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Suvi Bayr

Biogas Production from Meat and Pulp and Paper Industry By-Products

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ABSTRACT

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Diss.

In this thesis, the anaerobic digestion of industrial by-products from meat and pulp and paper industry were studied. These materials, which are treated mainly like wastes, were seen to be potential substrates for anaerobic digestion. Methane yields of ca. 700 dm^3 kg volatile solids $(VS)_{added}^{-1}$ were obtained for slaughterhouse wastes, alone and in co-digestion with rendering wastes. On the other hand, methane yields of 190–240 dm^3 $kgVS_{added}^{-1}$ were obtained in the monodigestion of pulp and paper industry primary sludge, and 150–170 dm^3 $kgVS_{added}^{-1}$ in the co-digestion of primary sludge with secondary sludge. In the digestion of slaughterhouse and rendering wastes, mesophilic conditions were more stable, and the feasible organic loading rates (OLRs) were 1–1.5 kgVS m^{-3} d^{-1} and hydraulic retention times (HRTs) were 50 d. For pulp and paper mill wastewater sludge, thermophilic conditions were more stable with the feasible OLRs of 1–2 kgVS m^{-3} d^{-1} and the HRTs of 14–32 d. The digestion processes of these industrial by-products can easily turn unstable. In the digestion of slaughterhouse and rendering wastes, process imbalance may occur due to inhibition from intermediate products like volatile fatty acids, long-chain fatty acids and ammonia. On the contrary, in the digestion of pulp and paper mill wastewater sludge, the low nitrogen content and low pH may cause problems. It was observed that the more stable digestion process of slaughterhouse waste is achieved by additive of Co, Ni, Se, W, Fe and HCl. In addition, in pulp and paper mill secondary sludge digestion, hydrothermal pre-treatment, among the studied 12 single or combined pre-treatments, increased the methane yield by 19–31 %. In conclusion, the digestion of two different kinds of industrial by-products was seen to be feasible, although operational conditions should be optimised and possibly enhancement methods used to ensure the stable digestion processes of these vulnerable organic materials.

Keywords: Anaerobic digestion; biogas; by-product; pre-treatment; pulp and paper mill wastewater sludge; rendering waste; slaughterhouse waste.

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-IV.

I planned the experiments together with my supervisors and I conducted the main part of the experimental work in articles III-IV. In articles I-II, I planned the experiments together with my supervisors and I conducted the experimental work together with my co-authors. I wrote the first drafts of all of the manuscripts and finalized them with my co-authors.

I Bayr S., Rantanen M., Kaparaju P. & Rintala J. 2012. Mesophilic and thermophilic anaerobic co-digestion of rendering plant and slaughterhouse wastes. *Bioresource Technology* 104: 28–36.

II Bayr S., Pakarinen O., Korppoo A., Liuksia S., Väisänen A., Kaparaju P. & Rintala J. 2012. Effect of additives on process stability of mesophilic anaerobic monodigestion of pig slaughterhouse waste. *Bioresource Technology* 120: 106–113.

III Bayr S. & Rintala J. 2012. Thermophilic anaerobic digestion of pulp and paper mill primary sludge and co-digestion of primary and secondary sludge. *Water Research* 46: 4713–4720.

IV Bayr S., Kaparaju P. & Rintala J. 2013. Screening pretreatment methods to enhance thermophilic anaerobic digestion of pulp and paper mill wastewater treatment secondary sludge. *Chemical Engineering Journal* 223: 479–486.

ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
BCTMP	Bleached chemi-thermo-mechanical pulp
BSE	Bovine spongiform encephalopathy
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
CTMP	Chemi-thermo-mechanical pulp
EPS	Extra-cellular polymeric substances
FID	Flame-ionization detector
FM	Fresh matter
GC	Gas chromatograph
HMF	5-hydroxymethylfurfural
HRT	Hydraulic retention time
ICP-OES	Inductively coupled plasma optical emission spectrometer
LCFA	Long chain fatty acid
MGWL	Monosodium glutamate waste liquor
MWWTP	Municipal wastewater treatment plant
NDF	Neutral detergent lignin
NH_3	Free ammonia nitrogen
NH_4-N	Ammonia nitrogen
OFMSW	Organic fraction of municipal waste
OLR	Organic loading rate
PID	Photo-ionization detector
SCOD	Soluble chemical oxygen demand
STP	Standard temperature and pressure conditions
TCD	Thermal conductivity detector
TSE	Transmissible spongiform encephalopathy
TVFA	Total volatile fatty acids
TKN	Total Kjeldahl nitrogen
TMP	Thermo-mechanical pulp
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids
WW	Wet weight

1 INTRODUCTION

1.1 Background

Renewable energy is important for future energy production due to environmental impacts from fossil fuels, like climate change, and due to decreasing fossil fuel resources. Renewable energy is energy that is produced in ways that can be regenerated in a human time scale. Renewable energy includes bioenergy, solar, wind, hydro, tidal and geothermal energy. Bioenergy is energy produced from biological sources, different biomasses, and includes wood, plants and biological wastes that are used in the production of heat, electricity or fuels. In the European Union, the promotion of renewable energy has been regulated by Directive 2009/28/EC of the European parliament and of the council on the promotion of the use of energy from renewable sources. This directive set the goal that 20 % of the gross final energy consumption should be derived from renewable energy sources by the year 2020 (Anon 2009a).

Among many other renewable energy forms, biogas production is one way to produce renewable energy from organic material. One advantage of biogas is that it can be produced from many organic materials which would otherwise be wasted. Biogas is a gas mixture of mainly methane (CH_4) and carbon dioxide (CO_2) that is produced by micro-organisms under anaerobic conditions. The digestate left over from the digestion process contains nutrients and can be used as a soil conditioner.

Industrial by-products are the secondary products formed in an industrial process, in addition to the primary product. Many of the industrial by-products are treated like wastes, but these materials also have a possibility of providing extra value, either in form of material or energy. Globally, many different types of industrial by-products and wastes are produced in industrial sectors that are globally general, and in sectors that are locally important. The meat industry is an example of a large worldwide sector of industry that is ever growing, as meat consumption is predicted to grow due to the growing world population

and increasing standard of living. On the other hand, pulp and paper industry is an example of a locally important industrial sector e.g. in Finland.

In the meat industry, slaughterhouse by-products are generated when animals are slaughtered for human consumption. During slaughtering, a large portion of the animal is wasted, and thus, there are slaughterhouse wastes available for further use. Traditionally, slaughterhouse wastes have been utilized for the production of animal feeds. After the bovine spongiform encephalopathy (BSE) scare, however, legislation has become more stringent and many traditional applications have been lost; thus there could be opportunities for new ways to take advantage of these by-products (Woodgate and van der Veen 2004).

In pulp and paper mills, wastewater sludge are produced in high quantities in the wastewater treatment processes. Sludge is a wet solid material formed in the wastewater treatment process. Pulp and paper mill wastewater sludge are difficult to handle because of the high water content and low dewaterability. Nowadays these sludge are generally dewatered and then either incinerated or composted (Hagelqvist 2013).

1.2 Anaerobic digestion

1.2.1 Biogas production

Anaerobic digestion is a process where micro-organisms convert organic material into biogas under anaerobic conditions. Biogas is a gas mixture containing methane (45–70 %), carbon dioxide (30–45 %), water vapour, nitrogen (N_2), oxygen (O_2), hydrogen sulphide (H_2S) and other minor compounds (Rasi 2009). The content of biogas depends on substrates and production method. Biogas can be used for production of heat or electricity, or as a vehicle fuel. Depending on the use of the biogas, some cleaning and upgrading is needed before use (Ryckebosch *et al.* 2011). Upgrading increases the heating value of the gas and makes it fulfil gas appliance requirements. The end product of biogas upgrading is called biomethane (Ryckebosch *et al.* 2011).

Anaerobic digestion is traditionally divided into four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 1). In each step, a different group of micro-organisms work. Three major groups of micro-organisms are primary fermenting bacteria, anaerobic oxidizing bacteria and methanogenic archaea (reviewed by Angelidaki *et al.* 2011). Although the digestion process is shown in the following steps, more than one of them, or even all, must co-occur to keep the process going (Argyropoulos *et al.* 2013a).

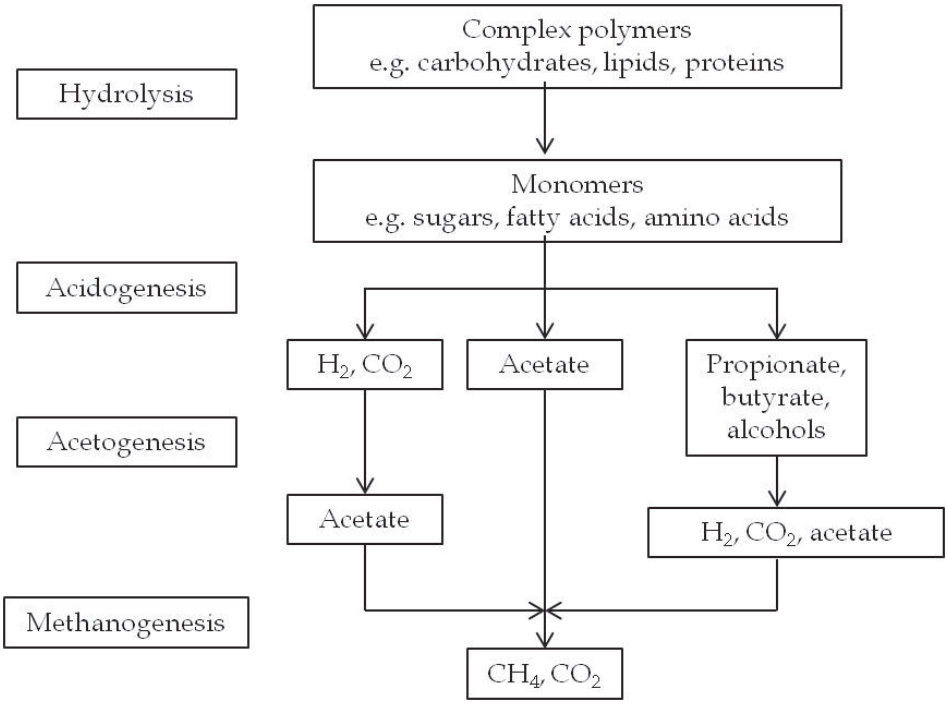

FIGURE 1 Anaerobic digestion pathways (adapted from Madigan *et al.* 2009, Madsen *et al.* 2011).

Hydrolysis is the first phase of anaerobic degradation. In hydrolysis, complex substrates like organic material that contains proteins, carbohydrates and lipids are degraded into soluble monomers (Angelidaki and Sanders 2004, Deublein and Steinhauser 2008). Polysaccharides are degraded to soluble sugars, proteins to amino acids and lipids to glycerol and long chain fatty acids (Angelidaki and Sanders 2004). Hydrolytic micro-organisms excrete hydrolytic enzymes, like cellulase, cellobiase, xylanase, amylase, lipase and protease (Weiland 2010), which are used in the process. Acidogenesis, also called fermentation, is a process phase were products of hydrolysis are converted to volatile fatty acids (VFAs), alcohols, hydrogen (H_2) and CO_2 (Deublein and Steinhauser 2008). Then, in acetogenesis the fermentative intermediates are degraded to acetic acid, CO_2 and H_2 (Argyropoulos *et al.* 2013b). The final step of the anaerobic digestion process is methanogenesis, in which CH_4, along with CO_2, is produced. There are two main pathways of methanogenesis: aceticlastic and hydrogenotrophic methanogenesis. In aceticlastic methanogenesis, CH_4 and CO_2 are produced from acetate and in hydrogenotrophic methanogenesis, hydrogen-consuming archaea produce CH_4 from CO_2 and H_2 (Angelidaki *et al.* 2011). Typically, 70 % of the methane is produced in aceticlastic methanogenesis and 30 % in hydrogenotrophic methanogenesis (Madsen *et al.* 2011).

Many factors affect the anaerobic digestion process (see e.g. review by Chandra *et al.* 2012). It is important to choose conditions according to the substrate to ensure high methane yields and a stable digestion process. Anaerobic digestion can be carried out in three temperature regimes: in psychrophilic (10–20 ˚C), mesophilic (30–35 ˚C) and thermophilic conditions (50–60 ˚C) of which mesophilic and thermophilic are used most often (Madigan *et al.* 2009, Chandra *et al.* 2012). The maximum growth rate of the thermophilic micro-organisms is higher than that of the mesophilic micro-organisms (Madigan *et al.* 2009); however, at a higher temperature, problems may arise, for example, due to the buffer capacity, as explained more specifically later on.

pH of the anaerobic digestion process is controlled by the bicarbonate acid-base system (Murphy and Thamsiriroj 2013). If the volatile acid concentration of the process increases, bicarbonate alkalinity will neutralize it. Temperature affects the buffering capacity through the solubility of CO_2; at lower temperatures, more CO_2 dissolves and more bicarbonate ions are formed, which increases the buffering capacity (Murphy and Thamsiriroj 2013). Ammonia also has a role in the buffer system, as the hydrogen ions formed in the ionization reaction of ammonia are used in bicarbonate production (Murphy and Thamsiriroj 2013). The pH requirement of the anaerobic digestion process is a compromise, as the optimal pH for acidogenic micro-organisms is 5.5–6.5, while for methanogens it is 7.8–8.2. Thus, the optimal pH for the digestion process is often near neutral at 6.8–7.4 (Khanal 2008a).

1.2.2 Substrates

Basically, all organic materials are possible substrates for biogas production. In practice, some materials like wood are hardly degradable in anaerobic conditions, and thus, are not considered to be feasible substrates. However, a large variety of organic materials, varying from different plant species to different waste materials, have been studied as substrates for methane production, as reviewed e.g. by Raposo *et al.* (2011).

Different organic materials degrade differently, thus having different theoretical methane potentials. One way to evaluate the methane potentials of different substrates is to calculate the theoretical methane potentials based on the total conversion of organic material, and H_2O to CH_4 and CO_2, by using Buswell's equation (reviewed by e.g. Angelidaki and Sanders 2004). For carbohydrates ($C_6H_{10}O_5)_n$, proteins ($C_5H_7NO_2$) and lipids ($C_{57}H_{104}O_6$), the theoretical methane potentials are 415, 496 and 1014 dm^3 $kgVS^{-1}$ (standard pressure and temperature conditions, STP), respectively. In addition to the basic composition of the substrate, other characteristics, such as the presence of nutrients, hardly digestible compounds like lignin or inhibitory concentrations of compounds, affect the degradation and methane yields of the substrate (Angelidaki and Sanders 2004). The by-products from the meat industry contain high concentrations of lipids and proteins, while pulp and paper mill wastewater sludge contain mainly carbohydrates in the form of lignocellulosic

substances. The structure and degradation of these basic components, relevant to this thesis, are introduced below.

Proteins account for more than half of the dry mass in most cells (Campbell and Reece 2002) and consist of polypeptides, which are polymers of amino acids. Each protein consists of one or more polypeptides. Proteins are said to have primary, secondary and tertiary structures (Campbell and Reece 2002). The primary structure is the unique sequence of amino acids, and all proteins are constructed from 20 different amino acids. The secondary structure is the bending of the polypeptide chain, attached with hydrogen bonds. The tertiary structure is the overall shape of the polypeptide, consisting of interactions between the side chains of the amino acids. The work of the proteins is related to the structural support, storage, transport of substances and enzymes (Campbell and Reece 2002). In the anaerobic digestion of nitrogenous compounds, mostly proteins, ammonia is formed (Kayhanian 1999). The proteins are hydrolysed to peptides and amino acids. Nitrogen is an essential nutrient for micro-organisms, and thus, for the anaerobic digestion process.

Lipids are hydrophobic molecules consisting mainly of hydrocarbons and the most important group of lipids is fats, which consist of glycerol and fatty acids. The main function of fats is energy storage (Campbell and Reece 2002). In the hydrolysis of lipids, like fats, oils and grease, long-chain fatty acids (LCFA) are produced. Some examples of LCFAs are palmitic, stearic, capric, linoleic and oleic acids (Khanal 2008a). Another group of fatty acids is VFAs, which are formed in the hydrolysis and fermentation of organic matter (Khanal 2008b).

Lignocellulosic substances represent one of the most abundant biological resources on the planet (Agbor *et al.* 2011). They include mainly cellulose, hemicellulose and lignin, but also extractives and ash (McKendry 2002). The contents of cellulose, hemicellulose and lignin vary in different lignocellulosic materials: for example, in hardwood stems, the cellulose content is 40–55 %, hemicellulose content is 24–40 % and lignin content is 18–25 %, while the respective contents in softwood stems are 45–50 %, 25–35 % and 25–35 % (Chandra *et al.* 2012).

Cellulose provides the structural support in the plant cell wall, and it is also present in bacteria, fungi and algae. Cellulose is a linear polysaccharide polymer of glucose, made up of cellobiose units linked to each other via β-1,4 glycosidic bonds (Agbor *et al.* 2011, Chandra *et al.* 2012) and cellulose chains are grouped together to form microfibrils, which together form cellulose fibre (Agbor *et al.* 2011). The structure of cellulose can be either crystalline or amorphous, depending on the orientation of the cellulose molecules in the cellulose fibre (Pérez *et al.* 2002, Agbor *et al.* 2011), and it is kept together by hydrogen bonds and Van der Waals forces (Pérez *et al.* 2002). The digestibility of cellulose depends on the cellulose's accessibility to cellulase and cellulose crystallinity (Agbor *et al.* 2011).

In plant cell walls, hemicelluloses are thought to coat cellulose-fibrils (Agbor *et al.* 2011). Hemicelluloses are a group of heterogenic polymers of pentoses, hexoses and sugar acids (Hendriks and Zeeman 2009, Agbor *et al.*

2011), which have branches with short lateral chains, and are easier to hydrolyse than cellulose (Pérez *et al.* 2002). Compared to cellulose, hemicellulose's molecular weight is lower (Hendriks and Zeeman 2009) and the composition of hemicellulose depends on the biomass, e.g. the main hemicellulose in grass is xylan, while in softwoods it is glucomannan (Agbor *et al.* 2011). Hemicellulose degrades to monomeric sugars and acetic acid (Pérez *et al.* 2002), and when hemicelluloses are removed, it has a positive effect on cellulose degradability (Hendriks and Zeeman 2009).

In the plant cell wall, lignin keeps the other substances together (Agbor *et al.* 2011); thus, the purpose of lignin is to give the plant structural support, impermeability and resistance to microbial attack and oxidative stress (Hendriks and Zeeman 2009). Lignin is a complex and irregular polymer consisting of phenyl propane units (Malherbe and Cloete 2002, Agbor *et al.* 2011). Lignin is insoluble in water (Pérez *et al.* 2002) and its enzymatic hydrolysis is difficult since no repeating similar subunits occur (Malherbe and Cloete 2002). Lignin does not degrade under anaerobic conditions (Angelidaki and Sanders 2004), or at least, degrades only slowly and incompletely (Deublein and Steinhauser 2008). Lignin is tightly bound to cellulose and hemicellulose in lignocellulosic biomass, which makes those carbohydrate polymers hardly available for enzymatic hydrolysis (Mosier *et al.* 2005). Products of lignin hydrolysis can have an inhibitory effect (Hendriks and Zeeman 2009), although methanogens can adapt to them to some extent (Barakat *et al.* 2012), as discussed more widely below. Thus, the digestibility of lignocellulosic biomass depends on diverse ratios of the three main biopolymers and, especially, lignin content, cellulose accessibility to cellulase and cellulose crystallinity (Agbor *et al.* 2011).

1.2.3 Nutrient requirements and inhibition

Since the anaerobic digestion process is a biological process carried out by different micro-organisms, nutrients needed for the growth of those micro-organisms are essential for the process. On the other hand, too high of concentrations of these nutrients, as well as some other compounds like digestion intermediate products, might be inhibitory for the microbial growth. The optimum concentrations of nutrients are hard to determine. A rough estimation of the nutrients needed can be made based on the composition of the anaerobic microbial biomass, which is estimated to be 50 % carbon (C), 20 % oxygen (O), 10 % hydrogen (H), 11 % nitrogen (N), 2 % phosphorus (P) and 1 % sulphur (S) (Drosg *et al.* 2013). In cells, carbon is the most abundant element as it is the major element in all classes of macromolecules. Nitrogen is a key element in e.g. proteins and nucleic acids. Phosphorus is needed in the synthesis of nucleic acids and phospholipids. Sulphur has a structural role in the amino acids cysteine and methionine, and it is also present in some vitamins. Additionally, potassium (K), magnesium (Mg), calcium (Ca) and sodium (Na) are essential for all, or many, micro-organisms (Madigan *et al.* 2009). In addition to the macronutrients, iron (Fe) and some other metals, like

cobalt (Co), copper (Cu), nickel (Ni), manganese (Mn), molybdenum (Mo), selenium (Se), vanadium (V), tungsten (W) and zinc (Zn), which are referred to here as trace elements, are needed. Iron has a major role in cellular respiration because it is a key element of the cytochromes and iron-sulphur proteins involved in electron transport reactions. Typically, trace elements are part of the enzymes (Madigan *et al.* 2009). Archaea have been suggested to be more sensitive to trace element concentrations than the bacteria participating in the anaerobic digestion process (Feng *et al.* 2010).

The carbon/nitrogen (C/N) ratio is one parameter used to describe the substrate suitability for anaerobic digestion. The optimal C/N ratio for anaerobic digestion is thought to be 20–30:1 (Banks and Heaven 2013). If the C/N ratio is higher, methanogens will consume nitrogen for protein production and some leftover carbon will remain in the process without reacting, thus leading to low biogas production (Chandra *et al.* 2012). A too high C/N ratio can also result in a too low N concentration for microbial growth (Banks and Heaven 2013). On the other hand, a low C/N ratio can lead to the accumulation of ammonia nitrogen, which can lead to the inhibition of the digestion process (Banks and Heaven 2013). A low C/N ratio can occur in the digestion of protein rich substrates like slaughterhouse wastes.

More important than the overall trace element concentration is the bioavailability of them. Trace elements form insoluble precipitates with sulphide, carbonate and phosphate (Banks and Heaven 2013). The risk for nutrient deficiency is higher in monodigestion processes (Drosg *et al.* 2013) than in the digestion of complex waste fractions, like the organic fraction of municipal solid waste (OFMSW), although even those have been observed to benefit from trace element additions, as discussed later on. Iron can be added to the reactors to reduce sulphur toxicity through precipitation, and at the same time, the bioavailability of the trace elements may increase (Banks and Heaven 2013).

Some substances can be inhibitory or toxic for the digestion process. Inhibition means a decrease in growth, while toxicity leads to the death of the micro-organisms (Drosg *et al.* 2013). Substantial concentrations of inhibitory substances are found to be the reason for many digester failures (Chen *et al.* 2008). Inhibition can be due to substances in the substrate, like ammonia or heavy metals, or by the metabolic by-products of the micro-organisms (intermediate products of digestion), like ammonia or VFAs (Khanal 2008a). Typically, the same elements can be essential for micro-organisms in low concentrations and inhibitory or toxic in higher concentrations. Common inhibitory substances for anaerobic digestion at certain concentrations are ammonia, sulphur compounds, VFAs, LCFAs, light metal ions (like Na^+, K^+ or Ca^+) and heavy metals (Chen *et al.* 2008, Khanal 2008a, Murphy and Thamsiriroj 2013).

As explained earlier, ammonia is formed in the anaerobic digestion of proteins. It has been assumed that ammonia concentrations between 50 and 100 mg l^{-1} are beneficial for the anaerobic digestion process (Khanal 2008a). The optimal ammonia concentration ensures a stable digestion process by

maintaining the buffer capacity (Rajagopal *et al.* 2013); however, a too high ammonia concentration is found to be inhibitory. It has been suggested, that ammonia concentrations of 1500–3000 mg l^{-1} can be inhibitory, and concentrations higher than 3000 mg l^{-1} can be toxic (reviewed by Khanal 2008a); however, e.g. adaptation may change these levels. Among the forms of ammonia, the free ammonia nitrogen (NH_3) is more toxic than ammonium nitrogen (NH_4-N, reviewed by Chen *et al.* 2008) as it can diffuse through the cell membrane (Kadam and Boone 1996). Temperature and pH affect the ammonia forms in the process as NH_3 concentrations are higher at a higher pH and temperature (Kayhanian 1999).

LCFAs and VFAs are formed in the digestion of lipids, and they are known to inhibit the digestion process. It is known that already low concentrations of individual LCFAs, such as 30 mg l^{-1} of linoleic acid, can inhibit digestion (Lalman and Bagley 2000). Additionally, VFAs can inhibit digestion, but for them higher concentrations (10 g l^{-1}) have been suggested (Aguilar *et al.* 1995). Individual VFAs have been suggested to be inhibitory in different concentrations; for example, no significant inhibition was noticed with acetic and butyric acids with concentrations of 2400 and 1800 mg l^{-1}, respectively, while propionic acid was inhibitory for methanogens at a concentration of 900 mg l^{-1} (Wang *et al.* 2009). On the other hand, VFA accumulation has been claimed to be because of a process imbalance and not the reason for it (Ahring *et al.* 1995, Pullammanappallil *et al.* 2001).

1.2.4 Methods to improve anaerobic digestion

The highest possible methane yields of the substrates are rarely obtained; therefore, some methods have been used to improve the anaerobic digestion process. The target of the improvement is usually higher methane yield, a more stable digestion process or the possibilities of using a higher organic loading rate (OLR) or a shorter hydraulic retention time (HRT). When considering improvement options, important factors include e.g. the effectiveness of the process (increased methane yield or more stable process), low capital and operational costs, and low energy need.

The simplest way to improve the anaerobic digestion process is to optimise the process parameters, like temperature or pH, to fit the substrate. One widely studied option is the co-digestion of several substrates at a time; for example, a high lipid and protein content substrate is co-digested with some more dilute material. Co-digestion may enhance the digestion process by the synergistic enhancement e.g. by balancing the nutrients and moisture conditions (Mata-Alvarez *et al.* 2000) as well as the buffer capacity (Zhang *et al.* 2013). Additionally, a better C/N ratio (Ward *et al.* 2008, Appels *et al.* 2011) and lower concentrations of inhibitory substances (reviewed by Mata-Alvarez *et al.* 2000) may be the enhancements of co-digestion. However, the specific impacts in each case depend on the substrates and process conditions; for example, during co-digestion of pig waste (slurry) with paper sludge, 1.5 fold methane yields were noticed in co-digestion, when compared to pig waste

monodigestion (Parameswaran and Rittmann 2012). The benefits of the co-digestion should be carefully thought when talking about materials from animal origin (e.g. meat industry by-products) or from processes with different chemicals (like pulp and paper mill wastewater sludge) to properly manage quality requirements of digestates for different uses. Pre-treatments of substrates are also widely studied to improve anaerobic digestion processes. Because of the wide variety of methods and their process parameters, as well as the wide variety of substrates, comparison and generalisation are difficult. Trace element additions are one interesting, and not so well understood, enhancement method. Pre-treatment methods, as well as additives, are discussed more widely below.

1.2.5 Substrate pre-treatment methods

Pre-treatments aim to alter the substrate composition to make it more suitable for digestion (Appels *et al.* 2011). In the case of lignocellulosic substances, the objective is to break the lignin seal and dissolve the hemicellulose to make the cellulose more accessible to hydrolysis (Mosier *et al.* 2005, Hendriks and Zeeman 2009). In the case of activated sludge, the goal of the pre-treatment is to disintegrate the sludge cells, and thus, release and solubilise the intracellular matter (Appels *et al.* 2008). Pre-treatment methods can be physical, chemical or biological, or combinations of them (Agbor *et al.* 2011). Physical methods include e.g. mechanical, thermal and ultrasound pre-treatments (Taherzadeh and Karimi 2008, Agbor *et al.* 2011). Chemical methods include alkaline or acid hydrolysis, organosolv, wet oxidation and ozonolysis pre-treatments, while biological pre-treatments include micro-organisms like fungi or bacteria, or purified enzymes (Taherzadeh and Karimi 2008, Agbor *et al.* 2011). The main effects of pre-treatments on the substrates are particle size reduction, decrystallisation of cellulose, solubilisation of hemicellulose and alteration of the lignin structure (Hendriks and Zeeman 2009, Carlsson *et al.* 2012). However, pre-treatments may also lead to the formation of refractory compounds and loss of organic material (Carlsson *et al.* 2012). The pre-treatments relevant to this thesis are briefly introduced in Table 1.

Some pre-treatment methods can lead to the production of inhibitory compounds, which have been reported to have negative effects on microbial cell growth and metabolism (reviewed by Barakat *et al.* 2012). The breakdown of lignin may release phenolic compounds like syringaldehyde or vanillin, while furans like furfural and 5-hydroxymethylfurfural (HMF) may be produced in the hydrolysis of hemicelluloses (Barakat *et al.* 2012). In addition, during the pre-treatment of protein and carbohydrates, melanoidins are formed through Maillard reactions (reviewed by Carlsson *et al.* 2012). On the other hand, it has also been shown that micro-organisms can adapt to inhibitory compounds. In a previous study, compounds formed in lignocellulosic pre-treatment, such as furans (furfural and HMF), phenolic compounds (syringaldehyde and vanillin) and lignin polymers, were observed to produce methane with yields from 14 to 453 $dm^3\ kg^{-1}$ of compound (Barakat *et al.* 2012).

TABLE 1 Summary of the theory of the pre-treatment methods used in the present thesis.

Method	Details	Effects	Substrates used	Reference
Acid	E.g. H_2SO_4, HNO_3 or HCl, also with high temperature	Solubilises hemicellulose to make cellulose better accessible	Lignocellulosic materials, OFMSW, manure	Hendriks and Zeeman (2009), Agbor *et al.* (2011), Carlsson *et al.* (2012)
Alkali	E.g. KOH, NaOH or $Ca(OH)_2$, also with high temperature	Solubilises hemicellulose and parts of lignin to make cellulose better accessible	Lignocellulosic materials, WWTP sludge, OFMSW, manure	Hendriks and Zeeman (2009), Agbor *et al.* (2011), Carlsson *et al.* (2012)
Enzymatic	Addition of specific enzymes	Improves depolymerization of lignocellulosics, Disrupts cell wall and membrane and thus solubilises cell components	Lignocellulosic materials, WWTP sludge	Jørgensen *et al.* (2007), Appels *et al.* (2008), Bruni *et al.* (2010)
Thermal	Exposing to high temperature, Hydrothermal pre-treatment with high pressure, Steam explosion with high pressure saturated steam	Solubilises hemicellulose and parts of lignin to make cellulose better accessible, Disrupts cell wall and membrane and thus solubilises cell components	Lignocellulosic materials, WWTP sludge, OFMSW, manure	Appels *et al.* (2008), Hendriks and Zeeman (2009), Carlsson *et al.* (2012)
Ultrasound	Exposing to sound waves at frequencies of 20 kHz–500 MHz	Disrupts cell wall and membrane and thus solubilises cell components	WWTP sludge, OFMSW, manure	Pilli *et al.* (2011), Carlsson *et al.* (2012)

1.2.6 Additives in anaerobic digestion

In a study of full scale biogas plants in Europe, low trace element concentrations were seen widely, although the variation in concentrations was high. Low trace element concentrations were seen, especially when substrates included high amounts of energy crops and manure, or when glycerol was fed besides agricultural feedstock (Schattauer *et al.* 2011). In laboratory scale studies, Se and Co (Banks *et al.* 2012) as well as Co, Mo, Ni, Se and W (Facchin *et al.* 2013) were seen to enhance the anaerobic digestion of food waste, Co the digestion of grass-clover silage (Jarvis *et al.* 1997) and Co or Ni alone or mixed with Fe, Zn, Mn, B, Cu, Se, Mo and W the digestion of a defined model substrate for maize silage (Pobeheim *et al.* 2010). In addition to nutrient additions, other additives have also

been studied. Decreasing the pH from 8.0 to 7.6 or 7.8 by hydrogen chloride acid (HCl) addition was shown to increase methane yields from 400 to 600 dm^3 $kgVS^{-1}$ in the anaerobic digestion of slaughterhouse waste, manure and mycelium at 37 ˚C (Karlsson and Ejlertsson 2012).

1.3 Industrial by-products

1.3.1 Background

Industrial wastes are wastes generated during the production of industrial goods and products. In the European Union (EU-27), 342 million t of wastes were produced in the manufacturing sector in 2008, while the amount of waste from households was 221 million t (Anon 2012). Industrial by-products are formed in production processes in addition to the primary product and many wastes could be by-products if they were used further, instead of treating them as wastes.

1.3.2 Animal production

Meat production is a worldwide industry that is predicted to grow at an average of 1.8 % per year until year 2020, and the production increase is predicted to be mainly in developing countries and in the sectors of poultry and pig meat production (Anon 2011a). In EU-27, 7.9 million t of cattle, 22.0 million t of pigs and 11.6 million t of poultry were slaughtered in 2010 (Anon 2011b). In EU-27, the cattle livestock numbers fell slightly during the years 1995-2010, while the number of pigs was stable. In Finland, 203,000 t of pigs, 82,000 t of cattle, and 96,000 t of poultry were slaughtered in 2010 (Anon 2011b). The relationships between animal production, animal slaughtering and rendering are presented in Fig. 2.

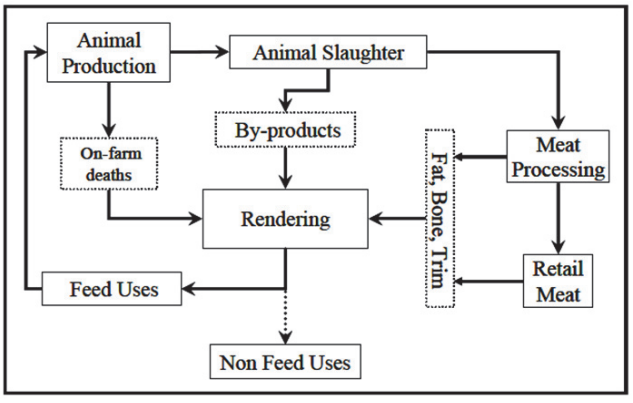

FIGURE 2 Schematic representation of animal production, animal slaughtering and rendering (Hamilton *et al.* 2006).

Animal by-products (ABP) are materials of animal origin that are unsuitable for human consumption, including e.g. dead animals, animal by-products from slaughterhouses and food waste (Anon 2009b). The handling of these materials is strictly regulated in the EU because of their possible health risks. This is largely because meat and bone meal was thought to spread transmissible spongiform encephalopathy (TSE) since the 1980s, mainly in the UK and from there, worldwide (Budka *et al.* 2008). The TSE disease, bovine spongiform encephalopathy (BSE), is untreatable deadly disease that was suggested to be the reason for Creutzfeldt-Jakob disease, which caused an epidemic in the mid-1990s (Budka *et al.* 2008). As a protective measure, the use of meat and bone meal for feed of animals farmed for human production has been abandoned (Budka *et al.* 2008).

The treatment of animal by-products has been divided into two regulations, Regulation (EC) No 1069/2009 and Commission Regulation (EU) No 142/2011 giving more specific information. Regulation (EC) No 1069/2009 lays down the rules for animal by-products and derived products in order to prevent risks to public and animal health (Anon 2009b, Anon 2011c) and in this regulation, animal by-products are divided into three categories based on their risks to public and animal health (Anon 2009b). Category 1 material includes entire bodies and all parts from animals suspected to be infected by TSE. This material must be incinerated or buried in an authorized landfill after pressure sterilization (Anon 2009b). Category 2 material includes manure and contaminated animal bodies, or other animal by-products, and in addition to incineration or burial, this material can be e.g. composted or digested into biogas (Anon 2009b). Category 3 material includes e.g. food waste and by-products from animals treated for human consumption, and in addition to alternatives in the Category 2 material, this material can be e.g. processed for raw pet food (Anon 2009b).

1.3.3 Meat industry by-products

In slaughterhouses, animals intended for human consumption are killed to produce meat products. When processing the meat from a slaughtered animal, one-third to one-half of each animal is not used by humans and ends up as by-products (Meeker and Hamilton 2006).

Slaughterhouse wastes are a large group of waste fractions with various characteristics and can include both solid and liquid materials. In addition, sludge originating from wastewater treatment plants at slaughterhouses is often included in slaughterhouse wastes. Some of the slaughterhouse wastes, like flotation sludge, can have a high water content of over 95 % (Luste *et al.* 2009) while others (like fat) may have a dry mass content of nearly 100 % (Pitk *et al.* 2012). On the other hand, even the same fractions are reported to vary in characteristics; for instance, the total solids (TS) content of the flotation sludge is reported to be between 4.3 and 22 % (Luste *et al.* 2009, Pitk *et al.* 2012). This high variation is because of the various animals slaughtered as well as the different processes.

Rendering is a process in which slaughtering by-products are stabilized with heat (Woodgate and van der Veen 2004). The main purpose of this process is to evaporate the water content and to sterilize the material and at the same time, some end products are produced for further use. The rendering process covers the treatment of both edible and inedible animal by-products (Woodgate and van der Veen 2004). Raw materials of the rendering process include whole carcasses, hides, skins, hair, feathers, hoofs, horns, feet, heads, bones, toe nails, blood, organs, glands, intestines, muscle and fat tissues, and shells (Meeker and Hamilton 2006), and originate from all farmed animals, of which cattle, pigs and poultry are the most common. Since raw materials from the rendering process vary, so do the characteristics of the rendering wastes. However, the approximate composition of rendering waste is 60 % water, 20 % proteins and minerals and 20 % fat (Meeker and Hamilton 2006).

The rendering process can consist of different processes which can include both physical and chemical parts, and thus produce different kinds of products (Woodgate and van der Veen 2004, Meeker and Hamilton 2006). However, the application of heat, extraction of moisture and fat separation are always included (Meeker and Hamilton 2006). The process can be either wet or dry (Fig. 3), depending on the raw material characteristics and it can be operated as a batch or a continuous process (Woodgate and van der Veen 2004). The dry process is the most commonly used nowadays. Materials produced in the rendering process contain high concentrations of proteins or lipids, and they are mainly used as animal feed ingredients, but also in the chemical, metallurgical, rubber and oleochemical industries as well as in the production of soap and personal care products (Meeker and Hamilton 2006).

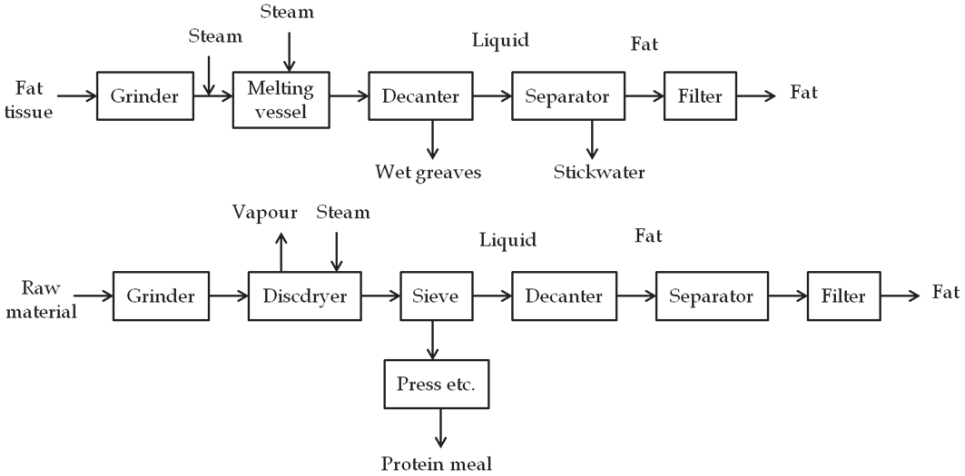

FIGURE 3 Examples of wet (above) and dry (below) rendering process concepts (adapted from Woodgate and van der Veen 2004).

1.3.4 Anaerobic digestion of meat industry by-products

Waste fractions and their characteristics, and thus methane yields, vary according to the type of animal slaughtered, and the rendering processes. Typically, the methane potential for meat-industry by-products is high, and can be up to 978 dm^3 $kgVS_{added}^{-1}$ (Pitk *et al.* 2012). The reason for the high methane potential is the high protein and lipid content in these materials. However, not all meat industry by-products have high methane potentials. For example, feathers are a waste fraction with a methane potential of only 210 dm^3 kg volatile solids $(VS)_{added}^{-1}$ (Salminen *et al.* 2003).

Previous results of the monodigestion of meat industry by-products are scarce. In monodigestion experiments, the applied HRT scale is large: 13–100 d with the OLRs ranging from 0.5 to 2.1 kgVS m^{-3} d^{-1} (Salminen and Rintala 2002, Alvarez and Lidén 2008, Cuetos *et al.* 2008). In the above studies, methane yields of 60–700 dm^3 $kgVS_{added}^{-1}$ were reported. This high variation is due to the differences in the types of animals slaughtered, waste fractions used and operational conditions; however, one more reason for the discrepancy is the inhibition in some studies due to the production of ammonia, VFAs and/or LCFAs.

Despite the scarce studies on meat industry by-products monodigestion, co-digestion has been studied more. In previous studies, slaughterhouse wastes have been reported to be feasible co-substrates, e.g. in co-digestion with sewage sludge, maize, manure or fruit and vegetable wastes methane yields between 40 and 645 dm^3 $kgVS_{added}^{-1}$ have been reported (e.g. Alvarez and Lidén 2008, Luste and Luostarinen 2010, Cuetos *et al.* 2013, Pitk *et al.* 2013).

1.3.5 Pulp and paper production

Globally, pulp production remained unchanged, with an annual production of around 175 million t, when comparing years 1999 and 2009 (Anon 2011d). On the other hand, the production of paper and paperboard increased nearly by 20 %, from 315 to 377 million t, during the same period (Anon 2011d). In EU-27, the production of pulp was 35 million t and the production of paper and paperboard was 88 million t in 2009 (Anon 2011d). In 2012, 7.9 million t of paper, 2.8 million t of cardboard and 10.2 million t of pulp mass were produced in Finland (Anon 2013a).

There are various pulp and paper production processes in use. Wood pulp processes can be divided into three groups: mechanical, chemical or combinations of these (Thompson *et al.* 2001). In the mechanical process, the wood block passes through a rotating grindstone where the fibres are stripped off and suspended in water (Thompson *et al.* 2001). In the chemical process, chemicals are used to break down the wood in the presence of heat and pressure (Thompson *et al.* 2001, Pokhrel and Viraraghavan 2004). Depending on the type of chemical used, the chemical process is divided into kraft and sulphite processes (Pokhrel and Viraraghavan 2004). In the widely used kraft process, sodium hydroxide (NaOH) and sodium sulfide (NaS_2) are used, while

in the sulphite process, sulphurous acid (H_2SO_3) and hydrogen sulfite ions (HSO_3^-) are used (Pokhrel and Viraraghavan 2004).

Pulp is used for paper making. First, the pulp stock is prepared by treating the pulp to the required degree of fitness (Pokhrel and Viraraghavan 2004). When making paper, the pulp is diluted to at least 99 % with water and other additives and to obtain different kind of paper products, dyes, coating materials or preservatives can be added (Thompson *et al.* 2001). The diluted pulp is distributed evenly along a continuously moving wire (Thompson *et al.* 2001, Pokhrel and Viraraghavan 2004), and most of the water drains through the wire, while the rest is removed with a vacuum drier, pressing and passing it through steam-heated cylinders, and the paper sheets are formed while pressing (Thompson *et al.* 2001).

1.3.6 Pulp and paper mill by-products

The widely used wastewater treatment process at the pulp and paper mills is the aerobic activated sludge treatment process (Hagelqvist 2013). This treatment consists of two main parts, primary clarification and the activated sludge process and in this treatment, two kinds of wastewater sludge are produced. In primary clarification, primary sludge is produced, while in the activated sludge process, secondary sludge (also called biosludge or activated sludge) is produced (Fig. 4). In Finland, 39,500 t TS of wastewater treatment sludge were produced in 2012 in pulp and paper mills (Anon 2013a).

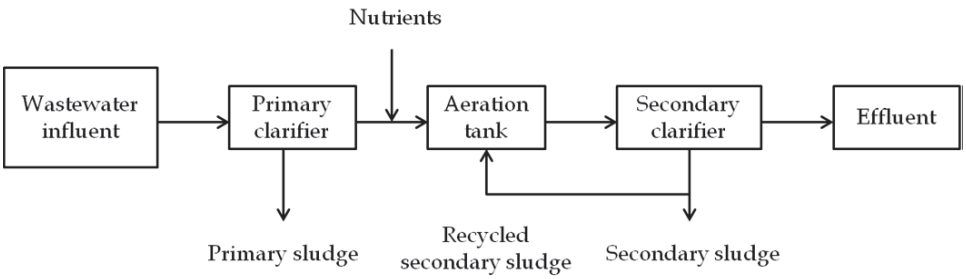

FIGURE 4 Process diagram of typical activated sludge wastewater treatment process.

Pulp and paper mill wastewater contains low levels of nitrogen and phosphorus, because their levels in the wood are also low (Slade *et al.* 2004). Thus, nutrients must be added to the activated sludge process to make the process work (Slade *et al.* 2004). Nowadays, pulp and paper mill wastewater treatment sludge are generally dewatered mechanically, and then either incinerated or composted (Hagelqvist 2013). However, the high water content makes the incineration energetically unfavourable, and the low nutrient content prevents the use of the compost as a fertilizer (Hagelqvist 2013).

The characteristics of the pulp and paper industry wastewater sludge varies, e.g. according to the production process, raw materials of the mill and

wastewater treatment process. Pulp and paper mill primary sludge consists of wood fibres (e.g. cellulose, hemicellulose and lignin), inorganic papermaking fillers like kaolin and $CaCO_3$, pitch (wood resin), lignin by-products and ash (Ochoa de Alda 2008). Secondary sludge consists of microbial biomass, cell-decay products and lignin precipitates (Puhakka *et al.* 1992). Typical for both sludges are low concentrations of VS and TS. In addition, they have low nitrogen, phosphorus and trace element concentrations (Ghosh and Taylor 1999).

In the activated sludge process, microbial flocs are formed. In addition to the microbial cells, microbial flocs include other organic material and inorganic substances. The organic materials other than cells are called extra-cellular polymeric substances (EPS), as reviewed by Park and Novak (2007). EPS composition is heterogeneous, but may include e.g. proteins, carbohydrates and humic compounds, uronic acid and DNA (Frølund *et al.* 1996). Of the activated sludge, 20–25 % of the TS or 33–42 % of the VS can be EPS (Frølund et al. 1996). The role of EPS is to bind cells and other particulate materials together (reviewed by Park and Novak 2007); however, the composition of the EPS is dependent on the type and composition of the wastewater coming to the activated sludge plant (Sponza 2003).

1.3.7 Anaerobic digestion of pulp and paper mill wastewater sludge

Previous studies on the anaerobic digestion of pulp and paper mill wastewater sludge are scarce. In the digestion of pulp and paper mill wastewater sludge, relatively low biodegradability has been suggested as VS removals in the batch digestion of secondary, and the mixture of primary and secondary sludge made up of bleached chemi-thermo-mechanical pulp mill (BCTMP), was reported to be 9–23 % at 35 and 55 °C (Saha *et al.* 2011). For secondary sludge, methane potentials between 100 and 200 $dm^3\ kgVS_{added}^{-1}$ have been reported in batch and continuous stirred tank reactor (CSTR) experiments (Karlsson *et al.* 2011). For primary sludge, 45 $dm^3\ kgVS_{added}^{-1}$ was reported at 35 °C (Jokela *et al.* 1997). Pulp and paper mill secondary sludge digestion was seen to be applicable in co-digestion with monosodium glutamate waste liquor (MGWL), with accumulative methane yields of 200 $dm^3\ kgVS_{added}^{-1}$ (Lin *et al.* 2011). The high water content of the sludge may affect the methane production, and higher methane yields per VS have been observed with dewatered sludge than in sludge without dewatering (Karlsson *et al.* 2011). For instance, the methane yield from sulphite mill sludge was 159 $dm^3\ kgVS^{-1}$ before dewatering and 176 $dm^3\ kgVS^{-1}$ after dewatering. Similarly, the methane yields for kraft mill sludge were 145 and 188 $dm^3\ kgVS^{-1}$, respectively (Karlsson *et al.* 2011). The above authors suggested this to be because soluble salts and other slowly or non-degradable constituents like humic and lignin residues were transferred into the reject water and thus the degradable organic matter fraction of the sludge concentrated (Karlsson *et al.* 2011).

Previous studies about anaerobic digestion and the pre-treatments of pulp and paper mill wastewater sludge are scarce. More results have been published about the anaerobic digestion of municipal wastewater treatment sludge; however, since the municipal wastewater differs from that of pulp and paper mills (e.g. in nutrient amounts), these results cannot be generalised (Bhathena *et al.* 2006). The increased methane yield of pulp and paper mill secondary sludge has been reported, at least with thermal, ultrasound, enzymatic and microwave pre-treatments (Wood *et al.* 2009, Karlsson *et al.* 2011, Saha *et al.* 2011).

2 OBJECTIVES

The objective of this thesis was to evaluate the feasibility of industrial by-products from the meat industry (slaughterhouse and rendering wastes), and pulp and paper industry (wastewater treatment plant primary and secondary sludge), as substrates for biogas production. This objective was divided into sub-objectives as follows:

- To characterise and determine the methane potentials of selected slaughterhouse and rendering waste materials, and to study the methane yields and process performance during the long-term co-digestion of the two materials at two different process temperatures (I).
- To study the monodigestion of pig slaughterhouse waste and to evaluate the effect of two additives on the anaerobic digestion process of pig slaughterhouse waste (II).
- To determine the methane potentials of the pulp and paper industry wastewater treatment plant primary and secondary sludge at two different process temperatures, and to study the methane yields and process performance during the long-term monodigestion of primary sludge and co-digestion of primary and secondary sludge (III).
- To evaluate the feasibility of 12 single or combined pre-treatment methods on the characteristics and methane yields of pulp and paper mill secondary sludge (IV).

3 MATERIALS AND METHODS

3.1 Experiments

In this thesis, the anaerobic digestion of meat and pulp and paper industry by-products was studied in batch experiments, as well as in semi-continuously fed CSTR experiments. In addition, methods to improve the digestion process of pig slaughterhouse waste using additives, and pulp and paper mill secondary sludge using pre-treatment methods were studied. The experiments of this thesis are summarised in Table 2.

TABLE 2 Experiments conducted in the thesis showing the substrates, objectives and operational temperatures of the systems.

Substrate	Objective	System	Temperature °C	Paper
Slaughterhouse waste and rendering wastes	Determine methane potentials	Batch	35	I, II
	Study methane yield and process parameters in two temperatures	CSTR	35 and 55	I
Pig slaughterhouse waste	Study methane yield and process parameters in mono-digestion	CSTR	35	II
	Study effect of two additives on methane yields and process stability	CSTR	35	II
Pulp and paper mill primary and secondary sludge	Determine methane potentials in two temperatures	Batch	35 and 55	III
	Study methane yield and process parameters	CSTR	55	III
Pulp and paper mill secondary sludge	Study effect of pre-treatment methods on methane yields and digestion	Batch	55	IV

3.2 Substrates and inocula

3.2.1 Meat industry by-products (I, II)

Meat industry by-products used in the present thesis included three fractions of slaughterhouse wastes and seven fractions of rendering wastes. Slaughterhouse wastes, that is, the contents of the stomach and intestines of cattle (without rumen and reticulum) and pig, were from a meat producing factory (Saarioinen Ltd., Jyväskylä). At the laboratory, cattle and pig slaughterhouse by-products were minced (5 mm) by using a meat mincer (Talsa W 22). Minced poultry (turkey) material was delivered by Honkajoki Ltd., Finland. The homogenized materials were stored at –20 °C until further use. Rendering wastes (fat from fat separation, separator sludge, melt, decanter sludge, biosludge, fat and boneflour) were collected from a rendering plant (Honkajoki Ltd., Finland) and stored at 4 ˚C until further use. The characteristics of the studied meat industry by-products are presented in Table 3.

3.2.2 Pulp and paper industry by-products (III, IV)

The pulp and paper industry by-products used in the present thesis were wastewater treatment primary and secondary sludge obtained from a pulp (kraft) and paper mill (Kaukas, Lappeenranta, Finland). The characteristics of the pulp and paper industry by-products used are presented in Table 4. The cellulose, hemicellulose and lignin contents were analysed from the third batch of the each sludge. For the primary sludge (III) cellulose, hemicellulose and lignin contents were 660, 103 and 88 g $kgVS^{-1}$ and for the secondary sludge (IV) 130, 250 and 190 g $kgVS^{-1}$, respectively. In the CSTR co-digestion study (III), primary and secondary sludge were used at a ratio of 3:2 upon VS basis.

3.2.3 Inocula (I–IV)

For the mesophilic experiments, digested sludge from a municipal wastewater treatment plant (MWWTP, Nenäinniemi, Jyväskylä, Finland, I) and digestate from a farm biogas plant treating cow manure and confectionary by-products (Kalmari, Laukaa, Finland, II) were used as the inocula. In the thermophilic experiments (I, III, IV), digested sludge from a full-scale thermophilic biogas plant (Stormossen, Vaasa, Finland), treating the putrescible organic fraction of municipal waste and sewage sludge, was used as the inoculum. The characteristics of the inocula used are presented in Table 5.

TABLE 3 Characteristics of the meat industry by-products used in the present study (I, II).

Substrate	Production method	Batch	TS % WW	VS % WW	TKN g kgFM^{-1}	Proteins g kgFM^{-1}	Proteins g kgVS^{-1}	Lipids g kgFM^{-1}	Lipids g kgVS^{-1}	Paper
Slaughterhouse wastes										
Pig		1	32	28	16	99	351	148	525	I
		2	32	31	15	nd	nd	nd	nd	II
Cattle		1	53	53	5.6	35	57	461	767	I
Poultry		1	38	33	26	164	483	151	453	I
		2	33	30	nd	nd	nd	nd	nd	
Rendering wastes										
Fat from fat separation	Fat separated with H_2O_2 from wastewater of production equipment and room	1	24	22	4.2	26	117	198	892	I
		2	78	76	nd	nd	nd	nd	nd	
Separator sludge	Water, protein and fat extracted in final purification by centrifuge from sterilised and solids separated fat	1	2.2	2.0	0.3	2	100	16	800	I
		2	22	21	nd	nd	nd	nd	nd	
Melt	Sterilised (133 ˚C, 20 min, 3 bar) mass	1	98	67	69	429	644	220	330	I
		2	98	75	nd	nd	nd	nd	nd	
Decanter sludge	Solids, separated by centrifuge from fat separated by pressing from sterilised mass	1	98	62	61	384	619	221	356	I
		2	99	75	nd	nd	nd	nd	nd	
Biosludge	Sludge from wastewater treatment	1	1.0	0.9	1.2	8	844	1	111	I
		2	2.3	2.0	nd	nd	nd	nd	nd	
Fat	Sterilised and purified fat	1	99	99	1.1	7	7	935	943	I
Boneflour	Solids separated by pressing from sterilised mass	1	99	56	72	450	804	100	179	I

nd = not determined

TABLE 4 Characteristics of the pulp and paper industry by-products used in the present thesis (III, IV).

Substrate	Batch	TS %	VS %	SCOD g l^{-1}	NH_4-N mg l^{-1}	TKN g l^{-1}	Paper
Primary sludge	1	3.4	2.9	1.8	<1	0.1	III
	2	3.8	3.2	1.5	<1	0.1	
	3	2.7	2.2	1.4	<1	0.1	
Secondary sludge	1	4.0	3.3	0.7	31	2.0	III
	2	3.6	2.9	0.4	8	1.9	
	3	4.7	3.9	1	37	-	IV
Reject water	1	0.2	0.1	0.9	0.4[a]	0.5	III

[a] unit g l^{-1}

TABLE 5 Characteristics of the inocula used in the experiments (I, II, III, IV).

Origin	Temperature °C	TS %	VS %	pH	Paper
MWWTP (Nenäinniemi)	35	1.5	0.8	7.9	I
Full scale (Stormossen)	55	2.7	1.2	8.5	I
Farm (Kalmari)	35	5	4	7.8	II
MWWTP (Nenäinniemi)	35	2.2	1.1	7.3	III
Full scale (Stormossen)	55	4.4	2.4	8.2	III
Full scale (Stormossen)	55	2.9	1.7	8.1	IV

3.3 Experimental set-up

3.3.1 Biochemical methane potential experiments (I–IV)

Biochemical methane potential experiments were conducted as batch experiments in 1 l glass bottles with 3 replicates (I, II, III), or in 118 ml glass bottles with 4 replicates (IV). The amount of inoculum added to each bottle was 250 ml (I), 300 ml (II and 55 °C III), 350 ml (35 °C III), and 30 ml (IV). To each bottle, the studied substrate was added at a substrate to inoculum VS ratio of 0.25 (I), 1 (II), 1.7–1.9 (35 °C III), 1.1–1.3 (55 °C III), and 2 (IV). Tap water was added in order to obtain the desired working volume of 750 ml (I, II), ca. 720 ml (III) and 60 ml (IV, except for 65 ml in the case of the enzymatic pre-treatments, due to a 5 ml addition of citrate buffer). In all assays (I, II, III, IV), $NaHCO_3$ (3 g l^{-1}) was added as a buffer.

The 118 ml glass bottles were closed with butyl rubber stoppers and aluminium crimps (IV). The prepared assays were flushed with N_2 gas for 3 min in order to create anaerobic conditions before (I, II, III) or after (IV) closing the bottles. The 1 l bottles were sealed with silicon stoppers (I, II). All assays were incubated statically at 35 ± 1 °C (I, II, III) or 55 ± 1 °C (I, III, IV) for 56–85 (I), 67 (II), 42 (III) or 140–152 (IV) days. The produced biogas was collected into

aluminium gas bags (I, II, III). The bottles were mixed manually before each gas analysis.

In all of the batch experiments (I, II, III, IV), bottles with the inoculum and water were used as blanks. Methane production of the inoculum was subtracted from the methane production of the respective sample assays. In the pre-treatment experiments (IV), assays with inoculum and water were used as blanks for all of the pre-treatments, except for the assays with enzymatic pre-treatment. For the enzymatic pre-treatments, inactivated enzymes and a citrate buffer (boiled for 10 min) were used, in addition to the inoculum and water. Additionally, assays with untreated sludge, inoculum and water were used as control assays.

3.3.2 Long-term digestion experiments (I, II, III)

Long-term digestion experiments were carried out in semi-continuously fed CSTR experiments with 5 l glass reactors (III, Fig. 5) and 13 l stainless steel reactors (I, II). The working volume of the reactors was 4 l (III) and 10 l (I, II), and the experiments were carried out under mesophilic (I, II) and thermophilic (I, III) conditions. The temperature in the reactors was maintained by a heating coil wrapped around the insulated reactor and controlled thermostatically (I, II), or by keeping the reactors in an incubator (III). The reactors were mixed mechanically with a timer (13 min on and 16 min off, I, II) or by magnetic stirrers (400–700 rpm, III). The produced biogas was collected into aluminium gas bags.

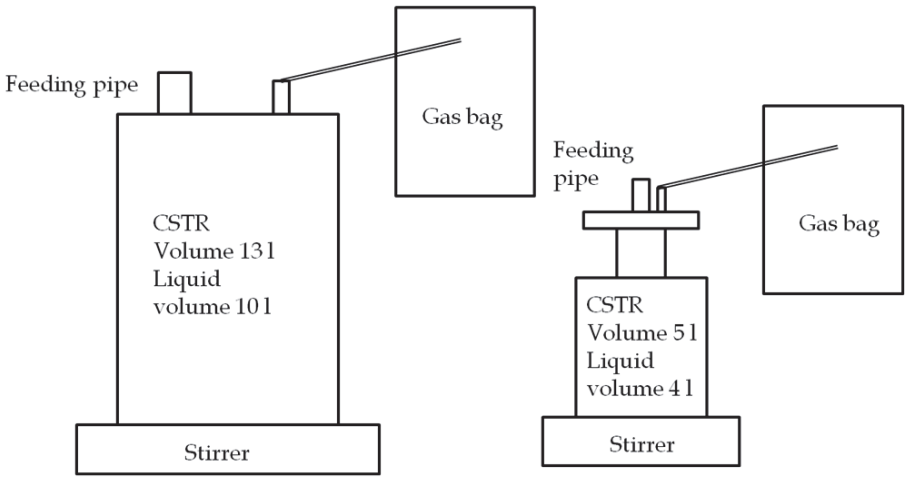

FIGURE 5 Schematic figure of CSTRs used in the long-term digestion experiments.

The start-up of the reactor was created with the inoculum and start-up material. To both CSTRs treating the slaughterhouse wastes and rendering wastes (I), 9.7 l of inoculum and 50 g of separator sludge were added. The separator sludge was added twice after that before the actual feeding was initiated on day 14. For

each reactor fed with pig slaughterhouse waste (II), 10 l of inoculum and 5 g of pig slaughterhouse waste were added during the start-up. The actual feeding was initiated on day 4. Similarly, for the reactors treating pulp and paper mill wastewater sludges (III), 4 l of inoculum was added at the beginning and on day 5, 500 g of feed was added to the reactors treating primary sludge and 320 g of feed was added to the reactor co-digesting primary and secondary sludge. The actual feeding was started on day 7. The process parameters of the experiments are summarised in Table 6.

TABLE 6 Process parameters in CSTR studies (I, II, III).

Substrate	Reactor abbrev.	Liquid volume l	Temp. °C	OLR kgVS m^{-3} d^{-1}	HRT d	Operation d	Paper
Pig, cattle, poultry slaughterhouse waste and rendering wastes	R1	10	35	0.5–2	50	178	I
	R2	10	55	0.4–2.25	50–109	168	I
Pig slaughterhouse waste and additives[a]	S1	10	35	1–2	30	284	II
	S2	10	35	1–2	30	242	II
	S3	10	35	1–2.5	30	242	II
Pulp and paper mill primary sludge	R1	4	55	1	23–32	122	III
	R2	4	55	1.4–2	14–16	122	III
Pulp and paper mill primary and secondary sludge	R3	4	55	1	25–31	122	III

[a] Additions presented in Table 7.

The reactors were fed manually with a gas tight plastic syringe (III) and through an airtight feeding tube (I, II) when the mixing was off. Before each feeding, an equal amount of digestate was taken out. The feeding was done five days per week (from Monday through Friday, I, II, III), except for seven days per week on d 49–178 (I) and four days per week (Monday, Tuesday, Thursday and Friday) in R2 on d 73–122 (III). Feed was prepared by diluting the waste materials with tap water or a liquid fraction of the digestate to get the desired OLR and HRT (I, II). Tap water was used in all experiments (I, II), except in the pig slaughterhouse waste monodigestion, where a recirculated liquid fraction of the digestate was used from day 18 onwards (32–38 % of liquid, 95 ml d^{-1}, II). Recirculation of the liquid fraction of the digestate was used to simulate better full-scale plant conditions and to reduce the amount of pure water usage. The liquid fraction was separated from the digestate by centrifuging (3000 rpm, 10 min). On day 82, the reactor contents were mixed to obtain identical digestate quality in each reactor before the additive feedings begun on d 98 (II). When the additives were used, they were added to tap water before mixing with the pig slaughterhouse waste (II). Since the wastewater sludge from the pulp and paper mill contain low

concentrations of TS, no extra water was added to them when preparing the reactor feeds (III). In the slaughterhouse and rendering waste experiments (I), slaughterhouse and rendering wastes were added to the feed in the following fractions (% of feed fresh matter (FM), water dilution not taken into account): pig 25.2 %, cattle 26.7 %, poultry 38.2 %, fat from fat separation 1.4 %, separator sludge 4.0 %, melt 1.1 %, decanter sludge 1.3 %, and biosludge 2.2 %, which were calculated based on the amounts available in the studied case.

Nitrogen (NH_4Cl) was added to the feed of the reactors treating primary sludge (R1 and R2, III) on days 46–122 in an amount such that the NH_4-N concentration of the reactor digestates increased to > 0.8 g l^{-1} around d 50. In addition, reject water from the dewatering of the digested sludge from the MWWTP (Nenäinniemi, Jyväskylä, Finland) was also added to the feeds of the same reactors (III) on days 60–95 as a nutrient source. The amount of reject water was 10 % of the volume of the feed (R1: 12.5 g d^{-1} and R2: 25 g d^{-1}) and this decreased the OLRs of the reactors R1 and R2 from 1 and 2 kgVS m^{-3} d^{-1} to 0.9 and 1.8 kgVS m^{-3} d^{-1}, respectively, during days 60–95.

When studying the effect of the additives on pig slaughterhouse waste monodigestion (II), two additive solutions delivered by Kemira Oyj, were used. Additive 1 (referred to A1 in this thesis) contained Fe and HCl and Additive 2 (referred to A2 in this thesis) contained Fe, HCl and trace elements. The composition of the additives and trace element contents of the substrate and inoculum are presented in Table 7.

TABLE 7 Composition of the additives, trace element contents of the substrate and inoculum and amounts added to reactors S1-S3 (II).

	Additive 1	Additive 2	Pig slaughterhouse waste	Inoculum
Fe_{tot} %	10 ± 2	9 ± 1	180[a]	621[a]
Fe^{3+} %	6.7 ± 1.4	6.0 ± 1.4	nd	nd
Fe^{2+} %	3.3 ± 0.7	3.0 ± 0.3	nd	nd
Free HCl %	2 ± 0.5	2 ± 0.5	nd	nd
Co mg $kgTS^{-1}$	No	Yes	1.7	2.6
Ni mg $kgTS^{-1}$	No	Yes	2.2	4.8
Se mg $kgTS^{-1}$	No	Yes	nd	nd
W mg $kgTS^{-1}$	No	Yes	4.5	3.1
Amounts added to the reactors g $reactor^{-1}$ d^{-1}				
S1 (d 218)	-	8.75	-	-
S1 (d 224–284)	-	0.67	-	-
S2 (d 98–125)	0.54	-	-	-
S2 (d 126–187)	0.76	-	-	-
S2 (d 188–242)	-	0.78	-	-
S3 (d 98–125)	-	0.67	-	-
S3 (d 126–187)	0.16	0.67	-	-
S3 (d 188–210)	0.21	0.89	-	-
S3 (d 211–223)	0.24	1.0	-	-
S3 (d 224–242)	-	1.1	-	-

[a] mg $kgTS^{-1}$
nd = not determined

3.3.3 Pre-treatments (IV)

All pre-treatments were performed in closed systems to prevent material losses during the pre-treatment. All pre-treatments, except for the hydrothermal pre-treatment, were carried out in ten parallel 118 ml serum bottles. After the pre-treatments, six of the ten parallel bottles were used for chemical analyses while the remaining four bottles were used for biochemical methane potential assays. For each pre-treatment, 260 g of secondary sludge was used, which meant 26 g $bottle^{-1}$. For hydrothermal pre-treatment, 260 g of the sludge was transferred to the hydrothermal reactor, and after the hydrothermal pre-treatment, the treated sludge was mixed and distributed among the four assay bottles (26 g $bottle^{-1}$), and the remaining amount was used for the chemical analyses. A summary of the parameters of the pre-treatments is presented in Table 8.

TABLE 8 Process parameters of the pre-treatments (IV).

Pre-treatment	Abbreviation	Process parameters
Control: Original	C1	-
Control: Enzyme+Buffer	C2	72 h, 50 °C
Ultrasound	US	30 min, 45 kHz
Alkali	AL	24 h, 22 °C, pH 12 with 5 M NaOH
Acid	AC	24 h, 22 °C, pH 3 with 5 M HNO_3
Enzyme	E	72 h, 50 °C
Ultrasound + Alkali	US+AL	30 min, 45 kHz + 24 h, 22 °C, 5 M NaoH
Hydrothermal 70 °C	HT70	40 min, 70 °C
Hydrothermal 150 °C	HT150	10 min, 150 °C
Ultrasound + Enzyme	US+E	30 min, 45 kHz + 72 h, 50 °C
Hydrothermal 150 °C + Enzyme	HT150+E	10 min, 150 °C + 72 h, 50 °C
Hydrothermal 70 °C + Enzyme	HT70+E	40 min, 70 °C + 72 h, 50 °C
Ultrasound + Hydrothermal 150 °C	US+HT150	30 min, 45 kHz + 10 min, 150 °C
Ultrasound + Hydrothermal 150 °C + Enzyme	US+HT150+E	30 min, 45 kHz + 10 min, 150 °C + 72 h, 50 °C

For the ultrasound pre-treatment, the ultrasonic apparatus, Branson 5210, was used with an operating frequency of 45 kHz for 30 min without heating. During the pre-treatment, the open ends of the bottles were closed with paraffin to prevent any loss of volatile compounds. For the alkali pre-treatment, 5 M NaOH was added to the samples to raise the pH to 12, and the samples were incubated at room temperature for 24 h. The amount of NaOH used was 0.38 g gVS^{-1}. After the pre-treatment, the pH was adjusted to 7 with 5 M HNO_3. For the acid pre-treatment, 5 M HNO_3 was used (0.48 g gVS^{-1}) to attain a pH of 3 and the samples were incubated at room temperature for 24 h. After the treatment, the pH was adjusted to 7 with 5 M NaOH. Hydrothermal pre-treatment was carried out using a high pressure (250 bars) and temperature (250 °C) reactor (Berghof with Berghof DTR 841 heating system, Germany). After sample (260 g) loading, the headspace of the reactor was flushed with nitrogen gas to exclude

oxygen and prevent any oxidation of the organic compounds. The reactor contents were continuously mixed by a built-in mixer. It took 35 min to reach 70 °C and 55 min to reach 150 °C. During this period, the reactor pressure progressively increased to reach a final pressure of 2 bars at 70 °C and 5–6 bars at 150 °C. Upon completion, the samples were allowed to cool to room temperature and the reactor was opened only on the following morning. In order to prevent the loss of organic volatiles, and to avoid accidents and the spillage of materials due to high pressure and temperature, the reactor was not opened immediately. Therefore, hydrolysis/solubilisation would have continued during this cooling period. In the enzymatic pre-treatment, the commercial enzyme Accelerase_1500, with an endoglucanase activity of 2200–2800 CMC U g^{-1} and the β-glucosidase activity of 525–775 pNPG U g^{-1}, was used. Enzymatic hydrolysis was performed in ten 118 ml serum bottles. To each bottle, 26 g of sludge, 0.07 g gVS^{-1} of the Accelerase_1500 enzyme complex and 5 ml of sodium citrate buffer (50 mM, pH 4.8) were added. The prepared assays were incubated at 50 °C on a shaker (100 rpm) for 72 h in order to achieve 80–90 % cellulose to glucose conversion efficiency.

3.4 Analysis and calculations

The TS and VS were analysed according to the Standard Methods (Anon 1998). The pH was measured with a Mettler Toledo SevenEasy pH-meter (I, II, IV) or Metrohm 774 pH-meter (III) immediately after each sampling. The soluble chemical oxygen demand (SCOD) was analysed according to the Finnish Standard Methods (SFS 5504, Anon 1988). The ammonia nitrogen and total Kjeldahl nitrogen (TKN) were determined according to the Tecator application note (II, III, digestates in I, Anon 1995) or a standard method (substrates in I, method 984.13, Anon 1990). The VFAs were determined by using a gas chromatograph (Perkin-Elmer Autosystem XL GC, PE FFAP column 30 m × 0.32 mm × 25 μm). Helium was used as a carrier gas and the operation conditions were: oven 100–160 °C (25 °C min^{-1}), detector 225 °C and injector 230 °C. The samples for SCOD and VFA analyses were centrifuged (Sanyo Harrier 18/80 Refrigerated) at 2500 rpm (15 min), and for VFA, also at 12,000 rpm (10 min) and filtered through a glass microfilter (VWR Glass microfibers filter 691, particle retention 1.6 μm). The VFA concentrations were converted to SCOD equivalents with the following coefficients: acetic acid 1.066, propionic acid 1.512, iso-butyric and butyric acid 1.816, iso-pentanoic (valeric) and pentanoic (valeric) acid 2.036 and hexanoic (caproic) acid 2.204 (Ince 1998). The total volatile fatty acids (TVFA) were calculated by summing the individual acid concentrations, which were first converted to SCOD. Thus, all of the VFA values presented in this thesis have been converted to SCOD equivalents. In the liquid fraction separation (II), a Jouan centrifuge (3,000 rpm, 10 min) was used. For the SCOD analysis of the substrate, pig slaughterhouse waste was extracted

in a shaker for 1 h at 22 °C (II). The lipid content was analysed with ether extract after hydrolysis with 3 M HCl (Anon 1971). The results of the analyses are presented in the tables as the mean of the study period ± standard deviation.

Gas composition (CH_4, CO_2 and H_2) was measured by using a gas chromatograph (GC, Perkin-Elmer Arnel Clarus 500, Alumina column 30 m × 0.53 mm) equipped with a thermal conductivity detector (TCD, I, II) or a flame-ionization detector (FID, III, IV). Argon was used as a carrier gas and the operation conditions were: oven 100 °C, and detector and injection port 225 °C. Gas samples for gas composition analyses were taken through stoppers from the gas phase with a pressure-locked glass syringe (Supelco, Pressure-Lok® Series A-2 Syringe, Bellefonte, USA). The biogas volume was measured using the water displacement method (I, II, III) or the manometric method (IV). The biogas results were converted to STP conditions (T = 273 K, p = 1 bar). The hydrogen sulphide content of the biogas was measured with a portable gas chromatograph with a detection limit of < 0.5 ppm (Photovac GC/ photo-ionization detector (PID), II).

In the lignin, cellulose and hemicellulose concentration analyses, the acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to Van Soest *et al.* (1991), and neutral detergent lignin (NDF) was analysed according to Robertson and Van Soest (1981, III, IV). The cellulose concentration was calculated by ADF–ADL, and the hemicellulose concentration was calculated by NDF–ADF. The degradation of the cellulose, hemicellulose and lignin in the reactors was calculated by the equation 100 % × ((amount in the feed – amount in the digestate) (amount in the feed)$^{-1}$).

The concentrations of the trace elements and Fe were determined using a Perkin Elmer Optima 4300DV inductively coupled plasma optical emission spectrometer (ICP-OES). The device was equipped with a Scott-type double-pass spray chamber and a cross-flow nebulizer; the RF power was 1400 W, plasma gas flow 15 l min^{-1}, nebulizer gas flow 0.6 l min^{-1} and auxiliary gas flow 0.2 l min^{-1}. Before analysis, the samples were pre-treated using the ultrasound-assisted digestion method. A sample volume of 20 ml was used throughout. The digestion solution of 6 ml of aqua regia and 4 droplets of HF was introduced into the sample vessel (50 ml centrifuge tube supplied by Sarstedt) and placed into the ultrasonic water bath. The sonication procedure was performed at 40 ˚C and contained three steps: 3 min sonication, 15 min standing and 2 × 3 min sonication. After the sonication procedure the sample was centrifuged for 15 min at 3500 rpm and filtered using the (VWR GF/A Grade 691, 90 mm, 1.6 µm) filter. After the filtration, the addition of the internal standard, Yttrium, was performed, resulting in a concentration of 1 ppm in the final sample volume of 50 ml. The detection limits of Co, Fe, Ni, and W were 1.2, 4.9, 0.5, and 3.3 mg kg^{-1}, respectively.

In the CSTR studies, the methane yields were calculated as weekly averages (I, II, III). The OLR and HRT were calculated according to five feeding days per week (II, III), and VS removals were calculated by the equation 100 %

× ((VS_{in} –VS_{out}) (VS_{in})$^{-1}$). In the pre-treatment experiments (IV), the average methane yields of four replicates were used to calculate the methane yields as per $VS_{original}$ (the amount of methane produced per VS of the untreated sludge). The protein content was calculated by 6.25 × TKN (I), and the unionized fraction of the ammonia nitrogen (I, II) was calculated by the following equation (Watcharasukarn et al. 2009):

$$F_{NH_3} = (1 + 10^{(pK_w - pK_b - pH)})^{-1}$$

The energy potential of the material was calculated by the equation Energy potential = m × P × HV, in which the m = mass (amount produced per year in Finland, see Introduction), P = methane potential (determined in this thesis) and HV = heating value (10 kWh m^{-3}) for methane. For the slaughterhouse wastes, lower and higher methane and energy production potentials were calculated based on two different methane potentials determined for pig slaughterhouse waste. For the pulp and paper industry wastewater sludge, the production amount was not separated for the primary and secondary sludge. Thus, 60 % of it was evaluated to be primary sludge and 40 % secondary sludge, as was in the case study of the present thesis.

For the methane yield comparisons of the pre-treated wastewater sludge (IV), statistical analyses were done with the statistical program R. The Shapiro-Wilk's test was used to determine if the methane yields were normally distributed. Since they were, the ANOVA was used for evaluating if there were differences between the treatments. The ANOVA post hoc test, Tukey was used to test which treatments differed from the original sludge in the methane yields. $P \leq 0.05$ was chosen as the level of significance.

4 RESULTS

4.1 Methane potentials of the substrates (I–IV)

The methane potentials of all of the substrates used in the present thesis (I–IV) were determined in batch experiments, and the results are presented in Table 9. In general, higher methane potentials were obtained for the meat industry than for the pulp and paper industry by-products. The highest methane yield per VS was obtained for the pig slaughterhouse waste (630 ± 120 dm^3 $kgVS_{added}^{-1}$), while the lowest yield was obtained for the rendering plant biosludge (16 ± 23 dm^3 $kgVS_{added}^{-1}$). Both the primary and secondary sludge from the pulp and paper mill wastewater treatment plant produced higher methane potentials under thermophilic than mesophilic conditions.

4.2 Anaerobic digestion of meat industry by-products

4.2.1 Anaerobic co-digestion of slaughterhouse and rendering wastes (I)

The anaerobic co-digestion of the slaughterhouse and rendering wastes was studied in two CSTRs, one at 35 °C and another at 55 °C. The chemical analyses showed that the feed had high solids, SCOD and nitrogen contents (Table 10). The feed TKN concentration ranged from 1.5 to 5.1 g l^{-1}, depending on the applied OLR, with NH_4-N accounting for 3–7 % of the TKN. The feed pH was low, varying between 5.4 and 6.3.

TABLE 9 Methane potentials of the substrates used in the present study determined in batch experiments.

Substrate	Temperature °C	Methane potential $dm^3\ kgVS_{added}^{-1}$	Methane potential $dm^3\ kgFM_{added}^{-1}$	Paper
Slaughterhouse wastes				
Pig	35	428 ± 25	120 ± 7	I
	35	630 ± 120	195 ± 38	II
Cattle	35	572 ± 89	296 ± 46	I
Poultry	35	266 ± 74	90 ± 25	I
	35	262 ± 93	76 ± 27	I
Rendering wastes				
Fat from fat separation	35	275 ± 52	61 ± 12	I
Separator sludge	35	572 ± 187	12 ± 4	I
Melt	35	515 ± 54	343 ± 37	I
Decanter sludge	35	476 ± 164	295 ± 102	I
Biosludge	35	16 ± 23	0.1 ± 0.2	I
Fat	35	406 ± 39	403 ± 39	I
Boneflour	35	287 ± 123	161 ± 69	I
Pulp and paper mill wastewater sludge				
Primary sludge	35	210 ± 40	6 ± 1	III
	55	230 ± 20	7 ± 1	III
Secondary sludge I	35	50 ± 0	2 ± 0	III
	55	100 ± 10	3 ± 0	III
Secondary sludge II	55	108 ± 5	4 ± 0	IV

TABLE 10 Process parameters, methane yields and feed and digestate characteristics in CSTRs studying anaerobic digestion of slaughterhouse and rendering wastes at 35 (R1) and 55 °C (R2, I).

Temperature °C	Feed/ Digestate	35					55		
Days		14–20	21–68	69–106	107–153	154–178	14–106	107–111	112–168
OLR kgVS m^{-3} d^{-1}		1.5	0.5	1.0	1.5	2.0	1.5	2.25	0.4
HRT d		50	50	50	50	50	50	50	109
Methane yield dm^3 kg VS_{added}^{-1}		35	970 ± 490	730 ± 30	720 ± 50	640 ± 40	770 ± 170	170	400 ± 130
VS removal %		89	68	83	87	89	81	90	39
pH	F	6.0–6.1	5.8–6.3	5.7–6.1	5.4–5.9	5.5–5.7	5.6–6.1	5.9	5.4–5.9
	D	6.8–7.4	6.6–7.4	7.1–7.5	7.5–7.9	7.6–7.8	7.7–8.1	7.9	7.5–7.9
VS %	F	7.9	2.2 ± 0.4	4.2 ± 0.4	7.1 ± 0.1	9.4 ± 0.2	7.0 ± 0.9	10.8	2.8 ± 0.0
	D	0.9 ± 0.4	0.7 ± 0.0	0.7 ± 0.0	0.9 ± 0.1	1.0 ± 0.0	1.3 ± 0.2	1.1	1.7 ± 0.2
TS %	F	8.2	2.3 ± 0.4	4.5 ± 0.4	7.5 ± 0.1	9.9 ± 0.3	7.4 ± 0.8	11.6	2.9 ± 0.0
	D	1.4 ± 0.5	1.3 ± 0.1	1.1 ± 0.0	1.3 ± 0.1	1.4 ± 0.0	2.2 ± 0.5	1.7	2.4 ± 0.2
SCOD g l^{-1}	F	7.9 ± 2.4	3.3 ± 0.3	8.6 ± 0.0	12.3 ± 1.2	13.4 ± 4.3	10.1 ± 1.7	17.8	5.0 ± 0.6
	D	2.1	1.8 ± 1.4	0.7 ± 0.1	1.2 ± 0.2	3.0 ± 0.8	3.9 ± 0.8	6.9 ± 0.6	12.9 ± 1.8
TVFA g l^{-1}	F	0.7	0.2 ± 0.0	0.5 ± 0.1	0.8 ± 0.1	nd	0.6 ± 0.1	1.2	0.3 ± 0.0
	D	1.5 ± 0.5	0.6 ± 0.8	0.1 ± 0.0	0.1 ± 0.1	1.3 ± 0.5	0.6 ± 0.6	3.1 ± 0.4	5.6 ± 0.6
TKN g l^{-1}	F	2.7 ± 0.0	1.6 ± 0.4	2.8 ± 0.2	4.0 ± 0.1	5.2 ± 0.1	3.2 ± 0.5	5.1	1.6 ± 0.0
	D	1.2	1.4 ± 0.2	1.9 ± 0.2	2.7 ± 0.3	3.3 ± 0.1	2.8 ± 0.4	3.4	3.4 ± 0.3
NH_4-N g l^{-1}	F	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3	0.2 ± 0.0	0.3	0.1 ± 0.0
	D	0.9	0.9 ± 0.1	1.0 ± 0.1	1.6 ± 0.3	2.2 ± 0.2	2.1 ± 0.3	2.7 ± 0.1	2.8 ± 0.2
NH_3 mg l^{-1}	D	20 ± 10	20 ± 10	20 ± 10	70 ± 20	90 ± 10	460 ± 140	600	490 ± 120

nd = not determined

After the start-up period, the daily feeding was begun on day 14, with an OLR of 1.5 kgVS m^{-3} d^{-1}. However, no methane production was noticed in the mesophilic reactor; and therefore, the OLR of the mesophilic reactor was decreased to 0.5 kgVS m^{-3} d^{-1} on day 21. Thereafter, the methane production rapidly peaked, indicating methanation of the accumulated VFAs (Fig. 6). The OLR in the mesophilic reactor was then increased in a stepwise manner to reach an OLR of 2.0 kgVS m^{-3} d^{-1} on day 154. For the mesophilic reactor, the highest average methane yield of 970 dm^3 $kgVS_{fed}^{-1}$ was obtained when the reactor was operated with an OLR of 0.5 kgVS $m^{-3}d^{-1}$ (Table 10). However, some of the methane produced could be from the degradation of the accumulated solids in the reactor from the initial period, with an OLR of 1.5 kgVS m^{-3} d^{-1}. Average methane yields of 730 and 720 dm^3 $kgVS_{fed}^{-1}$ were obtained with OLRs of 1 and 1.5 kgVS m^{-3} d^{-1}, respectively. The methane content of the biogas during the stable period at days 25–178 was between 65 and 75 %. In the thermophilic reactor, methane production started immediately after daily feeding was begun on day 14 with an OLR of 1.5 kgVS m^{-3} d^{-1}. However, some low methane production was noticed around day 35 (Fig. 6). The OLR of the thermophilic reactor was increased stepwise to reach 2.25 kgVS m^{-3} d^{-1} on day 107. However, the methane production of the thermophilic reactor begun to decrease, and from day 112 onward, for rest of the run, the reactor was fed at 50–200 g d^{-1} on individual days (d 113, 119-120, 133-168) with an average OLR of 0.4 kgVS m^{-3} d^{-1} and HRT of 109 d. In the thermophilic process, the highest mean methane yield of 770 dm^3 $kgVS_{fed}^{-1}$, was obtained when the reactor was operated with an OLR of 1.5 kgVS m^{-3} d^{-1} (Table 10). The methane content of the biogas was between 55 and 72 % during days 7-168.

The SCOD concentration in the mesophilic reactor was below 4 g l^{-1} with slight variation during the experimental run. On the other hand, the SCOD concentration in the thermophilic reactor slowly increased from 2 to 4 g l^{-1}, and then began to increase sharply around d 100 to reach the maximum value of 16 g l^{-1} on d 150. The TVFA concentration in the mesophilic reactor increased steadily during the initial start-up period (days 1–39), slightly after day 140, and sharply after day 156, to reach 1.9 g l^{-1} at the end of the experiment (Fig. 6). On the other hand, TVFA concentration in the thermophilic reactor increased gradually from 0.03 g l^{-1} (day 17) to reach the highest concentration of 6.4 g l^{-1} on day 143; thereafter it dropped to 4.5 g l^{-1} at the end of the run (day 168). Acetic acid was the main VFA, accounting for > 80 % of the TVFA in the mesophilic reactor while in the thermophilic reactor acetic acid accounted for around 50 % and propionic acid less than 30 % of the TVFA (Fig. 7).

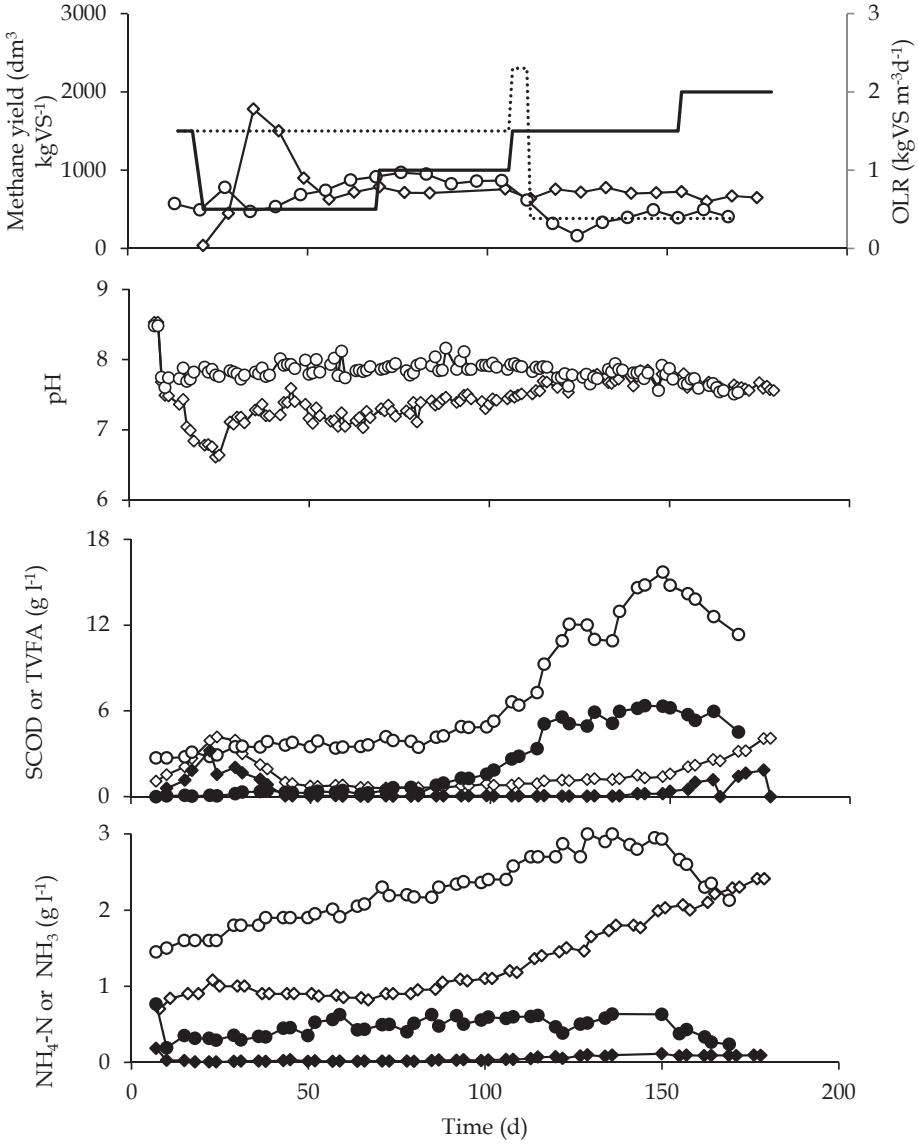

FIGURE 6 Methane yield and digestate characteristics pH, SCOD (open), TVFA (as SCOD, black), NH_4-N (open) and NH_3 (black) during semi-continuous co-digestion of rendering and slaughterhouse wastes in CSTRs at 35 ˚C (R1, — for OLR or ◊ and ♦ for the rest) and 55 °C (R2, ··· for OLR or ○ and • for the rest, I).

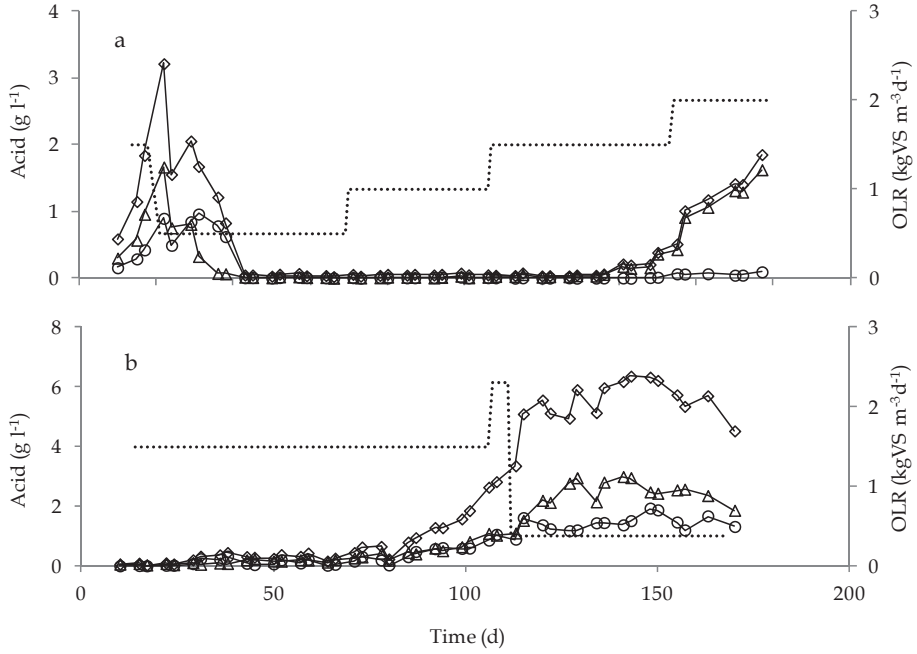

FIGURE 7 TVFA (◊), acetic (Δ) and propionic acid (○) concentrations (as SCOD) and OLR (···) during semi-continuous co-digestion of rendering and slaughterhouse wastes in CSTRs at 35 ˚C (a) and 55 °C (b). Concentrations of other acids were below 0.2 g l^{-1} in mesophilic and 0.9 g l^{-1} in thermophilic reactor throughout the experiments.

The digestate NH_4-N concentration in the mesophilic and thermophilic reactor was 54–75 % and 74–82 % of the TKN, respectively. As the feed TKN was changed according to the OLR (dilution) the digestate TKN was supposed to follow the feed changes with the delay related to applied HRT (Table 10). The concentration of the NH_4-N was higher in the thermophilic than in the mesophilic reactor. In the thermophilic reactor, the NH_4-N concentration increased throughout the experiment, from 1.6 to 3.0 g l^{-1}; on the other hand, the NH_4-N concentration in the mesophilic reactor was 0.8–1.1 g l^{-1} during days 1–101, but increased to 2.4 g l^{-1} (on day 178) when the OLR was increased to 1.5 kgVS m^{-3} d^{-1}. The calculated free NH_3 concentration was 3-6 times higher in thermophilic than in the mesophilic reactor and followed the same trend as that of the NH_4-N content. The calculated free NH_3 concentration in the thermophilic reactor was between 290 and 640 mg l^{-1} during the entire experiment (except days 1–14). The corresponding value for the mesophilic reactor was < 120 mg l^{-1} (except days 1–20). The pH of the thermophilic reactor remained more or less above 7.5, while the pH in the mesophilic reactor was more than 7 from day 28 onwards.

4.2.2 Anaerobic mono-digestion of pig slaughterhouse waste and improving anaerobic digestion of pig slaughterhouse waste by additives (II)

The results from the CSTR experiment (I) demonstrated that the anaerobic digestion of slaughterhouse and rendering wastes was feasible. However, the digestion process was shown to be highly vulnerable to instability because of the inhibition due to the digestion intermediate compounds. For this reason, the effects of additive feeding on process stability and methane yields were investigated. For simplicity and an easy comparison, only diluted pig slaughterhouse waste was chosen as the substrate for this experiment. Experiments were done in three CSTRs at 35 °C. The mesophilic process was chosen as it was seen to be more feasible than the thermophilic process for this kind of substrate in the previous study (I).

At first, all three reactors were operated under similar process conditions (OLR of 1 kgVS m^{-3} d^{-1} and HRT of 30 d). During this period (days 4–97), the methane production in all three reactors fluctuated between 600 and 800 dm^3 $kgVS_{fed}^{-1}$ (Table 11, Fig. 8). An increase in the OLR, from 1.5 to 2 kgVS m^{-3} d^{-1} (day 63), resulted in a decrease in the methane content and methane production in reactor S2, and thus, feeding was withheld in all three reactors on days 71–77, 81 and 82. On day 82, the reactor contents were mixed thoroughly and redistributed among the three reactors in order to ensure identical sludge content in each reactor, before starting additive feeding on day 98. After beginning the additive feeding, methane production in all three reactors continued at the level of 700 dm^3 $kgVS_{fed}^{-1}$. However, methane production in the control reactor S1 began to decrease around day 123 (OLR of 1.5 kgVS m^{-3} d^{-1}), indicating an unstable process, and thus, feeding was withheld on days 126–157. On the other hand, the methane production in the other reactors (S2 and S3) continued at the same level until it decreased in reactor S2 on day 152 (OLR 1.5 kgVS m^{-3} d^{-1}, additive 1) and in reactor S3 on day 235 (OLR 2.5 kgVS $m^{-3}d^{-1}$, additive 2). In reactor S1, the methane production increased again when feeding was resumed on day 218 with additive 2. The mean methane production in S1 was 760 dm^3 $kgVS_{fed}^{-1}$, with an OLR of 1.5 kgVS m^{-3} d^{-1} during days 218–284. Similarly, methane production in S2 also increased again when feeding was resumed with additive 2 with OLRs of 1–1.5 kgVS m^{-3} d^{-1} during days 188–242. The methane content in the biogas fluctuated between ca. 65 and 75 % in all three reactors.

TABLE 11 Process parameters, additive additions and digestate characteristics in CSTRs studying semi-continuous digestion of pig slaughterhouse waste at 35 °C (II). Additions are presented in Table 7. In whole experiment HRT was 30 d. nd = not determined

Substrate and additions	Days	OLR kgVS m^{-3} d^{-1}	Methane yield dm^3 $kgVS_{fed}^{-1}$	pH	VS %	TS %	SCOD g l^{-1}	TVFA g l^{-1}	NH_4-N g l^{-1}
S1: Pig	4–17	1	690 ± 150	7.6–7.8	3.3 ± 0.2	4.4 ± 0.2	8.9	nd	1.5 ± 0.1
slaughterhouse	18–62	1.5	680 ± 60	7.6–7.8	1.8 ± 0.5	2.4 ± 0.6	7.7 ± 1.0	0 ± 0	1.7 ± 0.1
waste	63–70	2	540	7.6–7.8	0.8	1.2	5.5	nd	1.8
	71–82	not fed	-	7.7–7.8	0.7	1.0	5.5 ± 0.8	0.4 ± 0	2.3
	83–125	1.5	720 ± 40	7.5–7.9	0.8 ± 0.3	1.2 ± 0.4	4.9 ± 0.2	0.8 ± 1.3	2.2 ± 0.1
	126–157	not fed	-	7.5–8.2	0.6 ± 0.0	0.9 ± 0.0	6.6 ± 0.1	2.1 ± 2.4	2.5 ± 0.3
	158–169	1	750 ± 10	7.7–7.9	1.8 ± 0.0	2.2 ± 0.1	4.7	0 ± 0	2.2
	170–217	1.5	630 ± 80	7.2–7.8	0.7 ± 0.3	1.0 ± 0.4	5.6 ± 2.0	1.8 ± 2.1	2.2 ± 0.1
+A2	218–284	1.5	760 ± 140	7.2–7.8	0.9 ± 0.6	1.2 ± 0.7	6.1 ± 2.8	nd	2.4 ± 0.1
S2: Pig	4–17	1	600 ± 10	7.7–7.8	3.3 ± 0.2	4.4 ± 0.3	9	nd	1.6 ± 0.1
slaughterhouse	18–62	1.5	680 ± 80	7.6–7.8	1.8 ± 0.5	2.4 ± 0.6	7.6 ± 1.1	0 ± 0	1.6 ± 0.1
waste	63–70	2	290	7.5–7.8	0.8	1.2	5.8	nd	1.7
	71–82	not fed	-	7.3–7.5	0.8	1.1	8.9 ± 0.6	4.6 ± 1.2	2.5
+A1 on days 98-187	83–155	1.5	670 ± 110	7.4–7.9	0.8 ± 0.4	1.2 ± 0.4	4.7 ± 0.9	0.8 ± 1.2	2.2 ± 0.1
	156–187	not fed	-	7.5–7.9	2.4	3.0	8.2	2.0 ± 2.5	2.7
+A2	188–195	1	580 ± 290	7.7–7.8	2.4	3.0	5.9	0.9	2.7
+A2	196–242	1.5	740 ± 50	7.6–7.8	1.9 ± 0.3	2.4 ± 0.4	4.4 ± 0.8	0.1 ± 0.1	2.5 ± 0.1
S3: Pig	4–17	1	620 ± 250	7.6–7.7	3.3 ± 0.3	4.4 ± 0.4	8.7	nd	1.6 ± 0.1
slaughterhouse	18–62	1.5	700 ± 50	7.6–7.8	1.9 ± 0.4	2.6 ± 0.5	7.2 ± 1.1	0 ± 0	1.7 ± 0.1
waste	63–70	2	560	7.7–7.9	1.2	1.7	5.1	nd	1.8
	71–82	not fed	-	7.6–7.8	1.3	1.7	5.4 ± 1.3	0 ± 0	2.4
+A2 on days 98-125	83–164	1.5	720 ± 20	7.6–8.0	1.7 ± 0.3	2.2 ± 0.3	4.4 ± 1.2	0 ± 0	2.5 ± 0.2
+A1 and A2 on	165–187	1.75	720 ± 20	7.7	1.4 ± 0.2	1.7 ± 0.2	2.7	0 ± 0	2.3
days 126-223	188–206	2	670 ± 10	7.7–7.8	1.3 ± 0.2	1.6 ± 0.2	2.5 ± 0.6	0 ± 0	2.6 ± 0.1
	207–224	2.25	710 ± 80	7.7–7.8	1.6 ± 0.1	2.0 ± 0.1	2.2 ± 0	0 ± 0	2.7
+A2	225–242	2.5	410 ± 160	7.5–7.9	1.4 ± 0.4	1.8 ± 0.5	6.8 ± 3.6	2.6 ± 3.4	3.2 ± 0.1

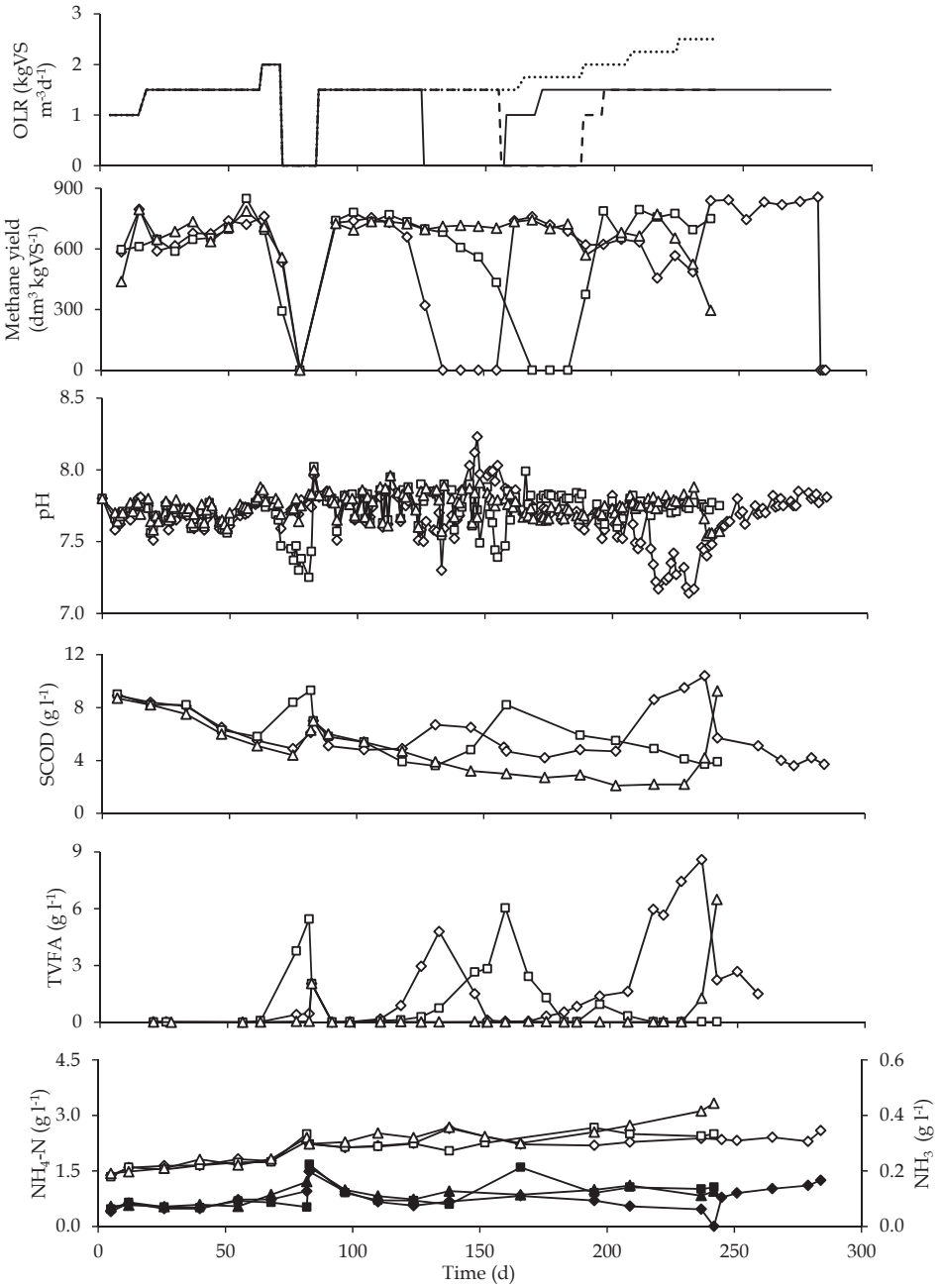

FIGURE 8 Methane yield and digestate characteristics pH, SCOD, TVFA (as SCOD), NH_4-N (open), NH_3 (black) during semi-continuous digestion of pig slaughterhouse waste in CSTRs ; S1 (— for OLR or ◊ and ♦ for the rest), S2 (-- for OLR or □ and ■ for the rest), S3 (··· for OLR or Δ and ▲for the rest, II). Additives were used as follows: S1 Additive 2 on days 218–284, S2 Additive 1 on days 98–187 and Additive 2 on days 188–242 and S3 Additive 1 on days 126–223 and Additive 2 on days 98–242.

During the first 70 days, the SCOD content in all three reactors decreased from ca. 9 g l^{-1} to 4–6 g l^{-1}, and the TS and VS decreased from 4.4 and 3.3 % to 1.2-1.7 and 0.7-1.2 %, respectively (Table 11). However, an increase in the SCOD and TVFA contents was observed in reactor S2 around day 70, and in the other two reactors somewhat on day 81, after the mixing and redistribution of the reactor contents. After mixing the reactor contents, the SCOD and TVFA contents first decreased in all three reactors, but increased again in reactor S1 (no additives) around day 120, and in reactor S2 (additive 1 addition from day 98 on) around day 135. Thus, reactors S1 and S2 were kept unfed during days 126–157 and 156–187, respectively. In reactor S3, where the feeding of additive 2 was begun on day 98, the SCOD and TVFA contents did not increase before day 235. The SCOD content in reactor S2 was stabilized to 4 g l^{-1} when the feeding was resumed with additive 2 instead of additive 1. Similarly, the SCOD concentration in reactor S1 first decreased, and then increased around day 210. On day 218, reactor S1 began receiving additive 2 at a higher one day dose, and from day 219 onward, a daily addition was fed. This led to a decrease in the SCOD and TVFA concentrations around day 240. In reactor S3, the concentrations of SCOD and TVFA increased sharply around day 235, when fed with an OLR of 2.5 kgVS m^{-3} d^{-1}. Among VFAs acetic acid was the main acid in all three reactors (Fig. 9).

The NH_4-N concentrations of all three reactors were around 1.5–2.5 g l^{-1} until increasing in reactor S3 around day 200 onward to reach 3.5 g l^{-1} on day 242. The TKN content in all three reactors was between 3 and 4 g l^{-1}, except in the last measurements for reactors S1 and S3, which were 4.5 g l^{-1} (day 284) and 5.7 g l^{-1} (day 242), respectively. The calculated free NH_3 concentrations fluctuated between 0.05 and 0.22 g l^{-1} in all three reactors during the operational period and the pH in all three reactors were mostly between 7 and 8. However, a pH higher than 8 was measured in reactor S1 during the unfed period (days 126–157).

The H_2S content in the biogas in all three reactors was between 1400 and 1800 ppm on day 67. After additive 1 was introduced to reactor S2 and additive 2 to reactor S3 on day 98, the content of the H_2S fell below the detection limit of 0.5 ppm. In reactor S1, the H_2S content fluctuated between 600 and 1900 ppm until it decreased to less than 100 ppm after additive 2 feeding was begun on day 218. Trace element contents in the reactors at the end of each feeding period are given in Table 12.

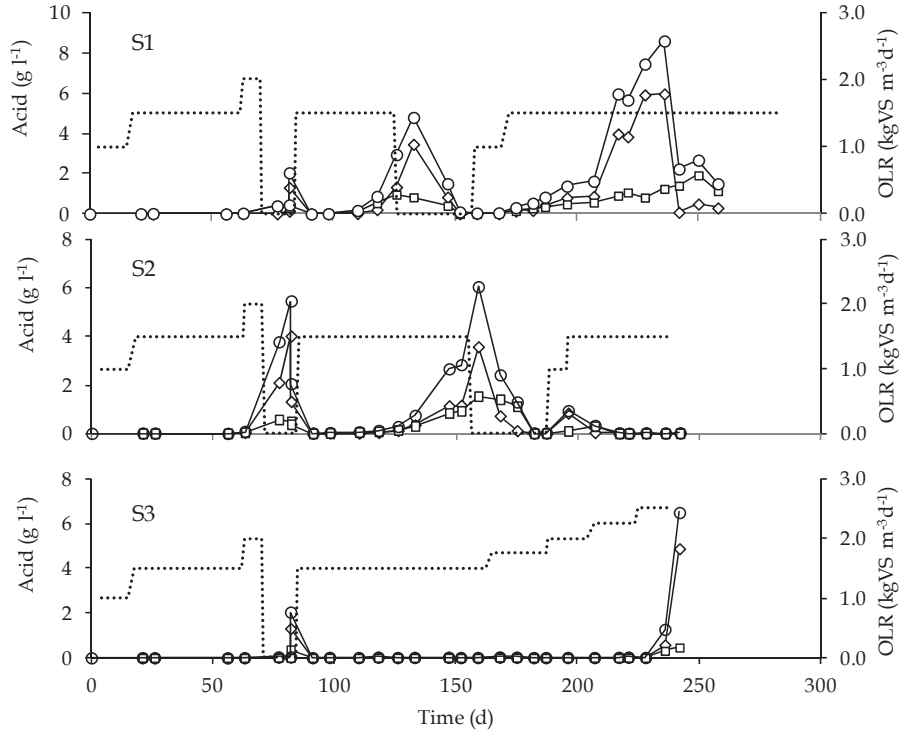

FIGURE 9 TVFA (○), acetic (◊) and propionic acid (□) concentrations (as SCOD) and OLR (⋯) during semi-continuous digestion of pig slaughterhouse waste in CSTRs. Concentrations of other VFAs were less than 0.7 g l^{-1} in all the reactors throughout the experiments. Additives were as follows: S1 Additive 2 on days 218–284, S2 Additive 1 on days 98–187 and Additive 2 on days 188–242 and S3 Additive 1 on days 126–223 and Additive 2 on days 98–242.

TABLE 12 Fe, Ni, Co, W concentrations in the reactors at the end of each feeding period during anaerobic digestion of pig slaughterhouse waste at 35 °C.

Reactor	Additive	OLR kgVS m^{-3} d^{-1}	Fe mg $kgFM^{-1}$	Ni mg $kgFM^{-1}$	Co mg $kgFM^{-1}$	W mg $kgFM^{-1}$
S1	-	1.5	8.1	0.38	0.02	0.69
S1	A2	1.5	64	1.1	0.03	0.49
S2	A1	1.5	69	0.17	0.01	0.39
S2	A2	1.5	251	0.68	0.07	0.84
S3	A2	1.5	170	0.45	0.07	0.60
S3	A2	2.5	243	0.49	0.15	1.13

4.3 Anaerobic digestion of pulp and paper industry by-products

4.3.1 Anaerobic digestion of primary sludge and co-digestion of primary and secondary sludge (III)

The anaerobic digestion of the pulp and paper mill primary sludge and co-digestion of the primary and secondary sludge were studied in three parallel CSTRs at 55 ˚C for 122 days. The digestion of the primary sludge was studied in two reactors (R1 and R2), and co-digestion of the primary sludge with the secondary sludge in one reactor (R3). The feeds in the present study, the pulp and paper industry primary sludge, and the mixture of primary and secondary sludge were low in solids (Table 4). Characteristically, both substrates were also low in nitrogen content: 0.1 g l^{-1} in the primary sludge and 0.8 g l^{-1} in the mixture of primary and secondary sludge.

Higher methane yields were obtained during the monodigestion of the primary sludge than during the co-digestion of the primary sludge and secondary sludge. For the primary sludge, methane yields of 190–240 dm^3 $kgVS_{fed}^{-1}$ were obtained and a higher methane yield was noticed when operating at an OLR of 1 kgVS m^{-3} d^{-1}, HRT of 23–32 d, than at an OLR of 2 kgVS m^{-3} d^{-1}, HRT of 16 d (Table 13, Fig. 10). No impact on the methane yield was observed when the NH_4Cl and reject water were added to reactors R1 and R2 (NH_4Cl on days 46–122 and reject water on days 60–95). Higher OLRs were not studied as the target OLR of 2 kgVS m^{-3} d^{-1} and HRT of 14–16 d resulted in a pH decrease. In the co-digestion of the primary and secondary sludge, methane yields of 150–170 dm^3 $kgVS_{fed}^{-1}$ were obtained with the studied OLR of 1 kgVS m^{-3} d^{-1} and HRT of 25–31 d.

The concentration of TVFA (expressed as SCOD) was less than 0.1 g l^{-1} in all three reactors throughout the experiment, except in the primary sludge digestion with OLR 2 kgVS m^{-3} d^{-1} (R2), when it was at the highest 0.34 g l^{-1} (Fig. 10). The SCOD contents in all three reactors were between 2 and 3 g l^{-1} throughout the experiments. Thus, the VFAs contributed to less than 5 % of the SCOD, except for the highest value of 10 % noticed in the primary sludge digestion with an OLR of 2 kgVS m^{-3} d^{-1} (R2).

TABLE 13 Process parameters, methane yields and feed and digestate characteristics in CSTRs studying digestion of pulp and paper mill primary sludge (R1 and R2) and co-digestion of primary and secondary sludge (R3) at 55 °C (III). nd = not determined.

Substrate	Feed/ Digestate	Primary sludge						Primary and secondary sludge	
N addition[a]		No	Yes	Yes	No	Yes	Yes	No	No
Days		1–45	46–95	96–122	1–45	46–95	96–122	1–93	94–122
OLR kgVS m^{-3} d^{-1}		1	1	1	2	2	1.4	1	1
Reactor		R1			R2			R3	
HRT d		29–32	32	23	14–16	16	16	30–31	25–26
Methane yield dm^3 kg VS_{added}^{-1}		240 ± 10	200 ± 20	230 ± 10	190 ± 10	190 ± 20	220 ± 10	150 ± 10	170 ± 10
VS removal %		37	40	30	40	37	25	32	29
pH	F	5.5–7.0	6.6–7.0	6.6–6.9	5.5–7.0	6.6–7.0	6.6–6.9	6.5–7.4	6.9–7.1
	D	7.3–8.2	7.1–7.4	6.8–7.1	6.9–8.0	6.5–7.0	6.6–6.8	7.2–8.2	7.2–7.3
VS %	F	3.0	3.2	2.3	3.0	3.2	2.3	3.1	2.5
	D	1.9 ± 0.0	1.9 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	2.0 ± 0.2	1.7 ± 0.1	2.1 ± 0.1	1.8 ± 0.1
TS %	F	3.4	3.8	2.7	3.4	3.8	2.7	3.7	3.1
	D	3.1 ± 0.2	2.8 ± 0.2	2.2 ± 0.2	2.9 ± 0.3	2.7 ± 0.2	2.2 ± 0.2	3.2 ± 0.3	2.5 ± 0.2
SCOD g l^{-1}	F	1.6	1.4	1.4	1.6	1.4	1.4	1.2	1.3
	D	2.9 ± 0.4	2.7 ± 0.2	2.1 ± 0.2	2.4 ± 0.4	2.4 ± 0.4	2.4 ± 0.3	3.0 ± 0.4	2.5 ± 0.4
TVFA mg l^{-1}	F	nd	nd	nd	nd	nd	nd	nd	nd
	D	30 ± 20	30 ± 20	50 ± 20	120 ± 70	190 ± 100	60 ± 40	30 ± 10	40 ± 20
NH_4-N g l^{-1}	F[b,c]	<1	<1	<1	<1	<1	<1	10	3
	D	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.5 ± 0.3	1.0 ± 0.4	0.7 ± 0.0	0.7 ± 0.3	0.4 ± 0.1
TKN g l^{-1}	F[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.8	0.8
	D	1.8 ± 0.3	1.4 ± 0.4	1.2 ± 0.1	1.1 ± 0.3	1.5 ± 0.8	1.2 ± 0.0	1.8 ± 0.5	1.2 ± 0.0
Cellulose removal %		nd	nd	71	nd	nd	73	nd	70
Hemicellulose removal %		nd	nd	21	nd	nd	27	nd	7
Lignin removal %		nd	nd	–53	nd	nd	–60	nd	–55

[a] N addition in form of NH_4Cl on days 46–122 and reject water on days 60–95.

[b] Analyses of the feed before nitrogen addition. [c] unit mg l^{-1}

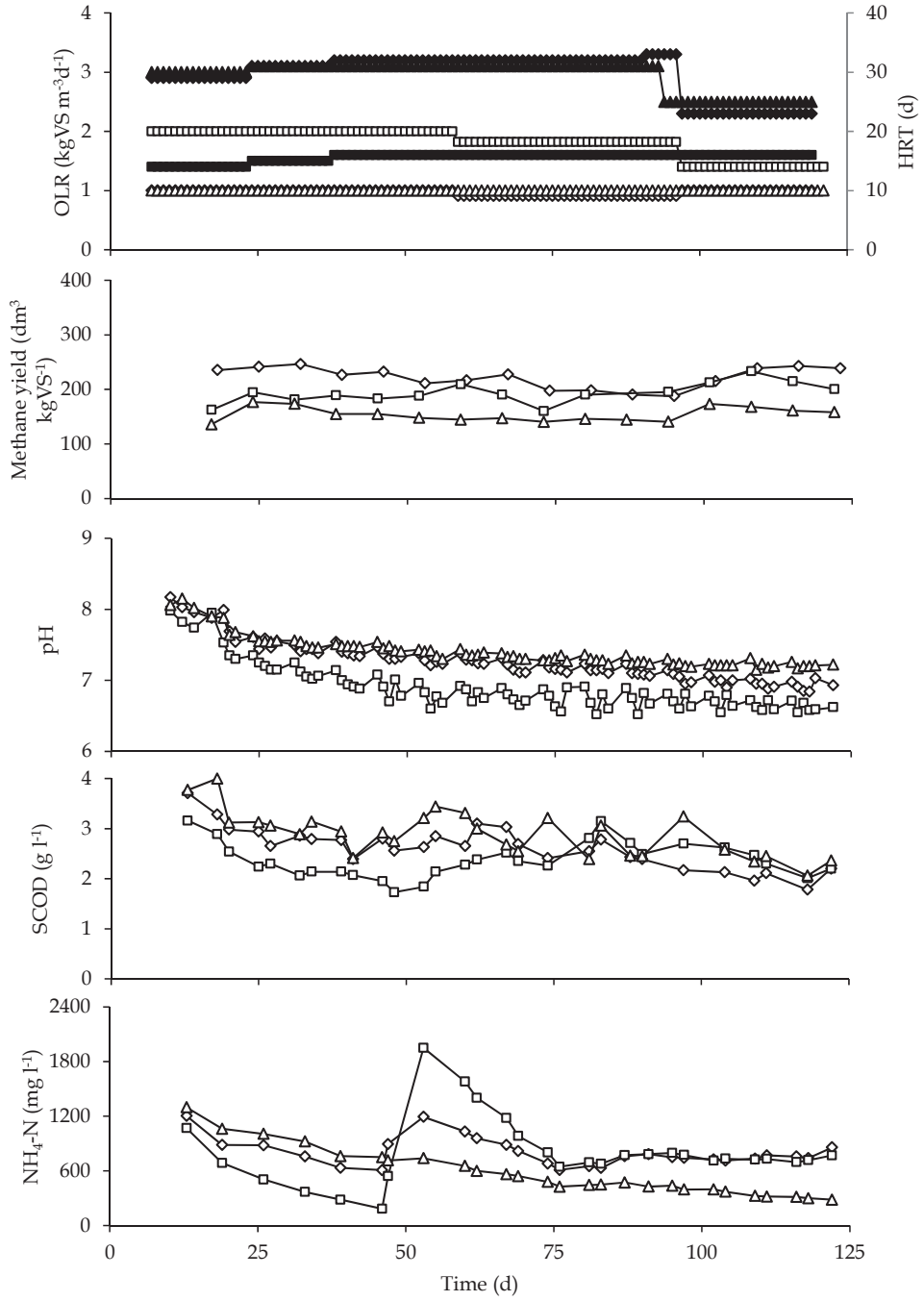

FIGURE 10 Operational parameters OLR (open) and HRT (black) as well as methane yields and digestate characteristics pH, SCOD, NH_4-N during digestion of pulp and paper mill primary sludge, R1 (◊ and ♦) and R2 (□ and ■), and co-digestion of primary and secondary sludge R3 (Δ and ▲) in CSTRs at 55 °C (III).

During primary sludge digestion, the pH decreased from an initial value of 8.0–8.2 to 7.3 in reactor R1, and to 6.7 in reactor R2, on day 47. In addition, the NH_4-N concentrations in these two reactors decreased as well, from an initial 1.1–1.2 g l^{-1} to 0.6 g l^{-1} (R1) and 0.2 g l^{-1} (R2) on day 46. The addition of NH_4Cl to reactors R1 and R2 was initiated on day 46. The NH_4-N concentrations increased up to 1.2 and 2.0 g l^{-1} in reactors R1 and R2, respectively, on day 53 and then subsequently stabilized to ca. 0.7 g l^{-1} around day 74. However, a steady decrease in pH continued in both reactors digesting primary sludge, and the pH at the end of the experiment was around 6.8 in R1 and 6.5 in R2. In the co-digestion of the primary and secondary sludge (R3), the pH decreased from 8.1 at the beginning to 7.5 during first HRT, and stabilized at 7.2 near the end of the experiment. The decrease in the NH_4-N concentration was also slower in reactor R3 than in the other reactors.

VS removal in the three reactors varied between 25 and 40 %, and there was no difference between the digestion of the primary sludge and co-digestion of the primary and secondary sludge. Of the main components of the substrates, the cellulose and hemicellulose removals were 70–73 % and 7–27 %, respectively. The lignin concentration, however, increased in all three reactors (Table 13).

4.3.2 Pre-treatments for improving anaerobic digestion of pulp and paper mill secondary sludge (IV)

In the batch and CSTR experiments (III), pulp and paper mill secondary sludge was seen to be hardly digestible. Thus, 12 different single or combined pre-treatments were studied to improve the anaerobic digestion of the pulp and paper mill secondary sludge. Thermophilic conditions were chosen as the previous study (III) showed that the thermophilic process was more feasible in digestion of this kind of material than the mesophilic process.

The TS and VS values of the untreated sludge were 4.7 % and 3.9 %, respectively (in all enzymatic pre-treatments, the TS and VS values were 4.0 % and 3.3 %, respectively, due to dilution with the buffer). Some pre-treatments were found to affect the TS and VS values (Table 14). The major impact was noticed with the combined ultrasound (US) + alkali (AL) pre-treatment which resulted in a decrease in the TS to 3.9 % and VS to 3.3 %. However, the ultrasound and alkali pre-treatments alone, or in other combinations, did not result in such high changes. The enzymatic pre-treatments did not affect the TS and VS values.

TABLE 14 Methane yields and characteristics of the untreated and pre-treated secondary sludge in pre-treatment experiments (IV). Abbreviations explained in Table 8.

Treatment	SCOD g l^{-1}	TS %	VS %	Methane yield d 11-12 dm^3 $kgVS_{original}^{-1}$	Methane yield final dm^3 $kgVS_{original}^{-1}$
Control	1	4.7	3.9	56 ± 1	108 ± 5
US	1	4.7	3.9	58 ± 3	114 ± 6
AL	5	5.1	3.7	–5 ± 1	86 ± 11[a]
AC	1	5.1	4.0	–5 ± 0	61 ± 3[a]
E	4	3.9	3.2	44 ± 4	114 ± 5
US+AL	5	3.9	3.3	23 ± 11	67 ± 17[a]
HT70	4	4.3	3.6	59 ± 9	112 ± 10
HT150	9	4.5	3.7	86 ± 1	134 ± 2[a]
US+E	4	4.0	3.3	48 ± 1	109 ± 1
HT150+E	9	4.0	3.2	79 ± 3	128 ± 6[a]
HT70+E	5	4.0	3.2	59 ± 4	124 ± 6
US+HT150	9	4.6	3.8	85 ± 5	141 ± 6[a]
US+HT150+E	10	3.7	3.0	88 ± 5	131 ± 5[a]

[a] Statistically significant, $p \leq 0.05$

The SCOD concentration in the untreated sludge was 1 g l^{-1}. All pre-treatments, except for the ultrasound and acid (HNO_3) pre-treatments, improved the COD solubilisation up to 4–10 g l^{-1}. The pre-treatment combination of ultrasound+hydrothermal at 150 ˚C+enzyme (US+HT150+E) had the greatest impact on the COD solubilisation. The pre-treatments affected the concentrations of the biomass constituents of the sludge, namely the cellulose, hemicellulose and lignin (Fig. 11). All pre-treatments, except the HNO_3 and NaOH pre-treatments, resulted in an increase in the cellulose content. The most effective pre-treatment was hydrothermal (HT150), and in the HT150 and US+HT150 pre-treated sludge the cellulose content increased by 1.8 times. Correspondingly, a decrease in the hemicellulose content was observed in all of the pre-treatments, especially in the hydrothermally pre-treated sludge (HT150+E and US+HT150+E). For lignin, the pre-treatments resulted in a high variation: from a very low value of 110 g $kgVS_{treated}^{-1}$ for the ultrasound+alkali (US+AL) pre-treatment, to a high value of 350 g $kgVS_{treated}^{-1}$ in the hydrothermal at 150 ˚C+enzyme (HT150+E) pre-treatment.

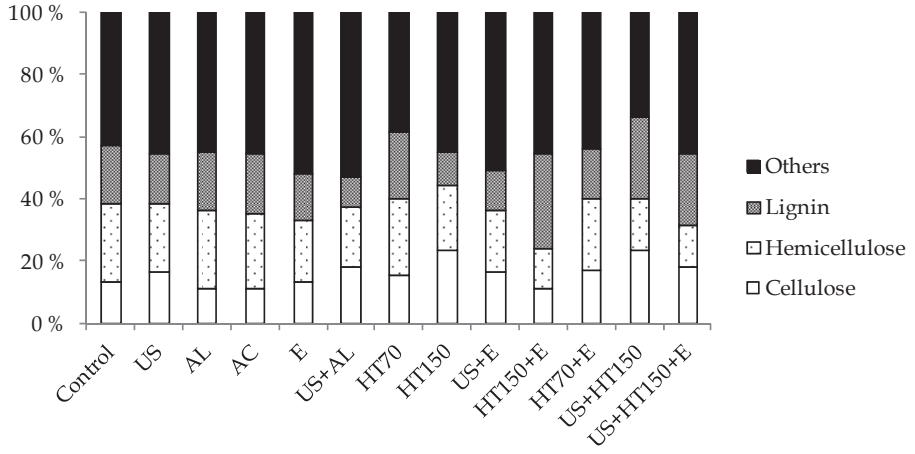

FIGURE 11 Cellulose, hemicellulose and lignin contents (% VS) of the untreated (control) and pre-treated sludges in the experiments studying the effect of pre-treatments on methane yield of the pulp and paper mill secondary sludge in batch assays at 55 ˚C (IV). Abbreviations explained in Table 8.

Methane production began immediately in all assays, except in the chemically pre-treated assays (Fig. 12 and Table 14), where a lag phase of 20–25 days was observed. Methane production rates of the HT150 pre-treatment, alone and its combinations with enzymes and/or ultrasound, were higher than those of the control and other pre-treatments, and resulted in 52–57 % more methane than the control (56 dm^3 $kgVS_{original}^{-1}$) during the initial 11–12 days of incubation.

Untreated sludge had a methane potential of 108 ± 5 dm^3 $kgVS_{original}^{-1}$ (Table 14). A statistically significant increase in the methane yields ($p \leq 0.05$) was noticed only in the pre-treatment combinations where hydrothermal pre-treatment at 150 ˚C was involved (HT150, HT150+E, US+HT150, US+HT150+E). Of the enzymatic pre-treatments, only those combined with hydrothermal pre-treatment at 150 ˚C (HT150) improved the methane yield; however, no statistically significant increase in the methane yields was observed when the enzymes were used alone (E) or in combination with ultrasound (US) or hydrothermal at 70˚C (HT70). Similarly, the ultrasound pre-treatment had no statistically significant impact on the methane production, as a methane yield of 114 ± 6 dm^3 $kgVS_{original}^{-1}$ was obtained from the pre-treated sludge. In addition, all chemical pre-treatments (AC, AL, US+AL) resulted in lower methane yields (20–44 %) than the untreated sludge.

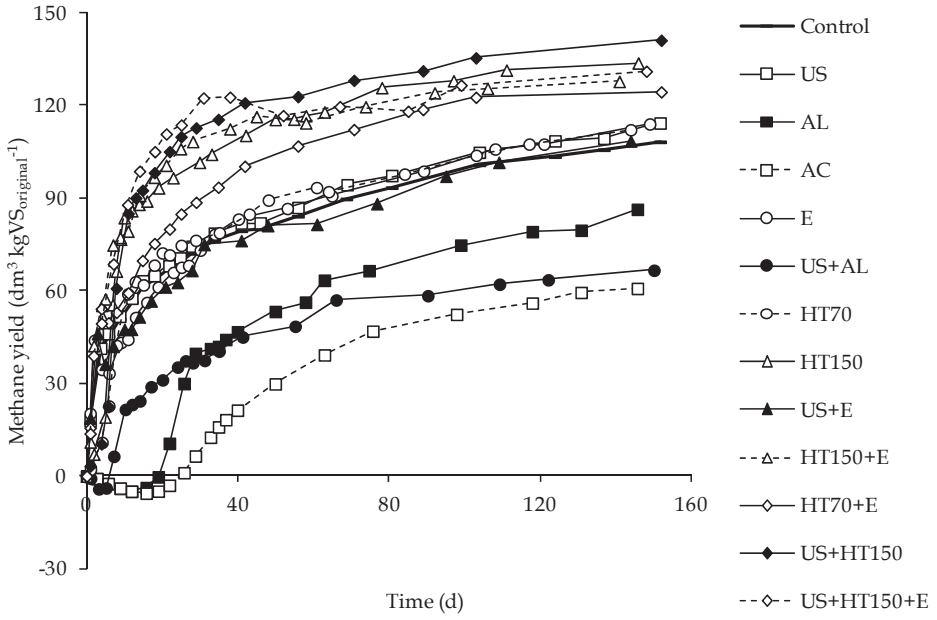

FIGURE 12 Methane yield (dm^3 $kgVS_{original}^{-1}$) of the original and pre-treated secondary sludge samples during the experiments studying the effect of pre-treatments on methane yield of the pulp and paper mill secondary sludge in batch assays at 55 ˚C (IV). Abbreviations are explained in Table 8.

5 DISCUSSION

5.1 Meat and pulp and paper industry by-products as substrates for biogas production

According to the characteristics of the meat and pulp and paper industry by-products, varying methane potentials were expected. Some of the by-products, like rendering waste fractions fat, fat from fat separation, melt, separator sludge or boneflour, and slaughterhouse wastes, had a high protein and/or lipid content and a high content of organic matter. This is supposed to lead to a high methane yield if no inhibitory substances like ammonia, VFAs or LCFAs are formed. On the other hand, pulp and paper mill sludge had low organic matter content, and more than half of it is hardly digestible lignocellulosic biomass. A rough estimation of the biodegradability of the substrate can be made from the VS/TS ratio. For pulp and paper mill sludge it varied between 80 and 85 % while the variation for slaughterhouse and rendering wastes was wider, between 57 and 100 %.

The methane yields obtained in the present study for the slaughterhouse wastes (262–630 dm^3 $kgVS_{added}^{-1}$) were similar or lower than the yields of 460–670 dm^3 $kgVS_{added}^{-1}$ reported previously for the poultry slaughterhouse waste and 580 dm^3 $kgVS_{added}^{-1}$ for the pig slaughterhouse waste (Table 15). On the other hand, somewhat lower methane yields obtained for rendering wastes in the present study (mainly 287–572 dm^3 $kgVS_{added}^{-1}$, for biosludge 16 dm^3 $kgVS_{added}^{-1}$) than reported in the literature (Pitk *et al.* 2012; Table 15) was due to differences in characteristics of the substrates, or different kinds of inoculum used in the studies. From Table 15, it can be seen that slaughterhouse and rendering wastes have varying characteristics and methane potentials. Materials often used as substrates for biogas production, like sewage sludge, manure, OFMSW and energy crops, have methane potentials of 100–600 dm^3 $kgVS_{added}^{-1}$ (reviewed by Ward *et al.* 2008, Appels *et al.* 2011). Thus, methane potentials obtained in the present study indicate that the studied industrial by-products seem to be feasible substrates for anaerobic digestion.

TABLE 15 Methane potentials and characteristics of slaughterhouse and rendering wastes in batch experiments reported in the literature.

Substrate	Temp. °C	VS %	TS %	N_{tot} g/kg	Protein g/kg	Lipid g/kg	C/N ratio	Methane potential $dm^3\ kgVS_{added}^{-1}$	Methane potential $dm^3\ kgFM_{added}^{-1}$	Reference
Poultry slaughterhouse waste	35	26.6	30.7	26.3	151.3	74.7	-	460	-	Rodríguez-Abalde *et al.* (2011)
	35	26.0	31.2	24.3[a]	152	100	-	550–670	-	Salminen *et al.* (2000)
	35	33	38	26[b]	483[c]	453[c]	-	270	90	This study
	35	30	33	-	-	-	-	260	80	
Pig slaughterhouse waste	35	48.9	50.7	20.7	120.9	363.4	-	580	-	Rodríguez-Abalde *et al.* (2011)
	35	28	32	16[b]	351[c]	525[c]	-	430	120	This study
	35	31	32	15[b]	-	-	-	630	200	
Cattle slaughterhouse waste	35	53	53	5.6[b]	57[c]	767[c]	-	570	300	This study
Melt	37.5	84	96	-	-	-	9.3	834	685	Pitk *et al.* (2012)
	35	67	98	69[b]	644[c]	330[c]	-	520	340	This study
Meat and bone meal	37.5	65	99	-	-	-	5.3	390	259	Pitk *et al.* (2012)
	35	-	-	-	-	-	-	351–381	-	Wu *et al.* (2009)
Bonemeal	35	25.4	37.0	30	190	83	-	600	150	Salminen *et al.* (2003)
	55					-	-	680	170	
Boneflour	35	56	99	72[b]	804[c]	179[c]		290	160	This study
Fat	37.5	99	99	-	-	-	371.6	978	966	Pitk *et al.* (2012)
	35	99	99	1.1[b]	7[c]	943[c]	-	410	400	This study
Fat from fat separation	35	22	24	4.2[b]	117[c]	892[c]	-	280	60	This study
Sterilized mass	37.5	84	96	59.8[d]	311[c]	559[c]	9.3	719	591	Pitk *et al.* (2013)
Feather	35 and 55	23.5	24.3	35	220	110	-	210	49	Salminen *et al.* (2003)
Blood	35	20.0	22.1	17	100	46	-	510	100	Salminen *et al.* (2003)
	55						-	460	92	
Offal	35	37.1	39.1	20	130	210	-	730–910	270–340	Salminen *et al.* (2003)
	55						-	890	330	

Substrate	Temp. °C	VS %	TS %	N_{tot} g/kg	Protein g/kg	Lipid g/kg	C/N ratio	Methane potential $dm^3\ kgVS_{added}^{-1}$	Methane potential $dm^3\ kgFM_{added}^{-1}$	Reference
Grease trap sludge	35	25.2	25.4	-	-	-	-	918	251	Luostarinen *et al.* (2009)
	35	11	11	-	-	-	-	900	99	Luste *et al.* (2009)
Greaves	37	86	88	70	440	406	-	707	-	Cavaleiro *et al.* (2013)
Rinds	37	65	65	73	458	379	-	756	-	
Digestive tract content	35	11	12	-	-	-	-	400	42	Luste *et al.* (2009)
Drumsieve waste	35	14	14	-	-	-	-	230	30	Luste *et al.* (2009)
Decanter sludge	37.5	75	99	-	-	-	7.2	607	459	Pitk *et al.* (2012)
	35	62	98	61[b]	619[c]	356[c]	-	480	300	This study
Separator sludge	35	2.0	2.2	0.3[b]	100[c]	800[c]	-	570	12	This study
Flotation sludge	37.5	19	22	-	-	-	16.2	650	131	Pitk *et al.* (2012)
Dissolved air flotation sludge	35	3.5	4.3	-	-	-	-	340	12	Luste *et al.* (2009)
Biosludge	35	0.9	1.0	1.2[b]	844[c]	111[c]	-	16	0.1	This study

[a] $g\ l^{-1}$
[b] FM
[c] VS
[d] TS

The methane yields obtained for the pulp and paper mill secondary sludge in the present study were similar or lower than the yields of 85–200 dm^3 $kgVS_{added}^{-1}$ reported in the literature at 35 ˚C (Jokela *et al.* 1997, Karlsson *et al.* 2011; Table 16). For primary sludge comparable results are scarce, but the yields obtained in the present study were higher than the 45 dm^3 $kgVS_{added}^{-1}$ reported previously under mesophilic conditions (Jokela *et al.* 1997). For the methane yields under thermophilic conditions, there are no comparable results for either of the sludge. During the digestion of the pulp and paper mill wastewater sludge, higher methane potentials were obtained under thermophilic than under mesophilic conditions. Similarly, higher methane yields were reported for municipal wastewater treatment plant primary and mixed primary and secondary sludge under thermophilic than under mesophilic conditions (Zábranská *et al.* 2000). The above authors suggested that this is because of the higher activity of the thermophilic sludge compared to the mesophilic sludge, and thus, higher maximum methane production rates and methane yields. On the contrary, Puhakka *et al.* (1988) reported higher biogas yields at 35 °C (90 dm^3 $kgVSS_{added}^{-1}$) than at 55 °C (50 dm^3 $kgVSS_{added}^{-1}$) during the co-digestion of pulp mill (CTMP) primary and secondary sludge in flow reactor experiments.

TABLE 16 Sludge characteristics and methane potentials of pulp and paper mill wastewater sludges in batch experiments reported in the literature.

Substrate	Temp. °C	VS %WW	TS %WW	Methane potential dm^3 $kgVS_{added}^{-1}$	Reference
Primary sludge					
TMP	37	1.4–2.8	2.3–6.2	45	Jokela *et al.* (1997)
Kraft	35	2.9	3.4	210	This study
	55			230	
Secondary sludge					
Mechanical	37	-	1.3	138	Karlsson *et al.* (2011)
	37	-	1.3	197	Karlsson *et al.* (2011)
Sulphite	37	-	0.9	159	Karlsson *et al.* (2011)
	35	-	1.1	320[a]	Wood *et al.* (2009)
CTMP/kraft	37	-	1.1	97	Karlsson *et al.* (2011)
Kraft/CTMP	37	-	0.7	167	Karlsson *et al.* (2011)
TMP	37	0.8–0.9	1.2–1.3	85	Jokela *et al.* (1997)
Kraft	37	-	0.6	145	Karlsson *et al.* (2011)
	35	-	2.4	90[a]	Wood *et al.* (2009)
	35	3.3	4.0	50	This study
	55			100	
	55	3.9	4.7	108	

[a]biogas dm^3 $kgVSS^{-1}$
TMP = thermo-mechanical pulp

Based on the amount of studied industrial by-products produced in Finland (see Introduction) and methane potentials determined in the present thesis, a methane potential of 28.8–36.5 million m^3 a^{-1} and 5.6 million m^3 a^{-1} could be produced from slaughterhouse wastes and pulp and paper mill wastewater

sludge, respectively. The corresponding values in terms of energy are 288–365 GWh a^{-1} and 56 GWh a^{-1}, respectively. As the consumption of natural gas in Finland in 2012 was 32 TWh, and the whole energy consumption was 381 TWh (Anon 2013b), methane from slaughterhouse by-products and pulp and paper industry wastewater sludge could replace 1.1–1.3 % of the natural gas, equalling 0.1 % of the whole energy consumption.

5.2 Digestion of industrial by-products in CSTRs

5.2.1 Process parameters and methane yields

The results from the present study show that anaerobic digestion of the studied industrial by-products was possible in long-term CSTR experiments. In the co-digestion of slaughterhouse and rendering wastes, OLRs of 1–1.5 kgVS m^{-3} d^{-1} with an HRT of 50 d were seen to be feasible under mesophilic conditions, while OLRs of 1.5 kgVS m^{-3} d^{-1} (HRT 50 d) were too high under thermophilic conditions. Thus, mesophilic conditions were suggested to be more feasible for these substrates than thermophilic conditions. On the other hand, for pig slaughterhouse waste, digestion at the same OLR of 1.5 kgVS m^{-3} d^{-1} with a shorter HRT of 30 d under mesophilic conditions, led to an unstable process. The feasible OLRs obtained in the present study are in the lower range of those reported in the literature while studying slaughterhouse wastes alone (0.5–2.1 kgVS m^{-3} d^{-1}), or in co-digestion with other substrates, like sewage sludge or OFMSW (1.1–4.5 kgVS m^{-3} d^{-1}, Table 17). Also, the feasible HRT of the present study (50 d) is in the range of HRTs used (13–100 d) during slaughterhouse waste mono- and co-digestion (Table 17). On the other hand, for thermophilic anaerobic digestion of pulp and paper mill primary sludge, feasible OLRs of 1–2 kgVS m^{-3} d^{-1}, with HRTs of 14–32 d were observed. However, in digestion with OLR of 2 kgVS m^{-3} d^{-1} with HRT of 14–16 d, nitrogen addition is required as discussed below. In the co-digestion of primary and secondary sludge, the studied OLR of 1 kgVS m^{-3} d^{-1} with an HRT of 25–31 d was seen to be feasible. These OLRs are in the lower range, while HRTs are similar to or somewhat longer than those reported for pulp and paper mill wastewater sludge (OLRs 1–4 kgVS m^{-3} d^{-1}, HRTs 8–24 d, Table 18). Although anaerobic digestion of the studied industrial by-products was seen to be possible, optimization of the process parameters was seen to be essential due to the emerging instabilities as discussed more widely below.

TABLE 17 Anaerobic digestion of slaughterhouse and rendering wastes in CSTR experiments reported in the literature.

Substrate	Temperature °C	Methane yield $dm^3\ kgVS_{fed}^{-1}$	HRT d	OLR $kgVS\ m^{-3}\ d^{-1}$	Reference
Poultry entrails and contents of stomach and intestines	34	600–700	25–50	0.9–1.7	Cuetos *et al.* (2008)
Poultry bone and trimmings, blood, offal and feather	31	90–550	13–100	0.5–2.1	Salminen and Rintala (2002)
Maize and poultry blood VS ratio 60:40	34	165	36	3.1	Cuetos *et al.* (2013)
Animal by-products and sewage sludge WW ratio 1:7	35	340–400	14–25	1.8–3.3	Luste and Luostarinen (2010)
Hygienized animal by-products and sewage sludge WW ratio 1:7	35	370–430	14–25	2.1–3.7	
Animal by-products and sewage sludge WW ratio 1:3	35	340–410	14–25	2.2–4.0	
Solid cattle and swine slaughterhouse waste	35	60	30	1.1–1.3	Alvarez and Lidén (2008)
Solid cattle and swine slaughterhouse waste and solid cattle and swine manure VS ratio 50:50	35	260	30	1.1–1.3	
Solid cattle and swine slaughterhouse waste and fruit and vegetable waste VS ratio 50:50	35	40	30	1.1–1.3	
Solid cattle and swine slaughterhouse waste, solid cattle and swine manure and fruit and vegetable waste varying VS ratios	35	270–350	30	1.1–1.3	
Slaughterhouse waste and organic fraction of municipal solid waste	34	400–500	25–50	1.85–3.7	Cuetos *et al.* (2008)
Mixture of slaughterhouse wastewater, fat, blood, animal by-products, manure and mycelium	37	370–380	24	3.5	Karlsson and Ejlertsson (2012)
Sewage sludge and sterilized mass (2.5–10 % WW)	37	396–645	22.5	2.1–4.5	Pitk *et al.* (2013)
Pig, poultry, cattle slaughterhouse wastes and rendering wastes	35	720	50	1–1.5	This study
Pig slaughterhouse waste and HCl, Fe, Co, Ni, Se, W	35	700	30	1.5	This study

TABLE 18 Anaerobic digestion of pulp and paper mill wastewater sludge in CSTR experiments reported in the literature.

Substrate	Temp. °C	Methane yield $dm^3\ kgVS_{fed}^{-1}$	OLR $kgVS\ m^{-3}\ d^{-1}$	HRT d	Reference
Secondary sludge, kraft	10–30	220[a]	1.5–5.2	24–8	Puhakka *et al.* (1992)
	37	120	ca 1–4	18–22	Karlsson *et al.* (2011)
Secondary sludge, mech	37	180	ca 1–4	18–22	Karlsson *et al.* (2011)
Primary and secondary sludge, TMP and municipal sludge	37	190	1.5–1.7[b]	ca 30	Jokela *et al.* (1997)
Primary sludge, kraft	55	240	1–2	14–32	This study
Primary and secondary sludge, kraft	55	170	1	25–31	This study

[a] biogas
[b] $kgTS\ m^{-3}\ d^{-1}$

As expected based on the substrate characteristics and batch assays, higher methane yields were obtained in the CSTR studies from slaughterhouse and rendering wastes than from pulp and paper mill wastewater sludge. In the digestion of pig slaughterhouse waste alone (II), and in the co-digestion of slaughterhouse waste with rendering wastes (I), average methane yields of ca 720 $dm^3\ kgVS_{fed}^{-1}$ were obtained. On the other hand, in the digestion of pulp and paper mill primary sludge, and co-digestion of primary with secondary sludge (III), the highest methane yields obtained were 240 $dm^3\ kgVS_{fed}^{-1}$ and 170 $dm^3\ kgVS_{fed}^{-1}$, respectively. For both groups of industrial by-products, previous results from CSTR studies are scarce. There are no previous CSTR studies about rendering waste monodigestion; however, in the co-digestion of sewage sludge with rendering waste sterilized mass (2.5–10 % WW), methane yields of 396–645 $dm^3\ kgVS_{fed}^{-1}$ were reported (Pitk *et al.* 2013). In slaughterhouse waste digestion alone, methane yields between 60 and 700 $dm^3\ kgVS_{fed}^{-1}$ have been reported (Table 17). Thus, the methane yields obtained in the present study are at the higher edge of those reported previously. Present and previous results indicate high possible methane yields of the substrates, but also both varying characteristics of the slaughterhouse wastes, and challenging characteristics for the digestion.

As in the case of rendering wastes, there are no previous studies reported on the anaerobic digestion of pulp and paper mill primary sludge in long-term CSTR experiments, and none of the CSTR studies on secondary sludge have been carried out under thermophilic conditions. The methane yields obtained in the present study for the secondary sludge at 35 ˚C were similar, while those of the primary sludge were similar or slightly higher than those reported in the literature. For instance, methane yield of 120 $dm^3\ kgVS_{fed}^{-1}$ was reported for kraft mill secondary sludge at 37 °C (Karlsson *et al.* 2011) and 220 dm^3 biogas

$kgVS_{added}^{-1}$ at 10–30 °C (Puhakka *et al.* 1992), as well as 180 dm^3 $kgVS_{fed}^{-1}$ (Karlsson *et al.* 2011) for mechanical pulp mill secondary sludge at 37 °C (Table 18). In addition, the methane yields in the present study for the pulp and paper mill wastewater sludge were at the same level (93–278 dm^3 $kgVS_{fed}^{-1}$) than reported previously for sewage sludge, in both the laboratory and pilot scale reactors (Davidsson *et al.* 2008, Luostarinen *et al.* 2009, Pitk *et al.* 2013).

Comparing the methane yields of the present CSTR and batch experiments, the methane yields of the slaughterhouse and rendering wastes were higher in CSTRs. Calculated according to the results of the batch assays, the methane potential of the feed of the slaughterhouse and rendering wastes should have been 422 dm^3 $kgVS_{fed}^{-1}$. Higher methane yields obtained in reactor experiments, compared to expectations according to batch experiments, could be due to the unadapted inoculum in the batch experiments, and/or due to the formation of some inhibitory or toxic compounds in the batch experiments (Angelidaki and Sanders 2004). For the pulp and paper mill wastewater sludge, similar methane yields were obtained in the CSTR and batch experiments, suggesting that all of the biodegradable material within the sludge degraded under the reactor conditions.

5.2.2 Nitrogen and buffer capacity

The two kinds of industrial by-products studied had large differences in nitrogen content, while in the feed of the pulp and paper mill sludge (III), the TKN concentrations were 0.1–0.8 g l^{-1} and in the slaughterhouse and rendering waste (I) feeds, the TKN concentrations were 1.6–5.2 g l^{-1}, depending on the applied OLR. This difference in TKN concentration between the studied substrates is due to the high protein and lipid contents of the slaughterhouse and rendering wastes, compared to the high carbohydrate content of the pulp and paper mill wastewater sludge. It should be kept in mind that because of the solid state of the slaughterhouse and rendering waste feed, the feed was diluted. Nitrogen was an important factor in both cases, as nitrogen is an essential nutrient for micro-organisms to grow, but too high ammonia nitrogen concentrations can be inhibitory to the process.

In the CSTR studies of the pulp and paper mill wastewater sludge, the low nitrogen content of the sludge resulted in low ammonium nitrogen concentration of the digestates, 0.2–0.8 g l^{-1} on day 46. Especially in the monodigestion of the primary sludge, the low nitrogen content of the sludge (0.1 g TKN l^{-1}) decreased the NH_4-N concentrations of the digestates to 0.2–0.6 g l^{-1} on day 46. These concentrations were thought to be too low for micro-organisms, as in a previous study, the concentration of 0.5 g l^{-1} was found to be too low for the digestion process, causing a low methane yield, loss of biomass and loss of the aceticlastic methanogenic activity. This was thought to be due to the decreased buffering capacity and deficiency in nutrient nitrogen (Procházka *et al.* 2012). In the present study in the digestion of primary sludge, the situation was thought to be similar, where the low NH_4-N concentration of the sludge was speculated to result in a lack of ammonia required for microbial needs, and

also to reduce the buffering capacity, which decreased the pH. On the other hand, in the microbioreactor study with *Methanosaeta concilii*, the optimum growth was found in the NH_4-N range of 250 to 1100 mg l^{-1} at 35 °C (Steinhaus *et al.* 2007). It must be kept in mind that the optimal NH_4-N concentration of the process depends on the origin of the inoculum, as well as type of substrate, and therefore, it differs according to each individual case. In the present study, the pH decrease slowed down after nitrogen additions were begun; however, since the addition of nitrogen did not increase the methane yields, and did not completely stabilize the pH, the impacts of the applied nutrient (nitrogen and reject water) remain undetermined. However, it seems probable that the pH would have decreased more if the nitrogen additions were not started; thus, the nitrogen addition, as well as the pH adjustment, must be evaluated when considering the anaerobic digestion of the pulp and paper mill wastewater sludge, which was also noted previously in the digestion of bleached kraft pulp mill waste activated sludge (Puhakka *et al.* 1992).

On the contrary, during the digestion of slaughterhouse and rendering wastes, the ammonia concentrations of the digestates were between 0.8–2.4 g l^{-1} in the mesophilic and 1.6–3.0 g l^{-1} in the thermophilic reactors. In previous digestion experiments with various substrates, inhibitory concentrations of NH_4-N have been suggested to be between 2.5 and 4.8 g l^{-1} at 35 °C, and between 3.5 and 5.6 g l^{-1} at 55 °C (Table 19). Compared to these results, ammonia could or could not have been inhibitory in the present study. On the other hand, the calculated free ammonia values in the mesophilic process (< 115 mg l^{-1}) in the present study were similar, or lower, than those reported from the digestion studies to be inhibitory (92–1100 mg l^{-1}, Table 19). The higher calculated NH_3 concentration in the thermophilic, than in the mesophilic, reactor was apparently due to the higher operating temperature, pH and NH_4-N concentration in the former reactor. Although the pH in the thermophilic reactor in the present study remained more or less above 7.5, process instability was noticed in the accumulating TVFA concentration and decreasing methane production. This is due to the fact that the high ammonia concentration provided the necessary buffering capacity against a pH drop (Procházka *et al.* 2012). On the other hand, the pH in the mesophilic reactor was more than 7 only from day 28 onward. This low pH during the initial experimental period in the mesophilic reactor was apparently due to VFA accumulation. However, VFA accumulation during the later period of the run did not result in a pH drop, most likely because the higher ammonia concentration of the digestate stabilized the VFA accumulation (Procházka *et al.* 2012).

TABLE 19 Ammonia inhibition in anaerobic digestion reported in the literature.

Substrate	Temp. °C	NH_4-N mg l^{-1}	NH_3 mg l^{-1}	Effects	Reference
Synthetic substrate (pet food) to simulate OFMSW	35	3860	215	50 % inhibition in CH_4 production	Benabdallah El Hadj *et al.* (2009)
	55	5600	468		
Swine manure	55	-	1100	Decreased CH_4 production	Hansen *et al.* (1998)
Cattle manure	55	4000	650	Decreased CH_4 production, increased VFAs	Angelidaki and Ahring (1993)
Biowaste slurry	37	3000	220	50 % inhibition in	Gallert and
	55	3500	690	CH_4 production	Winter (1997)
Chicken manure	35	4800	650	10 % inhibition in methanogenesis	Niu *et al.* (2013)
Glucose	35	4000	-	Decreased CH_4 production	Procházka *et al.* (2012)
Glucose solution	52	-	620	21 % decrease in biogas production	Siles *et al.* (2010)
Synthetic wastewater	55	4000	-	Inhibition of methanogenesis	Sung and Liu (2003)
		8000–13000	-	100 % inhibition of methanogenesis	
Corn stover	37	> 2500	-	Decreased CH_4 production	Wang *et al.* (2013)
		6000	-	Decreased CH_4 production by 50 %	

5.2.3 Digestion and inhibition

VS removals in the present CSTR studies were between 68 and 89 % during the stable periods of slaughterhouse and rendering waste digestion, while they were between 25 and 40 % in the digestion of pulp and paper mill wastewater sludge. This relatively low biodegradability of the sludge could partly be related to the lignin content, as lignin is not anaerobically degradable (Angelidaki and Sanders 2004). Another reason for lower degradation of the pulp and paper mill wastewater sludge compared to slaughterhouse and rendering wastes could be chemicals used in paper making process and inhibiting the degradation of the sludge. In the anaerobic digestion of pulp and paper mill sludge, cellulose and hemicellulose are the components that can be degraded. In the present study, cellulose removals of 70–73 % were obtained; although, a 22 % cellulose removal was reported in the batch digestion of primary and secondary pulp and paper mill sludge with monosodium glutamate waste liquor at 37 °C (Lin *et al.* 2009). The higher cellulose content of the primary sludge than that of the secondary sludge could explain its higher methane yield in the present study. Furthermore, secondary sludge mainly

consists of microbial biomass, which is poorly degradable due to the glycan strands cross-linked by peptide chains in the microbial cell walls (Appels *et al.* 2008). In the present study, 7–27 % of hemicellulose degraded, while in a previous study, the concentration of hemicellulose was seen to increase (Lin *et al.* 2009). In general, hemicellulose is considered to be more easily hydrolysed than cellulose (Pérez *et al.* 2002). Therefore, it can be speculated that some inhibitory compounds were the reason for the low hemicellulose degradation in the present study.

In the co-digestion of slaughterhouse and rendering wastes, VFA and LCFA inhibition may become a problem for the digestion process besides ammonia. In the present study, the TVFA concentration was higher in the thermophilic reactor than in the mesophilic reactor. Previously, it was concluded that the VFA accumulation in the reactors was because of process imbalance, and not the cause of the inhibition (Ahring *et al.* 1995, Pullammanappallil *et al.* 2001). However, inhibition due to VFAs has also been proven in reactor experiments were VFAs in concentrations of 10 g l^{-1} were found to be inhibitory for acetate degradation (Aguilar *et al.* 1995), and in pure culture experiments were propionic acid concentrations of 1.5–2.2 g l^{-1} were shown to inhibit methanogens and the concentration of 5.9 g l^{-1} had an effect over two orders of magnitude (Barredo and Evison 1991). In the present study, the highest TVFA concentrations noticed were 6.4 g l^{-1} on day 143 in the thermophilic reactor and 1.9 g l^{-1} in the mesophilic reactor at the end of the study. The TVFA accumulation observed in both reactors indicates that the OLR of 2 kgVS m^{-3} d^{-1} in the mesophilic reactor and OLR of 1.5 kgVS m^{-3} d^{-1} in the thermophilic reactor were too high. On the other hand, the VFAs might not have been the reason but the consequence of the process imbalance, although this cannot be ensured. Acetic acid was the main VFA, accounting for > 80 % of the TVFA presented in the mesophilic reactor, indicating that aceticlastic methanogenesis was the rate-limiting step in this reactor. A similar observation was also reported during the mesophilic digestion of poultry slaughterhouse waste (Salminen and Rintala 2002). As in thermophilic reactor acetic acid accounted for around 50 % of the TVFA, aceticlastic methanogenesis could have been the rate-limiting step also in here. However, propionic acid concentration of around 30 % indicates LCFAs inhibiting propionic acid degradation (Salminen *et al.* 2000). LCFAs have also previously been speculated to inhibit digestion of different slaughterhouse wastes in the mesophilic CSTR process (Cuetos *et al.* 2010). From the batch assays with individual LCFAs, it is known that already low concentrations of some individual LCFAs can be inhibitory (Lalman and Bagley 2000, 2001); for example, greater than 30 mg l^{-1} linoleic acid completely inhibited aceticlastic methanogenesis (Lalman and Bagley 2000). As discussed above, ammonia concentrations in the present study could or could not have been inhibitory, and role of VFAs cannot be ensured. Thus, LCFAs could have been the reason for the failure of the thermophilic reactor, possibly in addition to ammonia and VFA inhibition. However, since the LCFA concentrations were not measured in the present study, this cannot be ensured.

5.3 Improving anaerobic digestion of industrial by-products

5.3.1 Additives to improve anaerobic digestion of pig slaughterhouse waste

In a previous chapter, it was concluded that meat industry by-products are feasible substrates for anaerobic digestion. However, the process is vulnerable to instability due to ammonia, VFA and/or LCFA inhibition. Thus, the use of additives containing trace elements, Fe and HCl were studied as methods to improve methane yields and to achieve a more stable digestion process. The effect of additives on the digestion of pig slaughterhouse waste was studied in three semi-continuously fed CSTRs at mesophilic conditions. In pig slaughterhouse waste digestion, the methane yields were mainly between 600 and 800 dm^3 $kgVS_{fed}^{-1}$. Thus, the methane yield was similar to the yield obtained in co-digestion of rendering plant and slaughterhouse wastes (720 dm^3 $kgVS_{fed}^{-1}$, I) and in the digestion of poultry slaughterhouse waste (600–700 dm^3 $kgVS_{fed}^{-1}$, Cuetos *et al.* 2008) at 35 °C. Use of additive 2 increased the methane yield due to a more stable process, compared to the digestion without additives; however, the additives did not increase the methane yield above the yields obtained during the similar stable processes (I, Cuetos *et al.* 2008).

As was expected from the good degradation of slaughterhouse and rendering wastes (VS removals of 68–89 %), good degradation was also noticed when studying the effect of the additives on the digestion of pig slaughterhouse wastes. However, the effect of additive 2 was seen in experiments comparing the digestion with additive 1, or without additives, in decreasing the SCOD and TVFA concentrations. The results of the experiments were ensured by changing the additives after the process imbalance was noticed in the reactor receiving additive 1 (S2), and in the control reactor (S1). It was concluded that without additives, and with the use of additive 1 (Fe and HCl), the process became unstable when operated at an OLR of 1.5 kgVS m^{-3} d^{-1} and HRT of 30 d. On the other hand, use of additive 2 (trace elements, Fe and HCl) improved the process stability and enabled to operate the reactor with OLRs up to 2.5 kgVS m^{-3} d^{-1}. However, operation with additive 2 with OLRs of 1.75–2.5 kgVS m^{-3} d^{-1}, lasted for less than 1 HRT each. Therefore, it cannot be ensured that an OLR as high as 2.25 kgVS m^{-3} d^{-1} would be stable in the long run.

In the digestion of pig slaughterhouse waste (II), the pH in all three reactors remained over 7 throughout the run, although process imbalances were noticed due to VFA accumulation. As in the previous experiments (I), the high ammonia concentration (1.5–3.5 g l^{-1}) buffered against a pH drop (Procházka *et al.* 2012). The NH_4-N concentrations, as well as the calculated free NH_3 concentrations (0.05–0.22 g l^{-1}) in the present study, were most probably below inhibitory when compared to previous results from reactor experiments (Table 19). In a previous study, the HCl addition improved the methane yield by decreasing the pH from 8.0 to 7.6–7.8 (Karlsson and Ejlertsson 2012). This led to a decrease in the calculated free NH_3 concentration, from 0.7 to 0.3–0.5 g l^{-1}, in

the digestion of slaughterhouse waste, manure and mycelium at 37 °C (Karlsson and Ejlertsson 2012). In the present study, the pH was not affected by the additives, despite the added HCl. The present results show that the studied additive 2 enabled the stable operation with a higher OLR than without the additive. The use of additive 2 (Fe, HCl and trace elements) prevented the build-up of VFAs, thus enabling acetogenesis and further improving the aceticlastic methanogenesis, which was seen to be the rate-limiting step in the digestion of slaughterhouse and rendering wastes in the previous study (I). It can be concluded that Fe and HCl together with the trace elements, Co, Ni, Se and W, were essential for improving the process, while the additive without the trace elements (additive 1) did not facilitate better performance of the reactor than that obtained without the additives (control).

The H_2S content of the biogas in all three reactors treating pig slaughterhouse waste was between 1400 and 1800 ppm on day 67, before any additives were fed. When the additives were fed, the H_2S content of the biogas was seen to decrease. The decrease in the H_2S content of the biogas in all three reactors upon use of the additives was due to fact that the added Fe reacts with H_2S to form FeS. The decreasing H_2S content in the biogas is important, as H_2S is corrosive to process equipment (Ryckebosch *et al.* 2011). Added Fe was seen in Fe concentrations measured at the end of each feeding period. In reactor S1 without additives, the Fe concentration was 8 mg $kgFM^{-1}$, while after additive 2 the concentrations were 64 mg $kgFM^{-1}$ in reactor S1 and 69–250 mg $kgFM^{-1}$ in the other reactors with additives, with the same OLR of 1.5 $kgVS\ m^{-3}\ d^{-1}$.

Previous results on the effects of Fe on anaerobic digestion are contradictory, as both enhanced methane yields in the digestion of fat containing wastewater (Ivanov *et al.* 2002) and food waste (Zhang and Jahng 2012), and decreased methane yields in the digestion of WWTP wastewater sludge (Smith and Carliell-Marquet 2008) have been reported. In the present study, no effect was observed on the methane yields or process stability by adding only Fe and HCl. As in Fe concentrations, in the concentrations of Ni, Co and W an increase was measured due to the additives in the present study. In a previous study with 10 species of methanogens, the composition of them was observed to vary quite a bit (Scherer *et al.* 1983). Methanogens were seen to consist of C (37–44 % WW), H (5.5–6.5 %), N (9.5–12.8 %), Na (0.3–4.0 %), K (0.13–5.0 %), S (0.56–1.2 %), P (0.5–2.8 %), Ca (85–4500 ppm), Mg (0.09–0.53 %), Fe (0.07–0.28 %), Ni (65–180 ppm), Co (10–120 ppm), Mo (10–70 ppm), Zn (50–630 ppm), Cu (<10–160 ppm), and Mn (<5–25 ppm, Scherer *et al.* 1983). It can be concluded, that the order of magnitude for the trace element requirement can be seen from the chemical composition shown above. However, every process is different and each process has its own trace element requirements. Also, previously recommended concentrations of trace elements vary (Table 20). Keeping this information in mind, compared to the previous results on the composition of methanogens (Scherer *et al.* 1983), Ni and Co concentrations, even after additions, were below these recommended concentrations. On the other hand, Fe concentration increased after the additions, to meet the limits. As

in the present study, HCl and Fe addition without trace elements did not improve the process stability, it seems that despite these concentration limits, trace element concentrations were high enough to effect the process. However, as the Se concentration could not be measured, the role of each trace element remains unclear.

TABLE 20 Recommended trace element concentrations, of the trace elements studied in this thesis, in previous studies about trace element additions in anaerobic digestion.

Fe	Co	Ni	Se	W	Reference
ns	0.22 mg $kgFM^{-1}$	ns	0.16 mg $kgFM^{-1}$	ns	Banks *et al.* (2012)
200 mg $kgCOD^{-1}$	6.0 mg $kgCOD^{-1}$	5.7 mg $kgCOD^{-1}$	ns	ns	Qiang *et al.* (2012)
ns	0.02–0.05 mg $kgFM^{-1}$	0.1–0.6 mg $kgFM^{-1}$	ns	ns	Pobeheim *et al.* (2011)
100 mg l^{-1}	2.0 mg l^{-1}	10.0 mg l^{-1}	ns	ns	Zhang and Jahng (2012)

ns = not studied

5.3.2 Pre-treatments to improve anaerobic digestion of pulp and paper mill secondary sludge

In the batch and CSTR studies, pulp and paper mill wastewater sludge were determined to be feasible substrates for anaerobic digestion. However, the methane yield of the secondary sludge remained relatively low, and thus, 12 different single or combined pre-treatment methods were studied to improve the digestion and methane yields. Hydrothermal pre-treatment at 150 ˚C alone, and in combination with ultrasound and/or enzymatic pre-treatments, increased the methane yield of the pulp and paper mill secondary sludge by 19–31 %. Conversely, no statistically significant increase in methane yields was noticed with hydrothermal pre-treatment at 70 ˚C, ultrasound or enzymatic pre-treatments. On the other hand, acid and alkaline pre-treatments resulted in lower methane yields than control. The higher methane production rates from the sludge pre-treated hydrothermally at 150 ˚C (HT150) compared to the untreated sludge suggests that shorter HRTs of 10–15 d could be used during the full scale digestion which would improve the economics as smaller digesters are needed. All studied pre-treatments, except for the ultrasound and acid pre-treatments, improved COD solubilisation. Previously, it has been observed that there is no direct correlation between the solubilisation and biodegradability seen as improved methane yields after pre-treatments (Ma *et al.* 2011).

As previous studies on pre-treatments of pulp and paper industry wastewater sludge are limited (Table 21), the results are often compared to those of municipal wastewater sludge. This has been claimed to be problematic as the composition of municipal wastewater, and thus, the wastewater sludge

differs from that of pulp and paper mills (Bhathena *et al.* 2006). In a previous study, municipal wastewater sludge from six different wastewater treatment plants were reported to consist of 0.43–0.82 g gVS^{-1} of proteins, 0.12–0.30 g gVS^{-1} of carbohydrates and 0.01–0.09 g gVS^{-1} of lipids (Mottet *et al.* 2010). In the above study, cellulose, hemicellulose and lignin contents were reported to be 0–3 % of TS, 7–27 % of TS and 3–27 % of TS, respectively (Mottet *et al.* 2010). In the present study, 57 % of the secondary sludge TS composed of cellulose, hemicellulose and lignin, while the corresponding value for municipal secondary sludge was mainly between 20 and 33 %, with highest value of 53 % (Mottet *et al.* 2010). Thus, differences between individual wastewater treatment plants seem to be higher than those between municipal and pulp and paper industry wastewater treatment plants categorically.

TABLE 21 Previous results from anaerobic digestion of pre-treated pulp and paper mill secondary sludge in batch experiments.

Pre-treatment	Parameters	Substrate	Digestion temperature °C	Change of methane yield %	Reference
Ultrasound	2–3 Wh l^{-1}	Kraft	37	19–21	Karlsson *et al.* (2011)
	5–30 Wh l^{-1}	Kraft/CTMP	37	2–5	
	20 kHz, 400 W, 15–90 min	BCTMP	35	33–51	Mehdizadeh *et al.* (2012)
			55	5–28	
	20 kHz, 400 W, 90 min	BCTMP	35	51	Saha *et al.* (2011)
			55	28	
Ultrasound +Enzyme	30 Wh l^{-1} + 40 mg gTS^{-1}	Kraft/CTMP	37	20	Karlsson *et al.* (2011)
Enzyme	40 mg gTS^{-1}		37	21	
Thermal	170 °C, 1h	Sulfite	35	50[a]	Wood *et al.* (2009)
		Kraft	35	280 [a]	
NaOH+ Thermal	pH 12, 140 °C, 1 h	Kraft	35	ca 280 [a]	
		Sulfite	35	18 [a]	
NaOH+ Ultrasound	0.206 g gTS^{-1}, 2 h + 40 kHz, 2 h	BCTMP/TMP	36	–16	Park *et al.* (2012)
	0.261 g gTS^{-1}, 40 kHz, 2 h	Thickened BCTMP/TMP	36	2	

[a] biogas

Previously, thermal pre-treatment has been studied to improve sludge dewaterability and solubilisation using a wide range of temperatures, from 70 °C up to 200 °C, with an optimum temperature range of 160–180 °C, and treatment times from 30 to 60 min (reviewed by Carrère *et al.* 2010). It has been reported that for municipal sludge pre-treatment at 70 °C is enough to increase the methane yield (Ferrer *et al.* 2008). However, it seems that thermal pre-

treatment at 70 °C may require long treatment times lasting for several hours to days in order to achieve a significant increase in the methane yield or COD solubilisation (Gavala *et al.* 2003, Ferrer *et al.* 2008). In the present study, in hydrothermal pre-treatment at 150 °C, COD solubilisation led to higher methane yields and somewhat increased solubilisation, but no increase in methane yield was observed in the hydrothermal pre-treatment at 70 °C probably due to the loss of organics during pre-treatment and the subsequent cooling time (reviewed by Carlsson *et al.* 2012).

The high COD solubilisation (4–10 times) was seen in all the enzymatic pre-treatments. However, only those enzymatic pretreatments combined with hydrothermal pre-treatment increased the methane yields. Thus, it seems that the sludge cellulose and hemicellulose were inaccessible for the enzymes. These results are similar to those reported by Bruni *et al.* (2010) who observed increased methane yield only when the enzymatic treatment was combined with steam treatment, but not alone. The above authors suggested that enzymes alone are not capable of digesting the tight association of lignocelluloses. In another previous study, no increase in methane yields from enzymatically (mixture of cellulases, proteases and lipases) pre-treated pulp and paper mill (kraft/CTMP) secondary sludge was noticed when the methane yield of the enzymes and chelating mixture was taken into account (Karlsson *et al.* 2011; Table 21). Nevertheless, the enzymatic pre-treatment of municipal secondary sludge with amylases and proteases was reported to increase methane yields, both alone and together (Yu *et al.* 2013). These results thus highlight the significance of use of suitable enzymes for each substrate.

The possible reason for no significant increase in methane yield or COD solubilisation noticed in ultrasound pre-treated sludge could be that the pre-treatment conditions used in the present study were not optimal to cause cavitation. Previously, low frequencies of 20–40 kHz have been seen to be most efficient in municipal sludge digestion (reviewed by Carrère *et al.* 2010), but the frequency used in the present study was around 45 kHz. Moreover, high solid concentrations have been shown to increase viscosity and thereby hinder cavitation bubble formation (Show *et al.* 2007). The solid loading used in the present study was slightly higher (4.7 % of TS) when compared to the optimal loading of 2.3–3.2 % of TS (reviewed by Carrère *et al.* 2010). Nevertheless, previously ultrasound pre-treatment have been reported to increase methane yield of pulp and paper mill secondary sludge more or less (Table 21).

The low methane yields with apparent long lag phase of ca 20 days noticed for both acid (HNO_3) and alkali (NaOH) pre-treated secondary sludge, was probably due to production of inhibitory compounds and/or sub-optimal pH. In a previous study with MWWTP secondary sludge, acid pre-treatment increased the biogas yields with pH between 1 and 4 (Devlin *et al.* 2011). Thus, it seems improbable that the pH of the present study (3) would have been too high. As COD solubilisation was not observed in the acid pre-treated sludge, another possibility for decreased methane yield could be inhibition due to N from HNO_3, or some other substance formed during pre-treatment. When

compared to inhibitory concentrations of NH_4-N (2.5–5.6 g l^{-1}, Table 19), the N concentration of the acid pre-treated sludge (1.8 g l^{-1}) is assumed to be below inhibitory. Other inhibitory substances could be furfural or HMF formed during the digestion of hemicelluloses (Barakat *et al.* 2012).

The possible reason for no improvement in the methane yield, despite a 5-fold increase in the SCOD concentration noticed for NaOH pre-treated sludge could be production of some inhibitory compound, e.g. Na or N or some other compound released during digestion. However, the concentrations of Na (3.6 g l^{-1}) and N (1.1 g l^{-1}) in the present study are low when compared to those reported to be inhibitory (IC_{50} for Na 5.6–53 g l^{-1}, reviewed by Chen *et al.* 2008 and NH_4-N 2.5–5.6 g l^{-1}, Table 19). Previously, with pulp mill secondary sludge, both increased and not increased methane yields have been reported (Table 21). On the other hand, when studying the effect of NaOH pre-treatment on MWWTP secondary sludge, the pH 10 was seen to be optimal based on biogas production, while a pH 12 was seen to decrease the biogas yield (Shao *et al.* 2012). This was suggested to be due to the formation of complex compounds under extreme pH conditions (Shao *et al.* 2012). These complex substances, like melanoidins, are formed in Maillard reactions (Penaud *et al.* 1999), which are chemical reactions between reducing sugars and the amino groups of proteins (Lan *et al.* 2010).

In the present study, the increase in cellulose with a corresponding decrease in hemicellulose content, as seen in most pre-treatments, except for chemical pre-treatments, indicates that the pre-treatments were effective in solubilising/removing hemicelluloses; thereby improving the accessibility of cellulose to further hydrolysis (Hendriks and Zeeman 2009). On the contrary, the decrease in carbohydrate content, in the case of chemical pre-treatments, indicates a conversion of these organic compounds to potential inhibitor compounds. For example, the breakdown of lignin may release phenolic compounds that can be toxic for anaerobic micro-organisms (Barakat *et al.* 2012). In a previous study on the effects of steam, lime and acid pre-treatments on switch grass, the hemicellulose concentration decreased while the cellulose and lignin concentrations either decreased or increased, depending on the treatment (Samuel *et al.* 2011). Besides analysed cellulose, hemicellulose and lignin, the organic matter of the sludges includes e.g. wood extractives, proteins and pectin (Jørgensen *et al.* 2007).

5.4 Further research needs

In this thesis, anaerobic digestion of meat and pulp and paper industry by-products was seen to be feasible. However, many unexplained questions remain and also some further research should be done.

About anaerobic digestion of substrates containing high concentrations of lipids and proteins, further research is needed with regard to the specific roles of nitrogen, VFAs and LCFAs in process inhibition. This could help to optimize

process conditions suitable for these substances. In addition, further research is needed to investigate the specific effect of the individual trace elements in the digestion of slaughterhouse wastes. Such a way additives could be planned to be proper for each substrate and process.

In the anaerobic digestion of pulp and paper mill wastewater sludge further research is required regarding to nitrogen additions and/or pH adjustment. Besides, need of other nutrients should be studied. These aspects should be studied to evaluate their possibility to increase methane yields and/or obtain more stable digestion process of pulp and paper mill wastewater sludge. About the pre-treatments of the pulp and paper mill secondary sludge, further research is needed with regard to the energy balance of the whole process from pre-treatment to biogas production, which should be evaluated as the energy demand of the pre-treatments was not considered in this study and as it effects both economical efficiency and sustainability of the digestion process.

For sustainability of the biogas production process it is important that the digestate can be further used e.g. as a soil conditioner. For this aspect, the possible effects of feeding additives, and subsequently increased trace element concentrations in the digestate should be studied. Similarly, in case of pulp and paper mill wastewater sludges, the effect of heavy metals and other possibly toxic constituents on further use of digestate should be studied.

6 CONCLUSIONS

In this thesis, the anaerobic digestion of two groups of industrial by-products, slaughterhouse and rendering wastes, and pulp and paper industry wastewater sludge, was seen to be feasible. Industrial by-products are formed in industrial processes as secondary products. Typical for both studied by-product groups is that they are thought to be difficult to digest. Anaerobic digestion is a way to combine the production of renewable energy, biogas, and waste treatment.

In the batch experiments, methane potentials of 16–630 dm^3 $kgVS_{added}^{-1}$ were obtained for the meat industry by-products and 50–230 dm^3 $kgVS_{added}^{-1}$ for the pulp and paper industry by-products. These methane potentials of the meat industry by-products are higher and the methane potentials of the pulp and paper industry by-products are at the same level or a bit lower than those of energy crops and sewage sludge, which are widely used as substrates for biogas production. Especially methane yields of the pulp and paper mill wastewater treatment primary sludge were promising as primary sludge digestion has been scarcely studied before. Based on these methane potentials, 288–365 GWh a^{-1} of energy could be produced in Finland from slaughterhouse wastes, and 56 GWh a^{-1} from pulp and paper mill wastewater sludge. Together, they could replace 1.1–1.3 % of the natural gas used in Finland in 2012, and the energy amount equals to 0.1 % of the whole energy consumption in Finland in 2012.

In the reactor experiments, methane yields of ca. 700 dm^3 $kgVS_{fed}^{-1}$ were obtained in the anaerobic digestion of slaughterhouse and rendering wastes. Correspondingly, methane yields of 170–240 dm^3 $kgVS_{fed}^{-1}$ were obtained for the pulp and paper mill wastewater sludge. In the digestion of slaughterhouse and rendering wastes, stable process was obtained with OLRs of 1.0–1.5 kgVS m^{-3} d^{-1} and HRT of 50 d. With OLR of ≥ 1.5 kgVS m^{-3} d^{-1} and an HRT of 30 d or OLRs of > 1.5 kgVS m^{-3} d^{-1} and an HRT of 50 d, the digestion processes became unstable due to high ammonia, VFA and probably also LCFA concentrations. Mesophilic conditions were seen to result in a more stable process in the digestion of slaughterhouse and rendering wastes, while in the digestion of pulp and paper mill wastewater sludge, thermophilic conditions were seen to

be better. OLRs of 1–2 kgVS m^{-3} d^{-1} and HRTs of 14–32 d were seen to be feasible for pulp and paper mill wastewater sludges, however, with an OLR of 2 kgVS m^{-3} d^{-1} and HRT of 14–16 d, nitrogen addition and/or pH adjustment must be considered.

According to the present study, more stable process in slaughterhouse waste digestion can be achieved by use of additive containing HCl, Fe, Co, Ni, Se and W. The use of this additive improved aceticlastic methanogenesis, which otherwise is thought to be the rate-limiting step during the digestion of meat industry by-products. An OLR of 1.5 kgVS m^{-3} d^{-1} and HRT of 30 d, which were seen to lead to instabilities without additives, were seen to be stable with HCl, Fe, Co, Ni, Se and W. Higher OLR and shorter HRT found to be possible in digestion with additive than without allow smaller digesters when planning full scale digestion. In addition, the H_2S concentration in the produced biogas decreased as the additive contains Fe which precipitates with S. This is important as H_2S is corrosive to process equipment. On the other hand, additive containing only HCl and Fe did not improve process stability.

Among the different pre-treatments studied, hydrothermal pre-treatment at 150 ˚C, alone or in combination with enzymes and/or ultrasound, improved the methane yield (19–31 %) and digestion rate of pulp and paper mill secondary sludge. Higher methane production rate would mean that shorter HRTs of 10-15 d could be used when planning full scale digestion. This could mean smaller digesters, and thus, be economically viable. On the other hand, other studied pre-treatments: acid, alkaline, ultrasound, enzyme and hydrothermal treatment at 70 ˚C, did not increase methane yields statistically significantly. However, further research is needed, as the energy balance of the pre-treatments was not calculated in this thesis.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Biokaasun tuotanto liha- ja sellu- ja paperiteollisuuden sivutuotteista

Uusiutuvan energian tuotanto on kasvussa maailmalla, koska sen avulla voidaan vähentää ympäristön kuormitusta, kuten kasvihuonekaasupäästöjä ja riippuvuutta fossiilisista polttoaineista. Yksi uusiutuvan energian muoto on biokaasu, jota muodostuu hapettomissa eli anaerobisissa olosuhteissa, kun mikro-organismit hajottavat orgaanista ainesta. Biokaasu koostuu pääasiassa metaanista (CH_4) ja hiilidioksidista (CO_2), ja sitä voidaan käyttää joko sähkön tai lämmön tuotantoon tai liikennepolttoaineena. Käyttötarkoituksesta riippuen biokaasu puhdistetaan ja jalostetaan biometaaniksi. Biokaasua voidaan tuottaa monenlaisista orgaanisista aineista, kuten energiakasveista, jätevesilietteistä ja biojätteistä. Biokaasun lisäksi hajotusprosessissa muodostuu ravinteikasta käsittelyjäännöstä, josta voidaan valmistaa maanparannus- tai lannoitevalmisteita, ja näin palauttaa ravinteet takaisin kiertoon.

Tässä väitöskirjassa tutkittiin mahdollisuutta tuottaa biokaasua liha- ja sellu- ja paperiteollisuuden sivutuotteista. Tutkitut sivutuotteet olivat teurastamon ja renderöintilaitoksen sivutuotteita sekä sellu- ja paperitehtaan jätevesilietteitä. Kaikille tutkituille sivutuotteille (12 jaetta) määritettiin metaanintuottopotentiaalit panoskokeissa. Sen jälkeen tutkittiin materiaalien käsittelyä jatkuvatoimisissa biokaasureaktoreissa erilaisissa prosessiolosuhteissa. Prosessien toimivuutta arvioitiin tuotetun metaanin määrän sekä käsittelyjäännöksen ominaisuuksien perusteella. Lisäksi tutkittiin lisäaineiden vaikutusta sian teurasjätteen käsittelyyn jatkuvatoimisessa biokaasureaktorissa sekä erilaisten esikäsittelyiden vaikutusta sellu- ja paperiteollisuuden jätevedenpuhdistamon sekundäärilietteen hajoamiseen panoskokeissa. Tutkituista materiaaleista teurasjätteiden hajotusta on aiemmin tutkittu pääasiassa yhteishajotuksessa muiden materiaalien kanssa ja renderöintijätteiden biokaasun tuottoa on tutkittu vain vähän jatkuvatoimisessa prosessissa. Metsäteollisuuden lietteistä primäärilietteen biokaasun tuottoa on aikaisemmin tutkittu vain vähän.

Tässä tutkimuksessa havaittiin, että lihateollisuuden sivutuotteiden metaanintuottopotentiaali on yleisesti sellu- ja paperiteollisuuden jätevesilietteitä korkeampi. Lihateollisuuden sivutuotteiden ominaisuudet, hajoaminen ja metaanintuotto vaihtelevat: metaanintuotto oli tässä tutkimuksessa 16 – 630 dm^3 kg $VS_{lisätty}^{-1}$ (lisättyä orgaanista kohti). Verrattuna yleisesti biokaasun raaka-aineena käytettyihin energiakasveihin ja yhdyskuntajätevedenpuhdistamolietteisiin, sellu- ja paperiteollisuuden jätevesilietteille tässä tutkimuksessa määritetyt metaanintuottopotentiaalit, 50 – 230 dm^3 $kgVS_{lisätty}^{-1}$, ovat samalla tasolla tai hiukan matalampia ja vastaavasti lihateollisuuden sivutuotteiden metaanintuottopotentiaalit ovat pääasiassa korkeampia.

Jatkuvatoimisessa prosessissa lihateollisuuden sivutuotteiden yhteishajotuksessa sekä sian teurasjätteen yksinhajotuksessa metaanintuotto oli keskimäärin n. 700 dm^3 $kgVS_{lisätty}^{-1}$. Lihateollisuuden sivutuotteilla vakaampi prosessi saavutettiin mesofiilisissä (35 ˚C) kuin termofiilisissä (55 ˚C) olosuhteissa.

Prosessin vakauden ja metaanintuoton perusteella prosessi toimi kuormituksella 1 – 1,5 kgVS m^{-3} d^{-1} ja viipymällä 50 d. Sen sijaan samalla kuormituksella 1,5 kgVS m^{-3} d^{-1}, mutta lyhyemmällä viipymällä 30 d prosessi oli epävakaa. Epävakautta prosessissa aiheutti ammoniumtypen, haihtuvien orgaanisten rasvahappojen sekä pitkäketjuisten rasvahappojen kertyminen.

Sellu- ja paperiteollisuuden primärilietteen hajotuksessa korkein metaanintuotto oli 240 dm^3 $kgVS_{lisätty}^{-1}$ ja primääri- ja sekundärilietteen yhteishajotuksessa 170 dm^3 $kgVS_{lisätty}^{-1}$. Paperi- ja selluteollisuuden jätevesilietteiden hajotukselle toimivammaksi havaittiin termofiiliset kuin mesofiiliset olosuhteet. Primärilietteen hajotuksessa toimivaksi kuormitukseksi havaittiin 1 – 2 kgVS m^{-3} d^{-1} ja viipymäksi 14 – 32 d, kun lietteiden yhteishajotuksessa kuormitus 1 kgVS m^{-3} d^{-1} ja viipymä 25 – 31 d olivat sopivia. Primärilietteen hajotuksessa typpilisäystä ja pH:n säätöä tulee harkita etenkin kuormituksella 2 kgVS m^{-3} d^{-1}, koska lietteen matalan typpipitoisuuden takia typen määrä saattaa laskea mikrobien kasvun kannalta liian matalaksi ja lisäksi prosessin puskurikyky voi heikentyä.

Sian teurasjätteen hajotuksessa lisäaine, joka sisälsi vetykloridihappoa (HCl), rautaa (Fe), nikkeliä (Ni), kobolttia (Co), seleeniä (Se) ja volframia (W) paransi hajotusprosessin vakautta kuormituksella 1,5 kgVS m^{-3} d^{-1} ja viipymällä 30 d. Tämä johtui asetiklastisen metanogeneesin paranemisesta, koska kyseisen hajotusvaiheen katsottiin olevan rajoittava tekijä teurastamo- ja renderöintilaitoksen sivutuotteiden yhteishajotuksessa. Sen sijaan lisäaine, joka sisälsi ainoastaan vetykloridihappoa ja rautaa, ei vaikuttanut prosessiin. Lisäaineen käytön lisäetuna oli biokaasun divetysulfidi (H_2S)-pitoisuuden lasku, koska rauta saostuu rikin kanssa. H_2S on syövyttävää tuotantovälineille.

Paperi- ja selluteollisuuden sekundärilietteen esikäsittelyistä höyrylämpökäsittely 150 ˚C:ssa yksin ja yhdistettynä muihin käsittelyihin lisäsi lietteen metaanintuottoa 19 – 31 %. Lisätutkimusta kuitenkin tarvitaan, koska tässä tutkimuksessa ei huomioitu esikäsittelyn energiankulutusta. Höyrylämpökäsittely 150 ˚C:ssa yksin ja yhdistettynä muihin käsittelyihin myös nopeutti hajotusta, mikä lyhentää prosessin viipymää ja täydenmittakaavan laitoksia suunniteltaessa voisi tarkoittaa pienempiä laitoksia.

Panoskokeissa määritettyjen metaanintuottopotentiaalien perusteella lasketut metaanintuotantopotentiaalit tutkituille teurastamon ja sellu- ja paperiteollisuuden sivutuotteille Suomessa ovat 28,8 – 36,5 miljoonaa m^3 a^{-1} ja 5,6 miljoonaa m^3 a^{-1}. Energiana tämä on 288 – 365 GWh a^{-1} ja 56 GWh a^{-1}, mikä yhteensä vastaa 1,1 – 1,3 % Suomessa vuonna 2012 kulutetusta maakaasusta ja 0,1 % Suomen vuoden 2012 kokonaisenergiankulutuksesta.

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ORIGINAL PAPERS

I

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II

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IV

SCREENING PRETREATMENT METHODS TO ENHANCE THERMOPHILIC ANAEROBIC DIGESTION OF PULP AND PAPER MILL WASTEWATER TREATMENT SECONDARY SLUDGE

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