased hydrolysis (except with DAF sludge), whereas with waste-activated sludge, hydrolysis has been reported to increase linearly and slow down only when Es > 10 000 kJ/kg TS (Bougrier et al., 2005). This reduction of hydrolysis with the higher Es inputs characterised the optimisation experiments and may partly be due to the higher TS content of the ABPs (7.9-17%) as compared to the ultrasound pre-treatments of different sludges from literature (TS 1.5-5.5 %; Bougrier et al., 2006b; ; Khanal et al., 2006; Nickel and Neis, 2007). This high TS content most likely prolonged treatment time and allowed for natural VFA degradation with subsequent decrease in COD_{sol} (Mendes et al., 2006). The reduction in hydrolysis with Es inputs > 8500 kJ/kg TS was presently also noticed with cattle slurry and ABP mixture + cattle slurry, though the TS content of these materials (5.9-7.6%) was similar to DAF sludge (4.3-7.9%), the hydrolysis of which was increased also by the highest Es inputs applied. This suggests that reduction in hydrolysis with the higher Es inputs may also depend on the different response of various materials.

Depending on the compounds, higher Es inputs (i.e. longer durations) increase opportunities to eliminate volatile compounds via evaporation (Vedrenne et al., 2008), via more reactions with the released molecules and formed intermediates (e.g. flocculation agents; Larrea et al., 1989; Bougier et al., 2005, 2008, sonication radicals; Wang et al., 2008 or Maillard reactions; Bougrier et al., 2008) and/or via pyrolysis (volatile and hydrophobic compounds) inside cavitation bubbles (Wang et al., 2008). However, due to the low ultrasound frequencies (24-30 kHz), formation of radicals was presently most likely low (Tiehm et al., 2001; Laurent et al., 2009) and no significant shifts in the nature of chemical groups occurred (Laurent et al., 2009).

In addition, at least the reduction of N_{sol} content (which occurred with the higher Es inputs), did not took place via evaporation (NH_3, N_2, N_2O) or pyrolysis, as reported by Wang et al. (2008), because N_{tot} content in ABPs remained relatively constant through the optimisation experiments. Thus, N_{sol} may have bound back to solids (Sayed et al., 1988; Zahedifar et al., 2002; Xu et al., 2005; Dewil et al., 2006; Bougrier et al., 2008).

The highest hydrolysis in grease trap sludge was achieved with Es of 8500 kJ/kg TS which also transferred the mixture of separate grease particles and water into a colloidal form. This may be due to inclusive lysis of the grease cells followed by enhanced hydrolysis of intracellular materials (i.e. LCFA and N_{sol}). The insoluble colloidal structure formed may bind liquids (Robinson et al., 1996) and molecules effectively (Sayed et al, 1988), which may also explain the present COD_{sol} decrease as compared to the Es of 6000 kJ/kg TS. This phenomenon may also clarify the observed re-flocculation of other grease cells content materials (DAF sludge, ABP mixture + ABP mixture + cattle slurry) with the Es inputs > 8500 kJ/kg TS.

The ultrasound optimisation via screening experiment revealed the relatively high hydrolysis of the low Es of 1000 kJ/kg TS. It has been previously reported adequate for degrading sludge flocs (Bougrier et al., 2005), and this phenomenon most likely also explains the presently reduced APSs of the pre-treated materials (Paper II). The Es of 1000 kJ/kg TS was also probably sufficient for hydrolysing weakly bound hydrophobic lipids and free protein and carbon hydrate molecules attaching easily on surface (adsorption) and between of flocs (absorption; Sayed et al., 1988; Rinzema et al., 1994; Cammarota and Freire, 2006; Dewil et al., 2006).

Cattle slurry (Paper V) and ABP mixture + cattle slurry (1:3; Paper IV) were hydrolysed the most using 6000 kJ/kg TS (hydrolysis parameters increased by 7.5-31%), but as high hydrolysis as in the optimisation experiments (27-130%; Paper

II) was not achieved. This may be due to the differences in the initial state of the materials, as e.g. VFA from COD_{sol} of the untreated the slurry varied from 29% to 41% (Papers IV and V). These changes in quality and/or content are usual in slurry and occur generally due to the changes in the seasonal storage conditions, diets of the cows and/or variations in sampling (Hindrichen et al., 2006).

These changes in the content of raw slurry probably also affected the hydrolysis of ABP mixture + cattle slurry during the semi-continuous experiment, as when Es input was declined from 6000 to 1000 kJ/kg TS, the hydrolysis parameters were increased from 8-23% to 17-33%. Moreover, Es of 6000 kJ/kg TS may have released flocculation agents, re-binding the previously solubilised material. Es input of 1000 kJ/kg TS increased especially the LRC_{sol}/VS and NH_4^+-N/VS (32 ±1%) ratios, when compared to those of 6000 kJ/kg TS (9.5 ±0.5%). Reduction in LRC_{sol} was earlier reported to correlate with the growing APS (see 6.2) and lignin compounds are known to react easily with N_{sol} compounds (Zahedifar et al., 2002), thus explaining the increase in these particular parameters. Accordingly, low Es inputs may provide a promising alternative to pre-treatment of easily flocculating material with high content of adsorbed molecules. Low ultrasound Es inputs have also been reported to assist the further efficiency of hydrolysing enzymes excreted by microorganisms (Chu et al., 2002).

In general, combined ultrasound pre-treatments of different raw materials, such as ABP mixture + cattle slurry (1:3, w.w.), intensified and stabilised the hydrolysis making the pre-treatment more easily controllable when compared to many of the separate ABP fractions. This may be due to the surrounding liquid matrix in the mixtures, into which the released compounds are dissolved instead of evaporating and/or directly re-flocculating with the solids.

6.2.2 Optimisation of bacterial product addition

The addition of bacterial product as a pre-treatment method was studied only with the separate ABP materials and their mixture (Paper I, II). It did not increase hydrolysis progressively with the increasing treatment time, and all the fractions had more than one optimal treatment time, depending on the observed hydrolysis parameters. Unlike in flocculation (indicated by the increase in APS), this variation in hydrolysis was probably because of heterogeneous bacteria populations (in different activation stages) consumed part of the dissolved compounds in their own growth. Moreover, the changing pH (due to the release of NH_3 or organic acids), proteolysis of bacteria and the change in the C/N ratio (indicated by the variation of 24% in COD_{sol}/N_{sol} relation, when compared to the similar ratios of untreated materials), may have affected the supplementation of the hydrolysing bacteria (Gombert et al., 1999).

Hydrolysis of digestive tract content + drum sieve waste started presently in three hours, though the hydrolysis of cellulose is reported to activate within 12 hours after the inoculation (Zhen-Hu et al., 2004). Fast activation may be supported by the previous ruminal hydrolysis of the digestive tract content and possible re-activation of cellulolytic rumen bacteria after re-exposing them to favorable anaerobic conditions. Moreover, the pH (7.1) of the treated materials was optimal for the highly sensitive cellulolytic bacteria (optimal pH at 6.9-7.3; Zhen-Hu et al., 2004, 2005). The bacterial product hydrolysed the smallest particles of digestive tract content and drumsieve waste effectively, while the larger particles were less accessible to enzymes, possibly due to tight lignin bond structures (Zhen-Hu et al., 2005). The larger cellulose particles were most likely inert to anaerobic degradation or their hydrolysis takes too long a period to be noticed within the treatment times presently applied.

The VS content in DAF sludge was momentarily reduced during the first three hours of treatment increasing the hydrolysis parameters. This reduction may be due to fast growth of the bacteria utilising the organic material to the exponential growth until the consumed substrate was covered with the increasing bacteria mass (Cirne et al., 2007). DAF sludge was hydrolysed the most during 3-6 hours, while the enzymatic treatment of 8-12 hours is reported to be the most suitable for hydrolysis of dairy and slaughterhouse wastewaters (Cammarota and Freire, 2006; Mendes et al., 2006).

Hydrolysis of the ABP mixture was notably lower with the shortest treatment times (3-6 h), when compared to the same treatment times with the separate ABPs. This may be because of the more heterogeneous content of the ABP mixture, generating a growth-limiting competition among the various prolific bacterial consortiums. This may be the case e.g. with lipid (LCFA/VS) and protein hydrolysis (N_{sol}/VS) which achieved the highest hydrolysis only in 48 hours, though protease synthesis should be the most active after the 20 hours (Gombert et al., 1999). The slow lipid hydrolysis could also be explained by inhibition of lipase production by amylase (Gombert et al., 1999), which is excreted by cellulolytic bacteria with the rather fast inoculation time of 12 hours (Zhen-Hu et al., 2005). Also the slower-rate protein hydrolysis may increase the pH as the result of deamination, which then restricts the lipase production (Gombert et al., 1999). Still, the mixture of different raw materials are usually well-buffered due to the high content of nitrogen compounds and organic acids maintaining the pH (6.0) more optimal for the lipolytic and proteolytic activity (5.0-6.0; Gombert et al., 1999) than for the cellolytic bacteria (6.8-7.3; Zhen-Hu et al., 2004).

Generally, the highest COD_{sol} releases from digestive tract content and drumsieve waste (130%), DAF sludge (71%) and the ABP mixture (29%) achieved in the optimization study are higher, when compared to the un-optimised (24 hours) bacterial product additions (35-62%; Paper I). COD_{sol} releases are also comparable to or higher than the reported hydrolysis to COD_{sol} by pure enzymes (Lipase G-1000; Pancreatic lipase PL 250) in the previous 4-24 hour studies on lipid-rich slaughterhouse wastewaters (6-40%; Massé et al., 2001; Mendes et al., 2006).

6.2.3 Hygienisation pre-treatments for semi-continuous digestion

Hygienisation pre-treatment was used in all the semi-continuous digestion experiments (Paper III-V) due to 1) hygienisation requirement for many ABPs in the EU ABP regulation, 2) its known potential to improve methane production and quality of digestate and 3) known energy-efficiency of the treatment in conjunction to biogas plant (utilisation of the produced heat, energy savings via heat exchangers).

APS in the hygienised batches containing cellulose-rich digestive tract content and drumsieve waste was notably higher when compared to the batches digesting untreated ABP fractions (Paper I). This may indicate inhibition via intermediates released by hygienisation and/or re-flocculation e.g. via heat stimulated Maillard reactions, in which carbohydrates react with amino acids and produce recalcitrant compounds for anaerobic digestion (Martins et al., 2001; Ajandouz et al., 2008; Bougrier et al., 2008; Dwyer et al., 2008). Hydrolysis of digestive tract content and drumsieve waste could not be monitored due to high evaporation of water (Paper I). Absence of carbohydrates may also explain the high APS reduction in pre-hygienised grease trap sludge digestate during the similar study, when compared to the digestates of other hygienised ABPs.

Pre-hygienisation of ABP mixture + cattle slurry separately prior to the mixing them together for feed preparation resulted in a higher TS of the ABP mixture. It also achieved a lower hydrolysis, than hygienisation of the combined fractions, probably due to the increased evaporation of volatile compounds (e.g. VFA, NH_3). Accordingly, when the pre-hygienisation was performed as a ready-made mixture, the hydrolysis parameters increased (from 5-27% to 35-100%) and NH_4^+-N content in N_{sol} decreased (from 80% to 50%) as compared to their separate hygienisation of ABP mixture + cattle slurry. The compounds were apparently dissolved into the slurry instead of possible evaporation or re-binding to solids. Moreover, the heat is spread more evenly into the liquid matrix. This may prevent regional high temperature peaks (> 140 °C) which enhance flocculation via Maillard reactions (Bochmann et al., 2010). Thus, hygienisation pre-treatment was more suitable for hydrolysis of cattle slurry alone and the ready-made mixture of APBs and cattle slurry.

Along with hydrolysis, hygienisation affected its quintessential purpose: to destroy pathogens. Hygienisation treatment alone decreased the number of enterococci to the acceptable level set in the ABP regulation (< 1000 CFU/g), while the reduction in *Clostridium* bacteria was less (1:400) than recommended (< 1:1000; 1774/2002/EC). *Clostridium* bacteria are capable of forming heat-resistant spores and thus survive during hygienisation (Sahlström, 2002).

6.3 CO-DIGESTION OF ABP MIXTURE + SEWAGE SLUDGE OR CATTLE SLURRY WITH AND WITHOUT PRE-TREATMENTS

Methane production started immediately in the batches with digestive tract content, drumsieve waste and DAF sludge. However, methane production from grease trap sludge started only after nearly 17 days, most likely due to high amount of intermediates (LCFA and VFA), as also noticed with other lipid-rich materials (Salminen et al., 2000; Vidal et al., 2000; Cirne et al., 2007; Climent et al., 2007). Raw materials such as grease trap sludge are most likely difficult, if not impossible to digest alone, but are highly suitable for co-digestion purposes (Luostarinen et al., 2009).

6.3.1 Effect of co-digestion

ABP mixture + sewage sludge were co-digested semi-continuously at 35 °C with the HRTs of 25, 20 and 14 days and in two different feed ratios (1:7 and 1:3 w.w.; Paper III). The starting HRT of 25 days was used to give the inoculum time to adapt to the feed material. The HRT of 14 days, in turn, increased the OLR to 3.3-4.0 kgVS/m^3 d and resulted in decreased methane production. Apparently, the OLR was too high or the process would have needed a longer period to adapt to it. The highest SMPs and steadiest quality of the digestates were achieved with the HRT of 20 days and these results will be discussed in more detail here. The results will also be compared to the second semi-continuous co-digestion experiment with APB mixture + cattle slurry (1:3 w.w., 35 °C, HRT 21 days).

Co-digestion of ABP mixture both with sewage sludge (Paper III) and with cattle slurry (Paper IV) resulted in significantly higher methane production (SMP 400 and 410 m^3 CH_4/tVS_{added}, respectively) than digesting sewage sludge (BMP 220-270 m^3 CH_4/t VS_{added}; Rintala and Järvinen, 1996; Ferrer et al., 2008; Luostarinen et al., 2009; Salsabil et al., 2009) or slurry (130-240 m^3 CH_4/tVS_{added}; Angelidaki and Ahring, 2000; Ahring et al., 2001; Møller et al., 2004; Nielsen et al., 2004; Amon et al., 2006; Mladenovska et al., 2006; Lehtomäki et al., 2007a) alone. Moreover, in the present batch experiment, the BMP of ABP mixture + cattle slurry was 300 m^3 CH_4/tVS_{added} and thus 31% higher than the BMP of cattle slurry alone (230 m^3 CH_4/tVS_{added}).

SMP of ABP mixture + sewage sludge (380-430 m^3 CH_4/tVS) and of ABP mixture + cattle slurry (260-270 m^3 CH_4/tVS) were comparable to the previously reported co-digestions (Table 1) with optimised feed ratios, such as digestive tract content/flotation tailings and sewage sludge (1:3 w.w.; 280-480 m^3 CH_4/tVS; Rosenwinkel and Meyer, 1999), slaughterhouse rejects, fruit and vegetable wastes and manure (1:3 w.w.; 270–350 m^3 CH_4/tVS; Alvarez and Liden, 2008) or municipal bio-waste and cow manure (1:4 w.w.; 210-250 m^3 CH_4/tVS; Paavola et al., 2006). This indicates that the materials studied presently and the feed ratios chosen were suitable for co-digestion.

SMA of inoculum when digesting ABP mixture + cattle slurry (64 m^3 CH_4/tVS_{added} d) was notably higher than SMAs of cattle slurry alone (24 m^3 CH_4/tVS_{added} d, Paper V) and of separate ABP fractions (13-25 m^3 CH_4/tVS_{added} d, Paper I), except for grease trap sludge (60 m^3 CH_4/tVS_{added} d, Paper I). Nonetheless, grease trap sludge had a significant lag phase, while ABP mixture + cattle slurry started to produce methane effectively approximately in 2-3 days. Higher SMA of inocolum enables shorter HRT and/or higher OLR which increases methane production and enables higher treatment capacity and/or smaller reactor sizes. SMA for the digested sewage sludge (collected from the same wastewater treatment plant) is reported to be relatively high (51 m^3 CH_4/tVS d; Luostarinen et al., 2009) and it is thus presumable that co-digestion with ABP mixture may have not enhanced the SMA as notably as with the cattle slurry. Still, in continuous digestion, the microbial consortium develops into a group especially suitable for that particular feed.

The present VS removal potential (i.e. VS removal from batches) of cattle slurry (27%; when inocolum is subtracted), was slightly lower than reported in literature (32-44%; Møller et al., 2004), which is notably lower when compared to the reported VS removal potential of sewage sludge 54 ±3% (Luostarinen et al., 2009). VS removal potential from the batches digesting ABP mixture + cattle slurry (64%) increased by 41%, when compared to that of cattle slurry alone (27%). This is because slurry has already passed through an digestive tract and most of the energy content has been utilised, and the remaining solids are recalcitrant cellulose (Lehtomäki et al., 2007a) and lignin acts as a glue between the polysaccharide filaments (Myint et al., 2007).

In the semi-continuous co-digestion experiments, VS removal from ABP mixture + cattle slurry was 31 ±1% (HRT 21 days) and from ABP mixture + sewage sludge it was 38 ±0.5% (HRT 20 days). In previous semi-continuous reactor studies (HRT 20 days) from literature sewage sludge removal is reported to be 27-40% (Bougrier et al., 2006a; Lu et al., 2008; Braguglia et al., 2010) and cattle slurry 20-26% (Lehtomäki et al., 2007a).

The optimal co-digestion ratio of ABP mixture + sewage sludge reported in literature is 1:3 w.w., Rosenwinkel and Meyer, 1999; Murto et al., 2004; Sosnowski et al., 2008). This did not substantially increase SMP per added VS (+3%), when compared to the other feed ratio applied (1:7 w.w., chosen as a case of middle-sized wastewater treatment plant and meat-production plant in Finland). However, SMP per w.w. added was increased by 20%, daily methane yield by 21% and N_{sol} content in digestate by 8.0%. The optimal feed ratio (1:3, w.w.) also resulted in a lower COD_{sol} (-11%) and VFA (-50%) in the digestate than using the feed ratio of 1:7, w.w. Correspondingly the co-digestion of ABP mixture + cattle slurry in the ratio of 1:3 (w.w.) produced less soluble compounds than digestion of cattle slurry alone. All this may result from higher hydrolysis in feed mixtures due to synergistic effects of the different raw materials (Mata-Alvarez 2003). High hydrolysis and suitable TS content reportedly enhances the contact between micro-organisms and molecules (Mata-Alvarez, 2003). Moreover, higher activity of the soluble material utilising bacteria may achieve subsequently higher SMP supporting the further hydrolysis (Palenzuela-Rollon, 1999; Miron et al., 2000).

These synergistic effects of co-digestion can be noted, when methane production calculated from the BMP of each fraction and its proportion in the feed mixtures is compared to the BMPs achieved from the co-digestion studies. E.g. BMP from co-digestion of ABP mixture + cattle slurry (1:3 w.w., 300 m^3 CH_4/tVS_{added}) was by 9.5% higher, when compared to the calculated potential of the separate fractions (270 m^3

CH_4/tVS_{added}). Also, SMP from co-digestion of ABP mixture + sewage sludge (1:7, 1:3 w.w., 400, 410 m^3 CH_4/tVS_{added}) in the continuous reactor studies (HRT of 20 days) was by 37 ±10% higher, when compared to calculated BMPs of separate fractions (270 - 310 m^3 CH_4/tVS_{added}; The BMP of sewage sludge 220-270 m^3 CH_4/tVS_{added} was achieved from the literature; Rintala and Järvinen, 1996; Ferrer et al., 2008; Luostarinen et al., 2009; Salsabil et al., 2009).

Co-digestion of ABP mixture + sewage sludge resulted in higher methane production (21-60%) and VS removal (15-21%), when compared to the co-digestion of ABP mixture + cattle slurry. However, alkalinity of slurry based feeds was significantly higher (97%), making a slurry based process most likely more robust towards changes in OLR or the characteristics of the raw materials. Moreover, higher content of nutrients (i.e. NH_4^+-N) and presumably lower content of harmful contaminants (e.g. heavy metals, pharmaceutical residues) makes slurry-based digestates more acceptable e.g. as fertiliser, when compared to sewage sludge based digestates.

6.3.2 Effect of pre-treatments on anaerobic digestion of ABPs

Nearly all the pre-treatments hydrolysed the separate ABPs (see 6.1; Paper I), but only few of them (acid, base, hygienisation) achieved the ultimate goal, to increase the methane production potential of some ABPs. The hydrolysis of these pre-treatments was less effective than those of ultrasound and bacterial product, which eventually decreased the BMP, when compared to the untreated ABPs. This was most likely because of high content of intermediate products inhibiting the digestion or release of flocculating agents. If all the pre-treatments applied with BMP measurements had been tested with semi-continuous digestion as well, the results may have been different due to e.g. longer adaptation of the microbial consortium to the feed.

All the pre-treatments studied decreased the BMP (-27 ±8%) of digestive tract content (originally 400 m^3 CH_4/tVS_{added}; Paper I). This is in agreement with the results of Angelidaki and Ahring (2000) and Lehtomäki et al. (2004), who pre-treated carbohydrate-rich materials (manure and energy crops, respectively) and gained little if any increase in methane production. This might be due to reduced VS during the pre-treatments and/or re-flocculation of cellulose after the pre-treatments, shrinking the surface area available for hydrolytic bacteria (Fan et al., 1981). Increased APS in the pre-treated digestates, when compared to the untreated digestates (+24 ±6%; excl. bacterial product: -58%) supports the possible re-flocculation of material.

Another possibility for the low BMP of the pre-treated digestive tract content is the high concentration of VFA after the pre-treatments (3.5-5.7 g/l). Climet et al. (2007) reported 3.9–4.9 g VFA/l to inhibit methane production in thermally treated (70 °C) sewage sludge, while Kalle and Menon (1984) reported methane production of acetate to decrease by 52% when VFA concentration is > 2.2 g/l. Based on these results, the high VFA content may have been the reason for the present lower BMP compared to untreated digestive tract content.

The BMP of drumsieve waste (230 m^3 CH_4/tVS_{added}) was improved after hygienisation (+48%). The SMA of inoculum was increased with all the studied pre-treatments (excl. bacterial product) and this probably supported the decreased duration of methane production phase, when compared to digestive tract content and grease trap sludge. The weak increase in BMP may be due to highly improved solubilisation (e.g. COD_{sol} increased from 0.9 to 4.3-9.0 g/l) and subsequent excess amount of soluble organic material, including recalcitrant intermediates as BMP presented a weak increase (Salminen et al., 2000; Lafitte-Trouqué and Forster, 2002; Cirne et al., 2007; Mendes et al., 2006).

The BMP of DAF sludge (340 m^3 CH_4/tVS$_{added}$) and the SMA of inoculum during its digestion improved (4-15%) with all pre-treatments studied. Though chemical treatments did not improve hydrolysis significantly, they gave the highest increase in BMP. Instead of direct solubilisation, the chemicals may have degraded the morphological structures, helping accessibility of hydrolytic enzymes during subsequent digestion (Sanders, 2000). Massé et al. (2001) reported that alkaline addition into slaughterhouse wastewater reduced especially the particles above size 500 μm with simultaneous APS reduction of 73 ±7% from the initial APS. In the present study, the APS in all pre-treated digestates of DAF sludge were notably higher (130-310%), when compared to the digestate of the untreated DAF sludge. The certain cellulose-based larger particles, which were monitored to be inert for ultrasound pre-treatment and bacterial product addition (Paper II), were probably also inert for anaerobic degradation (Mata-Alvarez, 2003). This could have increased the APS in digestate and support the lower VS removal (62-79%) noticed, when compared to the other ABPs studied. Bacterial product addition as a pre-treatment method and ultrasound pre-treatments may have also inhibited methane production due to effective hydrolysis and high release of intermediates resulting in inhibitive concentrations.

Hygienisation shortened the lag phase of grease trap sludge by three days, whereas ultrasound pre-treatment prolonged it by eight days as compared to untreated material (lag phase 9 days). This may be due to the different amounts of hydrolysed LCFA (Cirne et al., 2007). Moreover, hygienisation of feed mixtures (Paper III, IV) seemed to disintegrate the materials more than result in actual solubilisation (see 6.3.3).

As with DAF sludge, chemical additions decreased (base) or slightly increased (acid) the hydrolysis of grease trap sludge and still generated the highest BMPs (900 and 1010 m^3 CH_4/tVS$_{added}$, respectively), while the most effective hydrolysis after ultrasound and bacterial product addition decreased it. The LCFAs (oleic and palmitic acids) monitored during ultrasound and bacterial product pre-treatments may resolve the decrease in BMP (Paper II). It increased from 0.01 g/l up to 2.9 g/l (0.8 g oleic acid/l), and the total LCFA concentration of 4.0-6.3 g/l (Hwu et al., 1998; Pereira et al., 2005) and oleic acid content of 0.03-0.3 g/l (Broughton et al., 1998; Alves et al., 2001; Lalman and Bagley, 2001) are reported inhibitive.

The correlation between BMP from ABPs and SMA of inoculum was 0.92, indicating interconnection between the two, but also variation. The highest increases in hydrolysis parameters of ABPs caused reduction in the SMAs of inoculum, which may be due to the inhibition resulting from excess amount of released intermediates (VFA, LCFA). Thus, the changes in the SMA may represent the release of readily degradable material, while changes in the BMP may represent the hydrolysis (e.g. effect of re-flocculation) and the final degradable content of the substrate (e.g. improved BMP with low hydrolysis chemical treatments).

6.3.3 Effect of ultrasound pre-treatment and pre-hygienisation on anaerobic co-digestion

Ultrasound increased the BMP from cattle slurry (230 m^3 CH_4/t VS_{added}; Paper V) and from ABP mixture + cattle slurry together (300 m^3 CH_4/t VS_{added}; Paper IV) by 15 ±2% and SMA of inoculom by 16 ±1% (days 5-10). Hygienisation improved the BMP of ABP mixture + cattle slurry, and cattle slurry alone by 25 ±5%, while the SMP (HRTs of 20-21 days) and daily methane yields from ABP mixture + sewage sludge (1:7, w.w.) and from ABP mixture + cattle slurry (1:3, w.w.) increased by 10 ±1% and 22 ±2%, respectively, when compared to BMPs and SMPs from the untreated materials. Hygienisation of ABP mixture + cattle slurry did not improve the SMA of inoculum, but probably improved viscosity and the increased hydrolysis (29-96%) of cattle slurry alone resulted in 25% improved SMA as compared to the SMA of untreated slurry. Ultrasound pre-treatments and hygienisations improved the VS removal from the batches (7.1

±2%), while VS removal during the semi-continuous digestion was not significantly improved by pre-treatments.

Though there were no relative difference in hydrolysis parameters (9-28%) of pre-treated ABP mixture + cattle slurry, hygienisation achieved a notably higher BMP when compared to the ultrasound pre-treated material. Thus, hygienisation may have improved the further action of the hydrolysing enzymes excreted by anaerobic bacteria via more loosen particle structures (Chu et al., 2002). Moreover, hygienisation of ABP mixture + cattle slurry in a ready-made mixture improved hydrolysis (parameters improved from 0-23 to 35-100%; see 6.2.3), but the SMP from the co-hygienised ABP mixture + cattle slurry increased only slightly (+9%), when compared to separate hygienisation of the materials (+8%). Thus, despite of the lower direct solubilisation, it seems that especially the separate hygienisation of the fractions may have enhanced the further bacterial hydrolysis (Chu et al., 2002). This also suggests that concentration of material that increased notably after the separate hygienisation of fractions (Paper III, IV; VS increase of 16 ±2%) did not significantly affect to the methane production rate from ABP mixture + cattle slurry.

The effective batch digestion (i.e. period of significant methane production) of the ultrasound pre-treated and hygienised feeds (ABP mixture + cattle slurry and cattle slurry alone) was 1-2 days longer, when compared to the batches digesting untreated materials. Thus, both pre-treatments most likely managed to disintegrate or loosen the structures that would have otherwise been inert for the hydrolysis of anaerobic micro-organisms (Mata-Alwarez, 2003). SMP (HRT of 21 days) from the hygienised ABP mixture + cattle slurry was 78% from its total BMP (42 days), when the corresponding differences in methane productions of ultrasound pre-treated and of untreated feeds were 87% and 97%, respectively. Due to that, SMP from the hygienised ABP mixture + cattle slurry remained by 7% lower than that of the ultrasound pre-treated feed. This indicates that even if the hygienisation may have loosen the solid structures and enhanced the further hydrolysis by anaerobic micro-organisms, it did not increase the methane production rate (or SMA; see 6.3.2) from the rest of the slowly degrading lignin-bond materials, but that remained relatively similar.

The ultrasound pre-treatment of ABP mixture + cattle slurry enhanced the SMP of the semi-continuous digestion (300 m^3 CH_4/t VS_{added}), following the effect during BMP measurement. When Es input was decreased from 6000 to 1000 kJ/kg TS, hydrolysis increased (see 6.2.1) and the improvements in SMP remained similar (12 ±1%). This may be due to the possible release of re-flocculating agents during the higher Es input (6000 kJ/kg TS) and/or variation between the different samples. However, this suggests low Es input for materials with different qualities varies, especially in cellulose-rich material having aptitude for flocculation. Thus, the utilisation of low Es inputs would be a more reliable method to assure positive energy balance for the process.

Hygienisation enhanced the BMP of cattle slurry alone (300 m^3 CH_4/t VS_{added}) resulting in the same BMP as with untreated ABP mixture + cattle slurry (1:3, w.w.). At the case of the cattle slurry, increased concentration probably enhanced the activity and contacts in the reactor (Karim et al., 2005; Vedrenne et al., 2008). Therefore, higher methane yield is achievable from slurry alone without e.g. the need for co-substrates with potentially long transportation to the biogas plan or requiring other inputs for production (e.g. energy crops). Moreover, in some cases co-digestion may reduce the quality of digestate and hinder the fertiliser use of the digestate due to e.g. harmful contaminants, when compared to digesting cattle slurry. Co-digestion may also cause instability to the process, when the quality or accessibility of co-substrate varies.

Hygienisation enabled also a higher SMP (430 m^3 /kg VS_{added}) from ABP mixture + sewage sludge (1:7 w.w.; mixed according to production amounts of materials), when compared to the same mixture with the feed-ratio from the literature (1:3 w.w.) with corresponding materials (410 m^3 CH_4/t VS_{added}; Rosenwinkel and Meyer, 1999; Murto et al., 2004; Sosnowski et al., 2008). The present OLR was higher, partly explaining the result. The higher feed-ratio applied in the present study (1:3, w.w.) reduced the concentration of undigested soluble compounds in reactor, but the VFA content of both reactors was utilised effectively (≤ 0.1 g/l in digestates). However, pre-hygienised digestate contained a higher content of NH_4^+-N (+8%), when compared to the reactor digesting the optimal feed ratio of 1:3 w.w. without pre-treatments.

Despite being reported as 'optimal' feed ratio for ABP mixture + sewage sludge, the ratio of 1:3 (w.w.) may be too high for the present materials. The effect of possibly too high OLR was noticed in the semi-continuous digestion of ABP mixture + sewage sludge as the SMP (4000 m^3 CH_4/t VS_{added}) of feed ratio 1:3 (w.w.; OLR: 2.2-4.0 kgVS/m^3 d) was compared to that (4100 m^3 CH_4/t VS_{added}) of hygienised ratio of 1:7 (w.w.; OLR: 2.1-3.6 kgVS/m^3 d). A reported optimal OLR for co-digestion of meat-processing wastes is 1.3-2.9 kgVS/m^3 d for the non-pre-treated materials (Rosenwinkel and Meyer, 1999; Alvarez and Liden, 2008; Kaparaju et al., 2010) and 3.9-4.2 kgVS/m^3 d for the mechanically pre-treated materials (Murto et al., 2004). If the experiment would have continued for a longer period than the present 175 days, the reactor may have adapted better to the higher OLR and resulted in improved SMP.

Ultrasound and hygienisation increased the N_{sol} content in ABP mixture + cattle slurry, but after the semi-continuous digestion (HRT 21 days) the effect was reversed. The content of 1.9 g N_{sol}/l ABP mixture + cattle slurry was the limiting value, since the loss of N_{sol} was 14 ±3% higher than the hydrolysis of it. As the N_{tot} value of the separate ABP fractions was 1.1-2.2 g/l (Paper II) and N_{sol} of the cattle slurry 1.6 g/l, it is possible that hygienisation and ultrasound pre-treatments (except for ultrasound treatment with 1000 kJ/kg TS) released already most of the nitrogen to the soluble matrix and was then reduced from the reactors via the nitrogen volatilisation (supported by the increasing NH_4^+-N content from N_{sol}), via reactions back to solids (e.g. with lignin compounds; Zahedifar et al., 2002) or via reactions to non-assayed compounds, e.g. via Maillard reactions (Khanal et al., 2006). Depending on the pH (7.5-7.9), approximately 3.5-8.5% of NH_4^+-N was in the form of NH_3-N (Eq. 3) and half of that is known to evaporate at 35 °C. Since the N_{tot} was not quantified, the amount of evaporated nitrogen or changes in N_{tot} cannot be estimated.

Ultrasound pre-treatment and hygienisation increased the NH_4^+-N content in the batches (ABP mixture + cattle slurry and cattle slurry alone) and in the digestate of hygienised ABP mixture + sewage sludge (11-20%). However, NH_4^+-N concentration in the pre-treated digestates from ABP mixture + cattle slurry remained similar to the NH_4^+-N contents of untreated digestates. This may due to the buffered pH of batch experiments (7.0) that may have decreased the conversion to volatile NH_3-N and different characteristic of sewage sludge and cattle slurry. Cattle slurry has already higher pH (supporting the volatilisation) and it contains more nitrogen bounding lignin and cellulose related compounds than sewage sludge. However, pre-treatments improved NH_4^+-N from N_{sol} of ABP mixture + cattle slurry digestates from 68 ±4 % to 85 ±3%, indicating improved fertiliser value of the digestate. This improvement was similar in batch and reactor studies of cattle slurry and ABP mixture + cattle slurry. Moreover, despite the N_{sol} reductions during the reactor studies, the NH_4^+-N concentration in the digestate from ABP mixture + cattle slurry remained higher or equal to content in the digestate of ABP mixture + sewage sludge. Moreover, cattle slurry based digestate contains presumably less harmful contaminants, than sewage sludge based digestate (i.e. heavy metals, pharmaceutical residues).

Pre-hygienisation alone decreased the number of enterococci in to ABP mixture + cattle slurry to the level set in the ABP-regulation (< 1000 CFU/g), while the reduction of *Clostridium* bacteria was less (1:400) than recommended (< 1:1000; 1774/2002/EC). However, it is known that clostridia are capable of forming spores, making them very resistant, and e.g. the spores of C*lostridium perfringens* -bacteria (which were the main clostridia analysed presently) cannot be destroyed even in thermophilic digesters (Sahlström, 2002). Clostridia form heat-resistant spores and will re-grow after the hygienisation. They are also an important microbial group for hydrolysis and acidogenesis during anaerobic degradation. Thus, their use as hygiene indicators may not be practical. However, the re-growth of salmonella and enterococci is unlikely after hygienisation (Ward et al., 1999). Ultrasound pre-treatment increased the ABP mixture + cattle slurry content of measured pathogens, but after digestions amount of CFUs were approximately similar to the digestate of untreated material.

6.4 INDICATIVE ENERGY BALANCES OF HYGIENISATION AND ULTRASOUND PRE-TREATMENTS

One of the most significant inputs of pre-treatments, environmentally and financially, is energy. The energy utilised in the pre-treatment should hopefully match or precede the energy produced by increases in biogas production (1 m^3 of methane ~ 10.4 kWh; Davidsson et al., 2007), though heat/hygienisation treatment is usually also used to destroy pathogens or concentrate the material. Indicative calculations (Eq.1, Eq.2, Eq.3) were made to observe the energy balances (energy input vs. energy content of increased methane production) for pre-treatment and digestion of ABP mixture + sewage sludge, of ABP mixture + cattle slurry, for cattle slurry alone and for DAF sludge alone (only feasible ABP fraction to digest alone as defined by suitability for anaerobic digestion;

Table 15). The energy consumption of the digestion process was not included into the calculations. Energy input of hygienisation includes the energy required to elevate the temperature from 35 °C to 70 °C, not maintaining this temperature for one hour. However, this energy need is estimated to be 10% of the total energy input (Paper III).

Table 15. *Input energies of ultrasound (Eq. 1) and hygienisation (Eq. 2) pre-treatments, energy outputs (Eq. 3) and indicative energy balances are calculated based on the present daily feeds of the semi-continuous reactors or feed content of the batches.*

Operational mode	Material	Pre-treatment (feed ratio)	Output energy (kJ)	Input energy (kJ)	Energy balance (kJ)
Batch	Cattle slurry	None	130	–	–
		US 6000 kJ/kgTS	170	140	-92
		Hygienisation	190	58	4.7
	ABPs + cattle slurry	None	190	–	–
		US 6000 kJ/kgTS	220	130	-91
		Hygienisation	250	54	7.2
	DAF sludge	None	110	–	–
		US 8500 kJ/kgTS	150	130	-83
		Hygienisation	160	41	7.3
Reactor	ABPs + sewage	None (1:7)	120	–	–
		None (1:3)	150	–	–
		Hygienisation	170	35	11
	ABPs + cattle slurry	None	100	–	–
		US 6000 kJ/kgTS	120	82	-70
		US 1000 kJ/kgTS	120	13	3.8
		Hygienisation	120	33	-10

A positive energy balance was achieved when hygienised ABP mixture + cattle slurry, cattle slurry alone and DAF sludge were digested in batches. The extra energy obtained then was averagely 6.5 (±1) kJ/ batch content. In the semi-continuous digestion (HRT 20 days), hygienised ABP mixture + sewage sludge (1:7, w.w.) achieved a positive energy balance of 10 kJ/d, while the hygienisation of ABP mixture + cattle slurry consumed more energy (-10 kJ/d) than it produced (HRT 21 days).

Cattle slurry has usually a lower BMP than sewage sludge (depending on TS), but slowly degrading cellulose and concentrating hygienisation treatment have probably also contributed to the longer duration of digestion. To improve slurry digestion further, a post-methanisation step is advisable. It will further stabilise the slurry by degrading remaining VS, produced more methane (5-10%, Møller et al., 2004) and thus also reduce possible methane emissions during digestate storage.

In practice, the energy input exhausted into hygienisation may be lower due to a lower specific heat capacity of the concentrated high viscosity feed-mixtures than that of water (0.00419 kJ/g °C; used in the calculations of Eq. 2). Thus, the surplus net energy with the use of hygienisation may be higher in real biogas plants than presently calculated. Calculation also presupposed that the material going through hygienisation would be pre-warmed close to the treatment temperature (35 °C) with heat produced from biogas and/or heat recovery from the process, thus reducing the heat losses from reactors (approx. 10% in mesophilic or 20% in thermophilic; Carrere et al., 2010). Most co-generation engines produce electricity at approx. 30–40% from biogas, and 40–50% as heat, when electricity is considered to be the primary product (the efficiency of the CHP –units are usually around 92 ±1%). Thus, the main-advantage of thermal pre-treatments (when compared to the other mechanical or physical pre-treatments) may in some cases be the possibility to utilise the heat produced, while the production of electricity could simultaneously be improved.

In order to achieve a profitable net energy production from the ultrasound batches, ABP mixture + cattle slurry, cattle slurry alone and DAF sludge alone should have been ultrasound pre-treated with an Es < 2100 ±100 kJ/kg TS (Eq. 1), while presently they were treated with the Es inputs of 6000-8500 kJ/kg TS optimised for hydrolysis, not energy-efficiency. However, a positive energy balance may be possible also using ultrasound, because both Es of 1000 and 6000 kJ/kg TS improved the SMP from ABP mixture + cattle slurry (1:3 w.w.; HRT 20 days) similarly and approximate to energy balances of -70 and +3.8 kJ/d, respectively. It was proved, that low Es input may not only have relatively high hydrolysis potential, but also offer the possibility to achieve a positive energy balance, especially with the easily re-flocculating materials.

If the VS based methane yields are extended to cover the feed mixtures according the yearly production rates of the studied ABPs (5400 t/ year) from the middle-size meat-processing plant in Finland, co-digestion (HRT 20 days) of ABP mixture + sewage sludge (1:7 and 1:3 w.w.) would produce approximately 5.2 and 7.4 GWh/a (1 m^3 CH_4 = 10.4 kWh; Davidsson et al., 2007) more energy than corresponding amount of sewage sludge alone. Moreover, pre-hygienisation of ABP mixture + sewage sludge (1:7, w.w.) would produce the surplus net energy production of about 0.6 GWh/a (60 m^3 of oil; 1 l of oil approximately equals to 10 kWh).

Similarly, the co-digestion of ABP mixture (5400 t/a) and cattle slurry (16 000 t/a; feed ratio 1:3, w.w.) would produce 1.9 GWh/year more energy than the corresponding amount of cattle slurry alone, while hygienisation would add to the net energy by 0.43 GWh/year. The ultrasound pre-treatment with 6000 kJ/kg TS might not be economically feasible. On the other hand, if no post-methanisation was applied (i.e. calculated according the SMP from reactor studies with HRT of 20 days), the 1000 kJ/kg TS would achieve a surplus net energy yield of 0.12 GWh/year (~12 m^3 of oil), while the hygienisation would consume about 0.25 GWh/year more energy than it produced via elevated methane production.

According to the national statistics (2010), there are over 290 000 dairy cows in Finland and they are estimated to produce 24 m^3 of slurry per cow yearly (931/2000/GC). With the present BMP (per w.w. added), this approximates to 800 GWh/a of energy (1 m^3 of methane ~ 10.4 kWh; Davidsson et al., 2007), which is equivalent to 80 t m^3 of oil (1 l of oil ~ 10 kWh). The net energy production from hygienised dairy cattle slurry would be 2.5 GWh/a (Eq. 2; ~250 m^3 of oil). Similarly, the greenhouse gas emission would be reduced approximately by 640 000 t CO_2/ year, when compared to the emissions from the untreated slurry (92.4 kg CO_2/m^3: Amon et al., 2006). It is notable that the annual reserve of animal manures in Finland suitable for anaerobic digestion is a multiple to yearly volume of dairy cattle slurry (appr. 7 million m^3 of slurry/ a). According to the national statistics (2009, 2010), there are: 930 t cows, 1.4 million pigs and 9.4 million heads of poultry, when in the corresponding numbers in the whole Europe scale are: 91 million, 160 million and 3 billion, respectively (> 1600 million m^3 of manure per year; Holm-Nielsen et al., 2009; FAO, 2010).

In summary, longer HRT or separate post-methanisation is recommended to ensure a positive energy balance when pre-treating the materials studied. In practice, hygienisation of manure is not required by law in farm-scale biogas plants or cooperatives of several farms in Finland and thus only the ABPs need to be hygienised. This would further decrease the energy input required for hygienisation (decreased amount of material with a reduced specific heat capacity, Eq. 2), though possibly the overall SMP would be somewhat lower due to not pre-treating the slurry. The utilisation of low ultrasound Es inputs would be a more reliable method to assure positive energy balance with the present materials than the values that obtained the optimal hydrolysis.

7 Incentives and limitations for implementing anaerobic digestion

Because of the possibility to utilise the biogas in energy production and the increasing interest in nutrient/material reuse, there is a growing interest on anaerobic digestion technology in waste(water) treatment plants and/or in industry producing or treating organic wastes and by-products. In Austria, nearly complete energy self-sufficiency of the slaughterhouse industrial complex have been obtained when ABPs produced (rumen, blood, grease trap waste, DAF sludge, colon and digestive tract content) are converted to the energy via CHP - unit (Waltenberger et al., 2010). The amount of anaerobic digestion plants in Europe and the size of the reactors have grown steadily during the past 20 years. At the beginning of year 2011, there are over 200 plants with the co-capacity of 600 000 t per year in 17 European countries (De Baere et al., 2010). In Finland there are approximately 30 anaerobic reactors running (2010) and increasing amount of license applications.

In Finland anaerobic digestion technology has already been used widely in wastewater treatment plants (about 20 biogas reactors), though many of the older reactors used are usually over-dimensioned and un-optimised operating with relatively low loadings. Thus, co-digestion of by-products from meat-processing industry (or corresponding material) with sewage sludge in municipal wastewater treatment plants offer an efficient way to increase the effectiveness of the existing biogas plants and finding a sound treatment for the ABPs.

In Finland, one of the main difficulties of co-digestion in practise is the long distances between materials and existing plants, which make the transportation uneconomical. Thus, in the future, the insertion of farm-scale digesters, usually treating animal manure and/or energy crops may offer a solution for smalle by-product streams. at the time of writing, the population density in Finland is low, distances in rural areas are long and amount of biogas reactors is inadequate (< 50 biogas plants) to utilise substrates available. Though the number of farms and cows has declined in Finland during the last decades, the farm sizes have simultaneously grown. Current farms are becoming more energy-intensive enterprises and there has been a growing interest in building biogas plants either on farm-scale or as co-operatives involving several farms, which might also increase the effective utilisation of organic waste streams in biogas production. In addition, becoming national change (1.9.2011) in the food legislation (854/2004/EC) facilitates the function of the farm-scale meat production, which is expected to increase the local food-production and amount of small-scale slaughterhouses in Finland.

Another difficulty in the co-digestion of several materials (even if it may increase the methane production and gate-fee incomes) is the quality of digestate and its utilisation as a fertiliser. Hygienisation of materials is an effective way to destroy pathogens and make the digestate more reusable. As discussed earlier, hygienisation treatment could be very beneficial for digestion of ABPs. In hygienisation both heat recovery and excess thermal energy from the CHP engines (approx. 40% electricity, 60% heat) of the biogas process can be utilised. In addition, a positive energy (or at least electricity) balance via increased methane production can be obtained. Still, the quality required for ensured reuse of the digestate should affect the selected substrates for digestionmore than merely the possible improvement in biogas production.

The current meat consumption in Finland is 72 kg per person per year, which is relatively low when compared to the many other industrial countries such as Japan (135 kg per person per year). However, the yearly increase in meat consumption in Finland is currently 12 000 t (2011). In 2030 the average meat consumption in industrialised countries is estimated to be 100 kg of meat per capita per year (FAO, 2010) and in Finland 66-75 kg, respectively (Vinnari, 2008). Also, highly increasing meat consumption in the develop world is believed to continue for several decades (De Haan et al., 2001; FAO, 2010) with the increasing economical growth (Capps et al., 1988). Total global need for meat is expected to grow by 56% between the years 1997-2020 (De Haan et al., 2001), which increases the need for safe treatment of ABPs. This same trend also relates to the amount of animal manure, but also to other food wastes (e.g. uneaten food, food preparation leftovers from residences and commercial establishments) further increasing the amount of suitable raw materials for anaerobic digestion. The amounts can be surprisingly high, for instance in the UK one third of the foodstuff bought in the households and in USA the 0.4 kg of food waste per person is daily thrown away (Sedláček et al., 2010).

Implementation of biogas technology is also affected by the environmental legislation and regulations. These offer tightening discharge limits for greenhouse gases and landfilling of untreated organic waste (31/1999/EC), and regulation for environmental safety (e.g. ABP regulation; 1774/2002/EC), with the trend toward more complete utilisation and reuse of wastes, (Industrial Emission directive IED; 75/2010/EC). The legislation may increase the use of anaerobic digestion in the treatment of organic by-products due to its multiple benefits. One of the aspects affecting the utilisation of biogas technology are the various economic incentives for renewable energy production, such as feed-in tariffs and green certificates, enabling the sale of the electricity produced to the grid. A feed-in tariff for large biogas plants is also expected to be launched in Finland during 2012.

It is estimated, that at least 25% of all European bio-energy originated from farming and forestry, which are the main bio-energy sources in Europe, could be produced via biogas process in the future (Holm-Nielsen et al., 2007). This estimation includes the biogas potential of manure with the implementation level of 40-70% (230 TWh/a) and energy crops produced on 5% of the arable farmland (570 TWh/a; Holm-Nielsen et al., 2009). However, when sewage sludge and other organic wastes and by-products as well as landfill gas are included, the available biogas potential would be significantly higher. According to general estimations in the statistics of EU energy programs, potential of biogas production in Europe (EU15) is between 600-1200 TWh/a, which would also mean reduction of 136 000-270 000 t/a in CO_2 emissions.

The estimated technical biogas potential of the animal manure, sewage sludge and other organic wastes and by-products produced in Finland is 14 TWh (reduction of CO_2 emissions: 3 200 t/a), which correspond to the yearly fuel consumption of 700 000 cars (Lehtomäki et al., 2007b). However, this biogas potential could be enhanced notably, if the potential of energy crops (20-30 MWh/ha on fallow field areas) are included into the calculations (Lehtomäki, 2006). According to another technical estimation (including energy crops), it would be possible to increase the yearly biogas potential in Finland up to 7-18 TWh by year 2015, while the maximal theoretical production potential is 40-150 TWh (Asplund et al., 2005). According to the Statistics in Finland, 0.5 TWh (< 0.2 % of the total energy consumption) of biogas energy was utilised in 2009. In a report considering the biogas potential in the Central Finland, reusable nutrient content was estimated to be 11 000 t of nitrogen and 2000 t of phosphorus in relation to the energy potential of 460 ±190 GWh from various organic materials including manure, sewage sludge, organic wastes and energy crops produced in the area (Vänttinen et al., 2009).

In summary, biogas technology is still relatively untapped form of energy production in Finland. However, it holds significant potential for processing different organic materials into valuable energy (biogas) and nutrient products (digestate). Still, more technological innovations and enhancement via research and development (De Baere et al., 2010) are needed. Also, local optimisation between the different areas of administration and practical execution is needed before anaerobic digestion has optimally reciprocated to the demands of sustainable development, allocated towards the environmental technology.

8 Conclusion

The ABPs studied (digestive tract content, drumsieve waste, DAF sludge and grease trap sludge) were highly bio-degradable and suitable for anaerobic digestion. The pre-treatments studied (ultrasound, chemical pre-treatments, hygienisation and bacterial product addition) hydrolysed ABPs effectively, but did not enhance the process or methane production potential notably. In fact, the more effective the hydrolysis, the less methane was produced (except for DAF sludge). Due to this and the high TS content of the ABPs, the materials studied are more feasible be utilised in co-digestion processes.

The co-digestion of the ABPs with sewage sludge and cattle slurry resulted in elevated methane production and fertiliser value of the digestate. Digestion of ABPs in wastewater treatment plants and/or in farm-based biogas plants may be beneficial for the stabilisation of the materials, but also for the process technique and for the improved production and reusability of the end-products. These enhancements were further improved by the pre-treatments studied. Process factors and parameters as well as the pre-treatments and their modalities studied and optimised, enhanced the digestion processes based on practical circumstances.

The responses of the different ABPs (e.g. lipid-rich and cellulose-rich materials) and feed mixtures on the various pre-treatments and their modalities were recognised. The most suitable pre-treatments (ultrasound, bacterial product addition) were found to enhance the complex degradation of materials, while hygienisation (recommended or demanded for the ABP materials) reduced the number of pathogens and was proven to have high synergy potential as a thermal pre-treatment. Positive energy balance (i.e. economical feasibility) of ultrasound and hygienisation pre-treatments is attainable. Pre-treatments effect on the whole process and on the end-products are depended on the specific hydrolysis values, but especially on the content of the materials and qualities of the solubilised compounds. The pre-treatments enabled disintegration of the structures that would have otherwise been inert for hydrolysis.

This thesis offers new information on the case-, treatment- and material-specific factors affecting process requirements, optimisation in practice and mechanisms involved in pre-treatments and co-digestion of ABPs. The information produced may be directly utilised in practical implementation of anaerobic digestion of the studied or corresponding materials and feed mixtures.

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Sami Luste

Anaerobic Digestion of Organic By-products from Meat-processing Industry

The Effect of Pre-treatments and Co-Digestion

Animal by-products (ABP) from meat-processing form an increasing group of materials with tightening treatment and disposal requirements. Many ABPs can be reused as energy- and nutrient-rich raw materials for anaerobic digestion process with multiple benefits for sustainable development in practice. This doctoral dissertation introduces new information about the case- and material-specific factors of anaerobic digestion process requirements and process optimisation, degradability of the ABPs and mechanisms involved in pre-treatments and co-digestion of ABPs.

UNIVERSITY OF EASTERN FINLAND

Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences

ISBN 978-952-61-0522-2