THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Biogas Production from Citrus Wastes and Chicken Feather:

Pretreatment and Co-digestion

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Cover: A schematic of biogas production from citrus wastes and chicken feather (Photographs by Solveig Klug) Printed in Sweden Repro-service, Chalmers University of Technology Göteborg, Sweden 2012

Abstract

Anaerobic digestion is a sustainable and economically feasible waste management technology, which lowers the emission of greenhouse gases (GHGs), decreases the soil and water pollution, and reduces the dependence on fossil fuels. The present thesis investigates the anaerobic digestion of waste from food-processing industries, including citrus wastes (CWs) from juice processing and chicken feather from poultry slaughterhouses.

Juice processing industries generate 15-25 million tons of citrus wastes every year. Utilization of CWs is not yet resolved, since drying or incineration processes are costly, due to the high moisture content; and biological processes are hindered by its peel oil content, primarily the D-limonene. Anaerobic digestion of untreated CWs consequently results in process failure because of the inhibiting effect of the produced and accumulated VFAs. The current thesis involves the development of a steam explosion pretreatment step. The methane yield increased by 426 % to 0.537 Nm³/kg VS by employing the steam explosion treatment at 150 °C for 20 min, which opened up the compact structure of the CWs and removed 94 % of the D-limonene. The developed process enables a production of 104 m³ methane and 8.4 L limonene from one ton of fresh CWs.

Poultry slaughterhouses generate a significant amount of feather every year. Feathers are basically composed of keratin, an extremely strong and resistible structural protein. Methane yield from feather is low, around $0.18 \text{ Nm}^3/\text{kg VS}$, which corresponds to only one third of the theoretical yield. In the present study, chemical, enzymatic and biological pretreatment methods were investigated to improve the biogas yield of feather waste. Chemical pretreatment with Ca(OH)₂ under relatively mild conditions (0.1 g Ca(OH)₂/g TS_{feather}, 100 °C, 30 min) improved the methane yield to 0.40 Nm³/kg VS, corresponding to 80 % of the theoretical yield. However, prior to digestion, the calcium needs to be removed. Enzymatic pretreatment with an alkaline endopeptidase, Savinase[®], also increased the methane yield up to 0.40 Nm³/kg VS. Direct enzyme addition to the digester was tested and proved successful, making this process economically more feasible, since no additional pretreatment step is needed. For biological pretreatment, a recombinant *Bacillus megaterium* strain holding a high keratinase activity was developed. The new strain was able to degrade the feather keratin which resulted in an increase in the methane yield by 122 % during the following anaerobic digestion.

Keywords: anaerobic digestion, pretreatments, co-digestion, economic analyses, citrus wastes, feather

List of publications

The thesis is mainly based on the results presented in the following articles:

- I. Pourbafrani, Mohammad; **Forgács, Gergely;** Sárvári Horváth, Ilona; Niklasson, Claes and Taherzadeh, Mohammad J. Production of biofuels, limonene and pectin from citrus wastes. *Bioresource technology*, 2010 *101*, 4246-4250.
- II. Forgács, Gergely; Pourbafrani, Mohammad; Niklasson, Claes; Taherzadeh, Mohammad J. and Sárvári Horváth, Ilona. Methane production from citrus wastes: process development and cost estimation. *Journal of Chemical Technology and Biotechnology*, 2012 87,250-255.
- III. Forgács, Gergely; Alinezhad, Saeid; Mirabdollah, Amir; Feuk-Lagerstedt, Elisabeth and Sárvári Horváth, Ilona. Biological treatment of chicken feather waste for improved biogas production. *Journal of Environmental Sciences*, 2011 23,1747-1753.
- IV. Forgács, Gergely; Lundin, Magnus; Taherzadeh, Mohammad J. and Sárvári Horváth, Ilona. Pretreatment of chicken feather waste for improved biogas production. Submitted.
- V. **Forgács**, **Gergely**; Niklasson, Claes; Sárvári Horváth, Ilona and Taherzadeh, Mohammad J. Methane production from feather waste pretreated with Ca(OH)₂: process development and economical analysis. Submitted.

Part of the work has been granted a Swedish patent (SE0901415-0) under the title "Framställning av mångahanda biprodukter från fasta citrusrester".

Statement of contribution

- Paper I: I was involved in the experimental work of the pretreatment experiments and in the data analyses. I was responsible for the anaerobic digestion experiments and I have participated in the preparation and organization of the manuscript.
- Paper II: I was responsible for the idea and for all experimental work and data analyses, but not the cost estimation. I was responsible for the manuscript preparation and its revision.
- Paper III: I was responsible for parts of the experimental work, i.e. cell cultivations, soluble protein measurements, and the anaerobic digestion procedures. I was responsible for the manuscript preparation and its revision.
- Paper IV: I was responsible for the major part of the idea, and for all of the experimental work. I was responsible for the manuscript preparation.
- Paper V: I was responsible for the major part of the idea, and all the experimental work and data analyses. I was responsible for the manuscript preparation.

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1. Introduction

1.1. Preface and scope

During the last decades, reduction of greenhouse emissions and protection of the environment, by using a green, efficient energy source able to replace the fossil fuels, has become the center of attention. Biogas production through anaerobic digestion (AD) of organic wastes has the advantage of valuable, renewable energy (methane) being produced, while the environmental impact of these wastes is diminished. Because of their high organic content, wastes from food processing industries hold the potential of producing biogas. Nonetheless, some characteristics of these wastes hinder their utilization as a biogas resource.

The present thesis investigated the feasibility of two different waste streams from food industry, namely citrus wastes (CWs) from juice-processing industry and chicken feather from poultry slaughterhouse, being utilized as substrates for anaerobic digestion. Biogas production from CWs is hampered by the inhibiting effect of D-limonene in the waste, while the main obstacle of anaerobic digestion of chicken feather is the complex structure of the feather. Different pretreatment strategies were investigated in order to solve the problems associated with anaerobic digestion of these materials.

The main goal of the present thesis was to develop suitable and economically feasible pretreatment methods for CWs and feather to be used in the production of biogas. To achieve this goal, the work was divided into four topics:

- Characterization of the wastes for a better understanding of the structure of the wastes, causing the difficulties of anaerobic digestion.
- Measuring the methane potential of the raw waste materials in a batch system, to determine the effect of D-limonene and the effect of feather structure.
- The long-term effects of the different pretreatments were also examined in semicontinuous anaerobic digestion systems, where the untreated and/or pretreated waste materials were subjected to co-digestion with the organic fraction of municipal solid waste

• Technical and economical feasibility studies, based on the results obtained by continuous digestion in continuously stirred tank reactors.

1.2. Outline of the thesis

The thesis comprises five chapters and five papers, summarized as follows:

- Chapter 1 introduces the thesis and the main objectives of the research.
- Chapter 2 provides information about the biogas market, and describes the anaerobic digestion process. The important process parameters are also discussed, and the different methods for determining the potential for biogas production are summarized.
- Chapter 3 presents the two raw materials studied, *i.e.* citrus wastes and chicken feather waste, and discusses the structure of these wastes in relation to production and application possibilities. (Papers I and IV)
- Chapter 4 begins with an introduction of the pretreatment methods, and the motivation for the choice of pretreatments in case of CWs and feather. Furthermore, this section describes the effects of different pretreatment methods on the biogas yield. The last part of the chapter explores co-digestion as a means to facilitate utilization of these wastes for biogas production. (Papers I-V)
- Chapter 5 overviews the economics of anaerobic digestion, and investigates the economical viability of using the developed pretreatment procedures in the biogas production process. (Papers I and V)

2. Anaerobic Digestion

2.1. Biogas, driving forces, and the biogas industry

Currently, around 80 % of the world's energy demand is covered by fossil fuels (oil, gas, and coal) [1]. These sources are not limitless, and moreover, the increasing price of the fuels accelerates the demands of replacing fossil fuels with renewable, green alternatives. Biogas is a gaseous biofuel manufactured by means of anaerobic digestion of organic material. Biogas holds a wide range of applications, it can be used as replacement of fossil fuels in the generation of power and heat, and it can also be upgraded to gaseous vehicle fuel [2, 3]. Thus, biogas has a great potential as an alternative to fossil fuels. In Europe, biogas is typically used for generating heat and electricity. In 2009, biogas was responsible for almost 1 % of the electricity produced in EU (Figure 2.1). However, in some EU countries, including Sweden, biogas is mainly utilized as vehicle fuel in the transportation sector, while in developing countries, biogas is utilized for cooking, heating, and lighting.





The main advantage of biogas, compared to other biofuels, is the wide range of suitable substrates that can be utilized for biogas production [5]. Biogas production can be considered

¹ European Commission Eurostat database

Website:http://epp.eurostat.ec.europa.eu/portal/page/portal/energy/data

a low-cost waste management technology, since it requires neither harsh conditions nor a complex process design. Moreover, the energy balance of the process is favorable compared to other processes, e.g. ethanol production or combustion [2, 6]. Under optimal conditions, the energy output/input ratio can reach 28 MJ/MJ, disclosing a very efficient use of the biomass [6].

Production of biogas in a controlled environment, significantly lowers the emission of green house gases (GHGs), since the captured methane is a potent greenhouse gas [7]. It is well known that emission of GHGs causes severe problems, in that the resulting global warming (GW) leads to climate changes. In 2009, carbon dioxide (CO₂) was accountable for the largest share (81.5 %) of the GHGs' effect on global warming (Figure 2.2). The main part of the CO₂ emission (94 %) was related to fuel combustion, while the remaining 6 % originated from other industrial processes. Methane had the second largest effect, with 9.0 % share of the total GHG emission. Half of the methane emission was produced by the agricultural sector, mainly related to rice cultivation and enteric fermentation. Furthermore, waste management industries (wastewater treatment, landfill) generated 31 % of the methane emission, while the remaining part emanated from the combustion sector and the oil and natural gas systems [8]. According to a report of the European Environmental Agency, a reduction of methane emission would have the largest impact on the climate change; with a life time of 20 years, methane has a 72 times higher potential of global warming than carbon dioxide over a 20 years period [8].



Figure 2.2. The total greenhouse emission in the European Union in 2009, in relation to different greenhouse gases [9]

Biogas production therefore holds a significant potential for lowering the methane emission, thereby decreasing the demand of fossil fuels, making biogas production a very attractive and rapidly growing industry [10]. Around 10 000 biogas plants are currently operated in Europe, producing biogas from animal manure, energy crops, sludge, and different types of wastes. According to a prognosis of the German Biogas Association, the number of the biogas plants will increase by a factor of five within the next 10 years in Europe (Figure 2.3).



Figure 2.3. The estimated development of the biogas industry in Europe $1995-2020^{1}$

More than 20 million biogas plants are installed worldwide, including small homemade biogas reactors. In China alone, the number of biogas plants is estimated to reach around 200 million by the year 2020 [11].

2.2. The anaerobic digestion process

Biogas is formed as a result of organic matter being anaerobically digested by different groups of facultative and obligatory anaerobic microorganisms. In nature, biogas is produced in oxygen-free environments like swamps (swamp gas), in the rumen of ruminants, in rice fields, and in landfills. Biogas is mainly composed of methane (CH₄) and carbon dioxide (CO₂) (carbon's most reduced and most oxidized forms, respectively), but it may also contain small amounts of nitrogen (N₂), hydrogen (H₂), oxygen (O₂), and hydrogen sulfide (H₂S). The

¹ German Biogas Association

Website: http://www.biogas.org

anaerobic digestion (AD) process of organic compounds into methane and carbon dioxide involves different kinds of microbial populations. Most of these do not produce methane, but entail an important step of the chain of reactions, leading to methane production. The main steps of the AD are hydrolysis, acidogenesis, acetogenesis, and methanogenesis as summarized in Figure 2.4.



Figure 2.4. Semantic figure of the anaerobic digestion process [12]

2.2.1. Hydrolysis

During the first phase of the AD, the undissolved macromolecules like proteins, fats, cellulose, and hemicelluloses are broken down to monomers by the action of extracellular enzymes of facultative and obligatory anaerobic microorganisms. The enzymes involved in the hydrolysis are mainly amylases, lipases, proteases, cellulases, and hemicellulases [12, 13]. The time required for the hydrolysis step depends on the substrate: the hydrolysis of carbohydrates takes hours, while the hydrolysis of protein and lipids requires days. Substrates with more complex structure, like cellulose, needs weeks to become degraded, and

degradation is usually not complete [14]. Hence, for substrates barely accessible to the enzymes, the hydrolysis step may be considered as the rate-limiting step [15, 16].

2.2.2. Acidogenic phase

In the acid-forming phase, the soluble monomers, formed by hydrolysis, are assimilated by obligatory anaerobic bacteria and further degraded to C1-C5 molecules, *i.e.* short chain acids, alcohols, hydrogen, and carbon dioxide [14]. The partial pressure of the hydrogen regulates what types of products that are formed. Generally, a high partial pressure favors acetate production [14]. In a well-balanced system, acidogenic bacteria mainly produce acetate, hydrogen, and carbon dioxide; and the methanogenic microorganisms readily utilize these products. If the conditions are not optimal, other intermediates are formed as well, such as alcohols and volatile fatty acids. These intermediates need to be further modified (acetogenic phase) before the methane-producing organisms are able to convert them to methane.

2.2.3. Acetogenic phase

The products from the previous phase, serve as substrates for the acetogenic microorganisms. In this phase, acetate, hydrogen, and carbon dioxide are formed by oxidation of intermediate products. Although acetogenic bacteria are hydrogen producers, they survive and function only at low hydrogen partial pressure (lower than 10^{-5} bar) [17]. This is the reason why acetogenic bacteria live in symbiosis with methanogenic microorganisms; the methane-producing microorganisms will assimilate the hydrogen, thus lowering the partial pressure of this gas. Regardless, homoacetogenic microorganisms are also present here, constantly forming acetate from H₂ and CO₂ [18]:

$$2CO_2 + 4H_2$$
 \leftarrow $CH_3COOH + 2H_2O$

In a well functioning biogas process, this step results in around 70 % of the carbon being in the form of acetate, while 30 % is in the form of carbon dioxide [19].

2.2.4. Methanogenic phase

In the methanogenic stage, methane and carbon dioxide are formed mainly from hydrogen, acetate, and other one-carbon compounds, by archaean species under strictly anaerobic conditions [20]:

$$CH_{3}COOH \Longrightarrow CH_{4} + CO_{2}$$
$$CO_{2} + 4H_{2} \Longrightarrow CH_{4} + 2H_{2}O$$

Hydrogenotrophic microorganisms convert hydrogen and carbon dioxide to methane. This pathway for methane formation is thermodynamically favorable during a high hydrogen partial pressure (above 10^{-6} bar). Consequently, the symbiosis between the acetogenic and methanogenic microorganisms discussed above, is only feasible within the narrow hydrogen pressure range, 10^{-6} – 10^{-5} bar. When the methane production works, the hydrogen is assimilated; thus the acetogenic organisms also function without problems. In a biogas digester, the methane-producing microorganisms comprise the group most sensitive to changed process parameters, such as pH, temperature, and substrate concentration. Also, they grow very slowly (generation time, 5–25 days); thus, this phase is usually the rate-limiting step.

2.3. Process parameters

The characteristics of the substrates and the operating conditions are the main parameters affecting the biogas production process. In some cases the substrate itself contains inhibitors, such as limonene (Papers I, II). In other cases, the accumulation of volatile fatty acids (VFAs) and ammonia (Papers III, IV) (which are toxic, particularly for the methanogens) will slow down the biogas production. The following subsections summarize the most important parameters influencing the efficacy of the anaerobic digestion process.

2.3.1. Temperature

Anaerobic digestion can be carried out in a wide range of temperatures, from psychrophilic (<20 °C) to thermophilic conditions (55 °C) [21, 22], but for industrial applications mesophilic and thermophilic processes are commonly used. Increasing the temperature holds

several advantages, e.g. increased solubility of the organic compounds, increased reaction rates, and higher methane yields [23]. Because of the faster reaction rate, anaerobic digesters are able to function at shorter hydraulic retention times (HRT). Moreover, in thermophilic digesters, operating at high temperature destroys the pathogens [24]. However, higher temperatures require more energy, and the process is more sensitive to changes in the operational conditions. For example, thermophilic methanogens are more sensitive to the accumulation of VFAs at high temperatures, and the increased pKa of ammonium at elevated temperature leads to an increased fraction of free ammonia, which is more toxic. Table 2.1 summarizes the differences in anaerobic digestion under mesophilic and thermophilic conditions.

Mesophilic (35 °C)	Thermophilic (55 °C)
Lower	Higher
Lower	Higher
Longer, or the same	Shorter, or the same
No	Possible
Low	High
Low	High
Higher	Lower
	Mesophilic (35 °C) Lower Lower Longer, or the same No Low Low Higher

Table 2.1. Comparison of mesophilic and thermophilic anaerobic digestion

In the present study, the anaerobic digestion processes were carried out at thermophilic conditions, for two main reasons. First, the economical benefits, *i.e.* the ability to use smaller reactors and obtain higher methane yields. Second, if the developed processes operate successfully under the more sensitive thermophilic conditions, this indicates that the process will work at mesophilic conditions as well.

2.3.2. Organic loading rate, and hydraulic or solid retention time

The control of the organic loading rate is very important to achieve a stable process and a high biogas production. Generally, the OLR of solid feedstocks is based on volatile solids (kg VS m⁻³day⁻¹), while for liquid substrates based on chemical oxygen demands, thus the OLR is expressed as kg COD m⁻³day⁻¹. Digesters with a low organic loading rate (underloaded) work

uneconomically, since the capacity of the digester is not fully utilized. On the other hand, overloading the system normally results in accumulation of VFAs or of other inhibitors, which may terminate the process.

There are two important retention times in anaerobic digestions: (1) HRT (hydraulic retention time) is the time that the substrate is present in the anaerobic digester, (2) SRT (solid retention time) is the average time that microorganisms are present in the digester [25]. The SRT and HRT are the same in suspended-growth digesters, if there is no recycling. HRT is considered more important for complex and slowly degradable feedstocks, while SRT is a significant factor for easily degradable biomass [26].

2.3.3. C/N ratio

Nitrogen is essential for the growth of microorganisms. Lack of nitrogen leads to insufficient utilization of the carbon source and consequently to insufficient growth [27]. On the other hand, high nitrogen concentrations result in an increased ammonia production, subsequently inhibiting the methanogens. In order to maximize biogas production, an optimal C/N ratio is necessary. The optimum C/N ratio in a biogas digester ranges between 15 and 30 [28]; hence, mixing different substrates with low and high C/N ratios in a co-digestion process may be beneficial to acquire optimal nutritional conditions.

2.3.4. Volatile fatty acids

Volatile fatty acids (VFAs) are some of the most important intermediates of the anaerobic digestion process. They exist partly in an undissociated and partly in a dissociated form in the biogas digesters. The dissociated form dominates at an elevated pH, while a lowered pH will cause an increase of the undissociated fraction. Typically, 99.9 % of the VFAs occur in the dissociated form at pH 8.0, while at pH 6.0, around 90.0 % is dissociated [14]. An increase of VFAs in anaerobic digestion may lead to inhibition of the methanogenesis [29]. Particularly the undissociated VFAs (free fatty acids) have an inhibiting effect, since they are able to diffuse into the cell, where they will cause denaturation of the proteins [14]. Beside the pH-value, the amount of VFAs is therefore commonly suggested as an indicator for the efficacy of anaerobic digesters [30]. Although the level of total VFAs is reported in most cases, it is

important to point out that the threshold levels for inhibition differ between individual VFAs [31]. The threshold level for inhibition by acetic acid is around 1000 mg/L at pH<7, while the threshold level of iso-butyric and iso-valeric acid is around 50 mg/L under similar conditions [14]. A monitored level of propionic acid is also an excellent process indicator, since decomposition of propionic acid works well only in a balanced system. Thus, increasing propionic acid concentrations in anaerobic digesters indicate unstable processes [32].



Figure 2.5. The effect of increasing VFAs on methane yield during co-digestion of the organic fraction of municipal solid waste and citrus wastes (Paper II)

In the present study, the organic fractions of municipal wastes (OFMSW) and citrus wastes (CWs) were co-digested using a semi-continuous process under thermophilic conditions (Paper II). The untreated citrus wastes contained limonene, which is a strong inhibitory agent. The presence of limonene led to an accumulation of VFAs during the anaerobic digestion process. As shown in Figure 2.5, the methane production slightly decreased during the first 20 days. At day 22, when the level of total VFAs exceeded 6.5 g/L, a concentration that the buffer capacity of the system was not able to handle anymore, the pH dropped from 7.3 to 5.5 (data not shown) causing a stop in the production of methane. The main component of the VFAs comprised propionic acid, with a final level of 2.0 g/L (Paper II).

2.3.5. Ammonia

Ammonia is produced by degradation of proteins and other nitrogenous matter [33]. Ammonium ion (NH_4^+) and free ammonia (NH_3) are the two forms found in biogas digesters. The free ammonia is the main source of inhibition, since it is able to diffuse into the cell, creating a proton imbalance, or leading to a loss of potassium [23]. The state of chemical equilibrium between ammonium and ammonia is temperature and pH-dependent. With rising temperature or increasing pH, the equilibrium is shifted towards NH_3 . Typically, the threshold for inhibition is around 4–6 g total ammonial N per liter, but in the case of NH_3 , the inhibition appears at around 80 mg/L [14, 34, 35], although microorganisms are able to adapt to higher levels [14].

Anaerobic digestion of chicken wastes (including feather) produce high amounts of ammonia [36, 37], with process failure as a consequence. With this in mind, the feather waste in the present study was co-digested with the organic fraction of municipal solid waste to avoid high ammonia production and the concomitant process problems.



Figure 2.6. Changes in the ammonium concentration during anaerobic co-digestion of feather with the organic fraction of municipal solid waste (Paper IV)

Figure 2.6 shows the ammonium nitrogen concentration during the 115 days operating period. Both reactors were operated with 80 % OFMSW and 20 % feather (based on the VS content of the substrate mixture) (Paper IV). In digester 1, where untreated feather was digested, the ammonium concentration continuously increased until day 70, when it stabilized around 3.0 g/L. Digester 2 operated with the same type of substrate, but with an alkaline endopeptidase (Savinase[®]) added to the feedstock in order to reinforce the degradation of feather. The addition of this enzyme speeded up the degradation of the feather protein, and the subsequent ammonium production. As a result, an ammonium concentration of 4.2 g/L was obtained in day 20, which afterwards slowly decreased until it reached 3.2 g/L (Paper IV).

2.4. Methods for determining the biogas potential

The anaerobic digestion potential (expressed as the biogas volume per unit substrate) can be used to evaluate different possible substrates. It can be determined by using theoretical as well as practical methods.

2.4.1. Theoretical methods

The theoretical methane potential can be calculated in three different ways. The methods presume that the substrate will be completely degraded, and the microorganisms' utilization of the substrate as carbon (energy) source, is negligible.

Elemental composition: The theoretical methane potential can be calculated from the elemental composition (C, H, O, S, N) of the substrate, using the Buswell formula [38]:

$$C_{c}H_{h}O_{o}N_{n}S_{s}+yH_{2}O \longrightarrow xCH_{4}+nNH_{3}+sH_{2}S+(c-x)CO_{2}$$
Where: $x=1/8(4c+h-2o-3n-2s)$

Component composition: If the elemental composition of the substrate is unknown, the component composition, *i.e.* carbohydrate, fat, and protein, can also be used for the calculation of the theoretical methane potential [39]. Using the general chemical formulas, 0.42, 0.50, and 1.01 Nm³ CH₄/ kg VS can be acquired from carbohydrates ($C_6H_{10}O_5$), proteins ($C_5H_7O_2N$), and lipids ($C_{57}H_{104}O_6$), respectively [40].

Chemical oxygen demand (COD): Chemical oxygen demand provides information about the organic content, and can therefore be used for the estimation of methane yield; employing the

fact that 1 mole of methane requires 2 moles of oxygen for the oxidation (of carbon) to carbon dioxide and water. Each gram of methane thus corresponds to 4 grams of COD [41].

Carbon source of the substrate
$$\rightarrow$$
 CH₄ + CO₂
CH₄ + 2O₂ \rightarrow CO₂ + H₂O

The equation shows that each kilogram of COD equals 0.35 m^3 methane gas, at standard pressure and temperature [41, 42].

In Papers III and IV, the component composition method was used to calculate the theoretical methane potential from feather waste, while in Papers IV and V, the soluble COD content was used to evaluate the efficiency of different pretreatment conditions.

2.4.2. Practical methods

The theoretical methods discussed above hold two major problems. First, they presume complete degradation of the organic matter, but the actual digestibility is usually 27–76 % [14]. Second, several inhibitions may occur during the digestion process, and these are not considered in these methods. Therefore, performing digestion tests for each substrate, as a tool for evaluating the actual biogas potential, is widely used. Digestion tests can be performed at different scales, and their results are commonly used for designing full-scale plants.

In the present work, two types of digestion tests were performed. Batch digestion tests were conducted to determine the methane potentials of untreated and treated materials (Papers I-V), and a semi-continuous digestion method was used for determining the long-term effects in co-digestion processes (Papers II, IV).

Batch digestion

A batch digestion assay is the simplest method of the digestion tests and can be used for determining the methane potential, and for kinetic measurements. Certain amounts of substrate (VS, COD) and methanogenic inoculum are placed in the reactors, which then are sealed and placed in a thermostat until the substrate is degraded. The conditions are anaerobic

and the temperature is kept optimal during the experimental period. These tests usually require 50 days, since anaerobic digestion is a slow process, but one advantage of the batch method is that many parallel tests can be performed simultaneously. This makes it suitable for comparing the methane potential of different substrates, or for evaluating different pretreatment methods and conditions. Typically, only the production of gas (methane and carbon dioxide) is measured, but sampling liquid is also possible. This, however, makes the calculation more complex, since liquid sampling changes the total working volume.

In the present thesis, all batch experiments were designed in accordance with the method described by Hansen *et al.* [43]. An exact volume of glass bottle (118 mL or 2 L), equipped with a thick rubber septum, was used as reactor. The VS content of the substrate was between 0.75 and 2.0 %, and the VS ratio of inoculum/ substrate was adjusted to 1 or 2. The reactors were flushed with a gas mixture comprising 80 % N_2 and 20 % CO_2 , to secure anaerobic conditions, and incubated at 55 °C under thermophilic conditions. The biogas produced in the headspace was measured regularly by gas chromatograph, using a gastight syringe for sampling, which allowed calculation of the amount of methane and carbon dioxide produced, without measuring the actual pressure in the reactors.



Figure 2.7. Schematic diagram of set up of the batch digestion assays (Adapted from [44])

Semi-continuous method

The semi-continuous method entails a more advanced technology, and usually provides more information about the process performance, compared to the batch digestion tests. It requires daily supervision, and operating experience as well. This method usually requires a testing period of several months. The CSTR (continuously stirred-tank reactor) is a widely used technology for semi-continuous digestion, from lab scale to industrial scale [45, 46]. A CSTR system requires a relatively long (10–50 days) HRT, to avoid washing out the slow growing microbial population.

In the present research, CSTR reactors were used for the semi-continuous experiments, since solid wastes were used as substrate. The configuration of the reactors is presented in Figure 2.8.



Figure 2.8. Setup of the CSTRs used in the semi-continuous anaerobic digestion experiments

Three CSTRs used with a working volume of 5 L, and an OLR of 2.5–3.0 kg VS m⁻³day¹ was employed. The HRT was adjusted between 21 and 25 days to avoid washing out the slow growing methanogens, and to provide sufficient time for the breakdown of the difficult-to-degrade substrates used in this study. An online monitoring system coupled to the reactors was used for determining the daily gas production and the pH changes. Other process parameters, including total and volatile solids, alkalinity, VFAs, NH₄-N, were measured manually, usually once or twice a week.

3. Raw materials from the food industry: Citrus wastes and chicken feather

3.1. Citrus wastes

3.1.1. Production of citrus wastes

According to the Food and Agriculture Organization of the United Nations (FAO), the global consumption of citrus fruits has steadily grown over the past five decades (Figure 3.1). In 2010, the European consumption was around 11 million tons, which corresponds to 10 % of the worldwide production. Approximately 33 % of the citrus crops, including oranges, mandarins, grapefruits, and lemons, are used for juice production [47]. During the juice production process, about 50–60 % of the crop ends up as waste [48, 49]. The estimated generation of these solid waste residues, here referred to as citrus wastes (CWs), ranges between 15 and 25 million tons per year [48].



Figure 3.1. Annual worldwide and European¹ citrus fruit production, 1980–2010

¹ Food and Agriculture Organization of the United Nations Website: www.fao.org

3.1.2. Structure of citrus wastes

CWs are mainly composed of peels, seeds, and segment membranes. Although considered as lignocellulosic materials, CWs contain soluble carbohydrates, small amounts of protein, fat [48], and peel oil as well (Table 3.1). Typically, 2–3 % of the dry matter in citrus wastes is peel oil. The major component of the peel oil is D-limonene (>90 %), a well known antimicrobial agent [50, 51]. The composition of CWs differs slightly, depending on the kind of citrus, and the process parameters. Table 3.1 summarizes the composition of CWs. CWs cause environmental problems in terms of odor, disposal problems, and methane emission due to uncontrolled anaerobic degradation [52, 53]; thus CWs comprise a major issue in the fruit processing industry.

Table 3.1. Composition of CWs acquired from juice-producing industries. Adapted from

 Paper I and [48]

Compound	Composition (% of DM)	
Ash	2.5-5.1	
Sugar	6.0-22.9	
Pectin	12.1-25.0	
Protein	6.1-9.1	
Fat	0.44-4.00	
Cellulose	22.0-37.1	
Hemicellulose	6.0-11.1	
Lignin	2.2-8.6	
Flavonoid	5.1-12.5	

3.1.3. Applications of CWs

Extraction of essential oils

Citrus oils are used in the food industry as aroma flavor, while pharmaceutical industries apply citrus oils to hide the unpleasant taste of drugs. Citrus oils are also commonly used in the cosmetic industry [54]. These applications make citrus oils the most widely used essential oils in the world [55]. Steam distillation is the traditional method to extract oil. During the distillation process, the steam vaporizes the volatile oils. Nowadays, however, research focuses on the development of new, green, and cheaper alternative techniques, like ultrasound extraction, supercritical fluid extraction, and pressure drop process [56-58].

Citrus wastes as animal feed

CWs are rich in sugar fibers which make them a suitable source as animal feed [59]. The rumen degradability is 75–95 % [59, 60]. However, drying is a necessary step before CWs can be utilized as animal feed; the animals will not eat CWs in raw form because of their distinctive smell and the strong taste. Unfortunately, the drying process makes this application of CWs very costly.

Ethanol production from CWs

CWs contain high concentrations of fermentable sugars, making them an interesting substrate for ethanol production. However, the presence of peel oil hinders the fermentation process [61]. This problem may be solved, either by removing the peel oil prior to fermentation, or by conducting the fermentation with yeast, protected by encapsulation [62, 63].

Other applications

CWs can furthermore be utilized for pectin, flavonoid, and dietary fiber production [64-66]. Pectin is a complex polysaccharide, composed of galacturonic acid. It is mainly used in the food industry as a gelling agent and a thickening stabilizer agent [67]. Flavonoids are secondary metabolites, well known for their antioxidant activity. Citrus flavonoids have been revealed as having beneficial effects against cancer as well as cardiovascular diseases [68, 69]. Consumption of dietary fiber from CWs may aid the prevention of certain diseases, e.g. hemorrhoids and colorectal cancer.

3.2. Chicken feather

3.2.1. Feather production

In 2010, chicken was the most common and widespread domestic species, with a consumption of more than 86 million $tons^1$ that year, and according to the Food and Agriculture Organization of the United Nations, the production and consumption of chicken meat are persistently growing. In Europe, the chicken consumption reached 20 kg/capita/year in 2007,

¹ Food and Agriculture Organization of the United Nations

Website: www.fao.org

according to FAO, while in the USA; the consumption of chicken has surpassed 50 kg/capita/year¹.

Deeming a mature chicken to weigh 1.8–1.9 kg (1.5 kg of meat) [37], with 5–7 % of its body weight comprising feathers [70], the generation of chicken feather waste is easily estimated. Figure 3.2 illustrates the estimated production of chicken feather waste over the last 30 years. According to the European legislation, chicken feathers are regarded as an animal byproduct; hence they must undergo strict treatment before they may be used or disposed of safely.



Figure 3.2. Annual generation of chicken feather between 1980 and 2010 (Data calculated based on FAO database¹)

3.2.2. Feather structure

Feathers are composed of 90–95 % of proteins and 5–10 % of lipids [72, 73]. The main protein component is keratin, a highly specialized fibrous protein with mechanical strength and protective abilities. Furthermore, keratin is also the main component of hair, wool, nails, horn, and hoofs [74]. Keratin is distinguished from the other structural proteins by its relatively high cysteine content, which enables it to form disulfide bonds, that serve as structural elements, thereby stabilizing the molecule [75]. The amino acid composition of feathers is presented in Table 3.2. The amounts of different amino acids in feather depend on the age of the bird age, and data vary in the literature [76, 77]. While feathers generally have a

¹ Food and Agriculture Organization of the United Nations Website: www.fao.org

high cysteine content along with high concentrations of serine, proline, and acidic amino acids, they are deficient in some essential amino acids, like methionine and histidine.

Protein and amino acids	Latshaw et	Bertsch and
$(g kg^{-1})$	al., 1994 [78]	Coello, 2005 [79]
Protein	922.0	948.0
Alanine	28.8	25.6
Glycine	51.8	41.7
Isoleucine	39.4	20.8
Leucine	56.9	56.0
Valine	53.0	37.0
Phenylalanine	34.6	19.9
Arginine	67.6	60.7
Histidine	2.3	2.8
Lysine	15.4	20.9
Aspartic acid	41.8	45.5
Glutamic acid	82.2	108.0
Serine	87.3	69.2
Threonine	34.5	75.8
Proline	73.9	40.0
Cysteine	65.8	57.8
Methionine	7.1	2.8

Table 3.2. Main amino acids present in feather and their concentrations

The secondary structure of feather keratin comprises 41 % α -helix and 38 % β -sheet configurations, and 21 % disordered regions [80]. Figure 3.3 shows a schematic model of α -helix and β -sheet configurations. The α -helix is a right-handed coil of amino acid residues, with 3.6 amino acid residues making up a complete turn of the helix. Hydrogen bonds are formed at every fourth amino acid residue. Usually, the polypeptide chain comprises between 4 and 40 residues. The β -pleated sheet is formed when 2 (or more) segments of the amino acid chain overlap each other. The strands are stretched out and lie parallel or antiparallel to each other (in Fig. 3.3, the chains are in antiparallel position). Hydrogen bonds are formed between the different polypeptide chains.

The secondary structure and the cysteine content are the two most important properties; they determine the physical and chemical qualities of feathers. Feathers are insoluble in water, weak acids, and alkalis. They are very resistant against attacks by most proteolytic enzymes, as a result of the numerous inter- and intra-molecular disulfide cysteine bonds, hydrogen bonds, and hydrophobic interactions [81, 82].



3.2.3. Feather applications

Feather as animal feed

Due to their high protein content, feather have been widely utilized as animal feed, particularly for poultry and swine [82]. However, the feather digestibility in rumen is low, around 18 % [81]; a suitable pretreatment method, increasing the feather digestibility is hence needed, to convert it into valuable feedstuffs [78, 84]. The pretreatment methods applied can be classified into two main groups. The first group includes physical, thermal, and chemical treatments. These treatments operate at a high temperature or a high pressure, and in some cases diluted acid or alkali is added as well. The disadvantages of these technologies are high running costs and that certain amino acids [85, 86] will be destroyed. The second group of pretreatments utilizes keratinolytic microorganisms to hydrolyze the proteins. Most keratinolytic microorganisms are fungi, but some bacteria are also able to degrade feather [84, 87]. These pretreatment methods are reported to be environmentally friendly and economically viable processes [88, 89].

Keratin-based materials for biomedical applications

During the last decades, the advanced technology in biotechnology and chemistry, along with the strong demand for environmentally friendly technologies, has led to the development of a keratin-based biomaterials platform. Extracted keratins have an intrinsic ability to self-assemble, and to polymerize into porous, fibrous scaffolds [90]. Keratin derivatives display

cell binding motifs, which support cellular attachments [91]. These qualities may prove to be important tools for using keratin-based materials for tissue engineering, wound healing, and drug delivery. The extracted keratin is in itself too fragile and has other undesirable mechanical properties as well, because of its low molecular weight [90, 92]. Therefore, to enhance and improve the mechanical properties, the keratin film needs to be blended with high molecular weight polymers [90]. Blending the keratin with synthetic polymers, such as polyethylene oxide (PEO), can also improve the properties of the film [93]. Several studies have investigated the positive effect of glycerol on mechanical properties [94]. Moreover, the addition of chitosan to the glycerol containing film guarantees antibacterial properties [95]. There are no keratin biomaterials in clinical use to date, but their unique properties, such as remarkable biocompatibility, and propensity for self-assembly, make them good candidates for future applications.

4. Pretreatments for improved biogas production

4.1. An overview of pretreatment methods

High biogas yield is essential for an economically viable operation of anaerobic digesters. However, the digestion of some substrates results in low biogas yields. The substrates are either very resistant against anaerobic digestion because of their compact, complex structure, or they contain inhibitors [96]. The degradation of complex materials is slow, and the AD process is therefore usually limited by the long retention times [25]. These limiting factors are associated with the hydrolysis phase of AD. In this case, the main purpose of applying a pretreatment is to enhance the degradation rate and efficiency, and to improve the bioavailability of the feedstock [16]. In other cases, the pretreatment aims at removing undesirable compounds. The choice of a suitable pretreatment method should always be based on the properties of the substrate, and the optimal pretreatment condition for the most efficient anaerobic digestion process, should be determined from an economical as well as an environmental point of view. Pretreatment methods can be classified as follows [16]:

- Physical pretreatments
- Chemical pretreatments
- Physicochemical pretreatments
- Biological pretreatments

Physical pretreatments

Physical pretreatments include milling, irradiation, and hydrothermal pretreatment processes [16]. The objective of milling is size reduction, which can be achieved with various milling processes, *i.e.* ball milling, two-roll milling, hammer milling, colloid milling, etc. Irradiation (gamma rays, electron beams, or microwaves) increases the accessible surface area and the pore size of the material, and also reduces the crystalline structure. Hydrothermal pretreatments require high temperature and/or high pressure to open up the complex organic structure [16].

Chemical pretreatments

Chemical pretreatments comprise acid and alkaline hydrolyses, wet oxidation, ozonolysis, and organosolv processes [16, 97]. Acid and alkaline hydrolyses are the most commonly used pretreatment methods. Strong acid pretreatment efficiently executes removal of hemicelluloses and lignin, and is usually chosen for pretreatment of lignocellulosic materials. Sodium hydroxide or calcium hydroxide is generally used for alkaline pretreatment, and can be applied for pretreatment of a wide range of substrates.

Physicochemical pretreatments

Physicochemical pretreatments combine physical and chemical processes in order to achieve a better efficacy. Steam-explosion with or without chemical addition, ammonia fiber explosion, CO₂ explosion, and microwave-chemical pretreatment, are the most important physicochemical pretreatment methods previously reported, leading to improvements of the subsequent biogas production [98].

Biological pretreatments

Biological pretreatments, using microorganisms or enzymes, can also be applied for enhanced biogas production. The main advantage of a biological pretreatment is that it does not require harsh pretreatment conditions and addition of chemicals. The pretreatment time required can, however, be very long under these mild conditions, compared to the other pretreatment processes [97].

4.2. Citrus wastes

4.2.1. Need for pretreatment

CWs have a high organic matter content, consisting of various soluble and nonsoluble carbohydrate polymers, making these wastes ideal to anaerobic digestion [52]. However, AD of CWs is hindered by the presence of D-limonene. D-limonene impedes the biogas production process by inhibiting certain microorganisms, which results in volatile fatty acids accumulation [52]. According to Mizuki *et al.* [50], inhibition occurs at loading rates from $65 \,\mu\text{L} \,\text{L}^{-1}\text{day}^{-1}$ when feeding peel oil to a mesophilic continuous system, and is caused by the

peel oil accumulation in the system from that loading rate, and final concentration of $400 \,\mu$ L/L leads ultimately to process failure.

The present work investigated the threshold level of D-limonene for inhibition of the AD process under thermophilic conditions, during co-digestion of CWs and the organic fraction of municipal solid waste (OFMSW) in a batch process (Figure 4.1).



Figure 4.1. Effect of D-limonene on the anaerobic digestion process

An initial lag phase was observed during the digestion of the mixture of OFMSW and CWs, 50 % of each, which indicated a disturbance of the system. Moreover, the final pH had slightly decreased to 7.38 (as compared to 7.81 when no D-limonene was present), indicating an increased concentration of VFAs. When CWs alone was digested, with an accompanying higher level of D-limonene, acidification dropped the pH level to 5.32, and the process stopped. These observations suggest that the threshold level of D-limonene for inhibiting AD under thermophilic conditions is between 450 and 900 μ L/L. Based on these findings, removal of D-limonene is recommended prior to the digestion process.

4.2.2. Steam explosion of CWs

Steam explosion has previously been reported to successfully increase the methane yield of different materials, such as wood, straw, sludge, cattle manure, and municipal solid waste [99-

101]. Steam explosion typically operates within a temperature range of 160–260 °C, from a few seconds to several minutes [16]. In the end of the treatment, the pressure suddenly drops, which causes an explosive decompression effect (Figure 4.2.). Steam explosion has previously been applied on CWs prior to ethanol production with great success [61]; hence this may be a potential pretreatment method for CWs prior to biogas production as well. The present study disclosed that steam explosion is able to remove the D-limonene, and to open up the lignocellulosic structure as well.



Figure 4.2. Schematic figure of the steam explosion unit

Steam explosion of CWs was carried out using a 10 L high-pressure reactor (Figure 4.2). Steam (provided by a power plant) was used at a pressure of 60 bar for heating the reactor. The CWs were hydrolyzed at 150 °C, with 20 minutes residence time. The hydrolyzed slurry of CWs was then discharged to an expansion tank at atmospheric pressure, while the D-limonene content was flashed out to the vapor phase (Paper II).

4.2.3. Physicochemical pretreatment of CWs

Currently, several investigations exist on combining steam explosion treatment with the addition of chemicals to obtain better results than with a thermal or a chemical pretreatment alone. Hydrothermal pretreatment requires high temperature or high pressure, and is usually combined with the addition of diluted acids, or alkali, such as sodium hydroxide. Addition of

chemicals reduces the required temperature and time, and also increases the degradation rate [102].

The current study examined the steam explosion treatment in combination with acid. The pretreatment experiments were carried out in the high-pressure reactor mentioned above (Figure 4.2.). Dilute sulfuric acid was added to CWs to a final concentration of 0.5 % (v/v), and the CWs were then hydrolyzed at various temperatures (130–170 °C), with different residence times (3–9 min) (Paper I).

4.2.4. Biogas production from CWs

Information on digestion of citrus wastes is limited. Kaparaju and Rintala [52] investigated thermophilic digestion of industrial orange waste at laboratory scale. They obtained a methane yield of 0.49 m³/kg VS in anaerobic batch tests. However, the organic loading was low, and the system was buffered by the addition of NaHCO₃, to keep the pH at an appropriate level for anaerobic digestion. In a semi-continuous system, with an OLR of 2.8 kg/m³/day and a 26 day HRT, anaerobic digestion of orange waste generated 0.60 m³ methane/kg VS. However, this system required a pH adjustment, using NaHCO₃ and NaOH [52]. The methane yield of untreated citrus waste in the present study was 0.10 m³/kg VS, which may be explained by the higher loading of D-limonene and the absence of buffer.

Figure 4.3 presents the methane yield obtained from anaerobic batch digestion assays of untreated *vs.* pretreated CWs. Production of multiple biofuels from CWs, *i.e.* ethanol and methane was investigated, using pretreatment with steam explosion in combination with sulfuric acid under various conditions. Since the ethanol production occurs before the AD, the purpose was to obtain maximal sugar yield in the liquid hydrolyzate, to ensure maximal ethanol yield. The highest sugar yield, around 41 %, was obtained after 6 minutes of steam explosion at 150 °C in combination with 0.5 % sulfuric acid (Paper I). The ethanol fermentation was subsequently followed by methane production, which utilized the stillage and the solid residues after the pretreatment, resulting in a yield of 0.36 Nm³ methane /kg VS.

When biogas was the major product, the main purpose of the pretreatment was to remove the D-limonene and open up the compact structure, which would maximize the biogas yield

(Paper II). Based on this assumption, steam explosion without addition of H_2SO_4 was explored, since during the subsequent AD process, presence of H_2SO_4 may trigger production of H_2S , lowering the methane yield (Figure 4.3.). The highest methane yield in this experiment was observed after 20 minutes of steam explosion treatment at 150 °C. Under these conditions, more than 94 % of the D-limonene was removed, resulting in the methane yield increasing by 426 %, acquiring 0.54 Nm³ methane/kg VS (Paper II).



Figure 4.3. Methane production of untreated CWs, CWs treated with steam explosion in combination with sulfuric acid (0.5 % conc., 150 °C, 6 min), and with steam explosion alone (150 °C, 20 min)

The acquired yield was slightly higher than the theoretical yield of CWs (calculated on the basis of the carbohydrate content of CWs), which may be explained by deficiencies of the measurement method. During batch digestion assays [43], the accumulated methane production of blanks (only inoculum) and samples (inoculum and substrate) are measured. The methane yields of the substrates alone are then calculated by subtracting the methane production obtained from blanks from the methane production obtained from samples. For this reason, it is assumed that the methane production from the inoculum is identical in each set up. This is not always true, since the substrate not only comprises a carbon source, but also contains other nutritional factors which may affect the CH_4 production from the inoculum. In this particular experiment, CWs had a high content of iron, nickel, zinc, cobalt, and magnesium [103, 104], all essential micronutrients for methanogens [105]. Presence of these
nutrients in the substrate during AD measurement assays may thus increase the biogas production from the inoculum.

4.3. Chicken feather

4.3.1. Need for pretreatment

Anaerobic digestion of poultry feather is a challenge, because of the complex, rigid, and fibrous structure of keratin, the main component of feathers. Under anaerobic conditions, poultry feather degrades poorly, which is the main obstacle for anaerobic digestion. Methane potential of feather waste has been reported to be 0.17–0.18 Nm³/kg VS, which is only one third of the theoretical value [24, 39], and consequently, anaerobic digestion of poultry feather is not recommended.

Fourier transform infrared spectroscopy (FTIR) is a technique that provides information about the secondary structure of proteins [106]. It can therefore be used to investigate structural changes of the keratin, caused by the different pretreatments applied and also by the AD process [107]. Amide I and Amide II bands are two major bands of the protein infrared spectrum. The Amide I band is located between 1600 and 1700 cm⁻¹. It is mainly associated with the C=O stretching vibration and is directly related to the backbone conformation. The Amide II band, on the other hand, located between 1545–1400 cm⁻¹, is sensitive to the N–H bending vibration, and to the C–N stretching vibration [108]. The secondary structure of a protein can be examined by the second order derivative of the Amide I absorption peak, because it is responsive to the secondary structure [109]. The secondary structures of β -sheet and α -helix proteins, and of undefined disordered regions, are represented by the absorption regions 1631–1621 cm⁻¹ and 1694–1680 cm⁻¹, along with 1657–1651 cm⁻¹ and 1679–1670 cm⁻¹, respectively [108, 109].

Feather degradation under anaerobic conditions was in the present study investigated after 100 days of digestion, by means of FTIR. The FTIR spectra of the feather before and after digestion, and the secondary derivative of the Amide I band, are displayed in Figure 4.4.



Figure 4.4. FTIR analysis of untreated and digested feather: A) FTIR spectra, and B) the second derivative of the Amide I band

As presented in Figure 4.4, the absorbance spectra and the secondary derivative of the Amide I band show no significant differences before or after the 100 days of digestion, since all secondary derivative peaks were represented, even in the feather residues after the digestion process. According to the FTIR analysis, the feather keratin contained 39.3 % α -helix, 37.6 % β -sheet, and 23.1 % disordered regions before the digestion, which changed to 42.4 %, 40.0 %, and 17.6 %, respectively, after the digestion. These figures indicate that untreated feather degrades poorly under anaerobic conditions. However, after suitable pretreatment, feather may be utilized for biogas production.

Many studies have been performed, and various pretreatment methods have been applied in order to improve the digestibility of feather meal, mainly for the production of a dietary protein, used as feedstuff for animals [88, 110]. The methods involve physical, physicochemical, enzymatic, and biological treatments [72, 81, 111, 112]. These pretreatments may also be used to enhance the biogas potential of feather, since their purpose is to break down the disulfide bonds, thus opening up the keratin structure.

4.3.2. Physicochemical pretreatment of feather

Among the chemical pretreatments, alkaline conditions are recommended prior to biogas production. At an elevated pH, sulfur-rich proteins such as feather will lose about half of their

sulfur content, which is liberated as H_2S [113], and as pointed out above, decreasing the sulfur content of the substrate aids anaerobic digestion. The production of H_2S during anaerobic digestion is toxic mainly for the methanogens, and when the dissolved H_2S concentration exceeds 200 mg/L [114], the AD process is inhibited. Also, H_2S present in the biogas should be reduced or removed before application of the biogas as fuel [115, 116].

Coward-Kelly *et al.* [81, 117] investigated alkaline hydrolysis of keratin-rich materials. They found that $Ca(OH)_2$, even at a concentration of 0.1 g/g lime, dramatically increases the degradation of protein at a temperature range of 100–150 °C. A step-wise process proposed for hydrolysis of protein-rich material under alkaline conditions is shown in Figure 4.5.



Figure 4.5. Degradation process of proteins containing many disulfide bonds, after alkaline pretreatment [117, 118]

Florence [108] suggested three different mechanisms for the breakdown of the S–S bond under alkaline conditions: hydrolysis, α -elimination, and β -elimination (Figure 4.6.). The hydrolysis of the disulfide bond entails a direct attack on the sulfur atom (the disulfide bond) by the hydroxyl anion, resulting in the forming of sulfenic acid (RSOH) and thiolate (RS⁻) [119]. The sulfenic acid is unstable, and probably reacts further to form thiolate or sulfinic acid (RSO₂⁻) [119]. The α -elimination reaction is initiated by the hydroxyl ion attacking the β -carbon, which produces a thiol and a thioaldehyde. It is unlikely that the classical α -elimination reaction takes place, since aldehyde groups never occur in the protein digest, and since the mechanism of that reaction cannot explain the other reaction products, e.g. dehydroalanine residues. The β -elimination is commenced by the hydroxide ion abstracting a proton from the α -carbon of Cys, followed by a cleavage of the disulfide bond. This pathway leads to the production of dehydroalanine and persulfide. The HS⁻ is formed from the persulfide by hydrolysis. The degradation rate significantly increases with elevated temperature and pH [118, 119].



Figure 4.6. Different mechanisms for disulfide bond degradation under alkaline conditions: 1. Hydrolysis, 2. α-elimination, 3. β-elimination [113]

In the present work, lime was applied as pretreatment prior to anaerobic digestion of feather waste (Paper V). After milling, Ca(OH)₂ was added to the material to a final concentration of 0.1-0.2 g Ca(OH)₂/g TS feather. The treatments were carried out in an autoclave within a temperature range of 100–120 °C. After the treatments, the calcium was precipitated and removed as CaCO₃ by adding CO₂ to the system. Solubilization of feather was evaluated by measuring soluble chemical oxygen demand (sCOD), based on the theoretical COD potential of proteins (*i.e.*: 1 g protein corresponds to 1.5 g COD) [120]. The alkaline treatment conditions applied in the present study resulted in a solubilization of 60–95% of the feather protein. In contrast, the thermal treatment with no addition of chemicals degraded less than 3 % of the feather keratin. Figure 4.7 illustrates the feather solubilization degree after chemical pretreatment under various conditions (Paper V).



Figure 4.7. The degree of feather degradation after chemical pretreatment (lime and Ca(OH)₂) at different temperatures, for 30 min., 1 h, and 2 hrs

4.3.3. Biogas from Ca(OH)₂ treated feather

The methane potential of lime-pretreated feather at the selected conditions was investigated, using batch digestion assays. As is illustrated in Figure 4.8, the assays conducted after lime

pretreatment produced about 0.40 Nm^3 methane /kg VS, independently of the concentration of Ca(OH)₂, hydrolysis time, and temperature. This yield denotes an improvement by 122 %, compared to the yield of untreated feather.



Figure 4.8. Accumulated methane produced from feather pretreated with lime under different conditions, and from untreated feather (Paper V)

4.3.4. Biological pretreatment of feather

In nature, a few bacteria, *Actinomycetes* and some keratinophilic fungi, are able to utilize keratin as a sole carbon and energy source. Bacteria with high keratin-degrading ability are known as keratinolytic bacteria, and they belong mainly to the genera [116] *Bacillus licheniformis* [121-123], *Bacillus subtilis*, and *Bacillus cereus* [84]. Keratinolytic species of *Actinomycetes* are represented predominantly in the genera *Streptomyces* [124] and *Thermoactinomyces* [87]. Keratinophilic fungi are dermatophytes and belong to the genus *Chrysosprium* [82]. They are able to express a specific kind of proteases called keratinases [70, 125]. Keratinases are mostly extracellular enzymes, although some keratinolytic bacteria and fungi produce intracellular keratinases as well, which are deposited on the cell surface [126]. Basically all keratinolytic proteases are inducible enzymes; thus, they are only expressed in the presence of keratin [127]. However, a small fraction of keratinases are expressed continuously [128]. Most keratinases act on a wide range of substrates, including bovine serum albumin, collagen, elastin, and feather keratin, but some of them are very

substrate specific. Table 4.1 summarizes the most important keratinolytic microorganisms and the characteristics of their proteases.

Species and strain	Molecular mass (kDa)	pH optimum	Temperature optimum (°C)	Substrate
Bacillus licheniformis PWD-1	33	7.5	50	BSA, casein, elastin, feather, keratin, azokeratin
Bacillus pumilis	31	10.0-10.5	60	keratin, casein
Stenotrophomonomas sp. D-1	40	7.0–10.0	40	keratin, collagen, elastine
Streptomyces pactum DSM 40530	30	7.0–10.0	40-75	feather meal, autoclaved chicken feather
Streptomyces thermoviolaceus SD8	40	6.5-8.5	55	muscle collagen, nail, hair, feather
Streptomyces gulbargensis	46	7.0–9.0	30-45	casein, BSA, chicken feather, hair, nails
Microsporum canis	33	8.0	_	azokeratin, hair keratin
Trichophyton rubrum	27–200	8.0	_	elastin, keratin synthetic peptide, collagen
Microsporum gypseum	33	7.0–9.0	_	BSA, human hair

Table 4.1. Keratinolytic microorganisms and main characteristics of their proteases [72]

Although the mechanism for keratin degradation by microorganisms is not fully known, some hypotheses have been proposed, particularly for keratin degradation by fungi. Kunert [129, 130] have suggested a two-stage (sulfitolysis and proteolysis) degradation process, based on long-term research on *Microsporum gypseum*. During sulfitolysis, the disulfide bonds between the polypeptide chains are cleaved by the sulfite, causing protein denaturation [131]:

 $cys - SS - cys (cysteine) + HSO_3^{-} \longrightarrow cysSH + cysS - SO_3^{-} (S-sulfocysteine)$

At the juncture of protein denaturation, the proteolysis is brought about by the enzyme (keratinolytic protease) attack. It is still not clear whether the sulfitolysis occurs prior to the proteolysis or if both steps occur simultaneously during the keratinolysis [132].

Keratin degradation by the action of prokaryotic organisms differs from keratin degradation by fungi. Prokaryotes decompose the keratin by producing enzymes. According to Sangeli and Brandelli [133], disulfide reductase is the enzyme responsible for disulfide reduction. Yamamura *et al.* [134] described a two-stage process, involving serine protease as well as disulfide reductase-like extracellular enzymes. Although none of the enzymes showed keratinolytic activity on their own, combined they were able to degrade the keratin protein. The disulfide reductase-like enzyme catalyzes the reduction of the disulfide bonds in the first step, after which the whole protein is degraded by the action of another protease, releasing soluble amino acids and peptides:

 $\begin{array}{ccc} \text{Disulfide reductase-like protease} & & \text{Protease 2} \\ \text{R-S-S-R} & & \longrightarrow & \text{R-SH} & & \longrightarrow & \text{Peptide / Amino acid} \end{array}$

Pretreatment with a recombinant Bacillus megaterium strain

In the current study, a recombinant *Bacillus megaterium* strain was developed, and used for the degradation of feather prior to biogas production. *Bacillus megaterium* is a gram-positive, rod shaped soil bacterium, used for the production of commercially important products, such as penicillin amidase and chitosanases, and it is also the major aerobic producer of vitamin B [135-137]. *Bacillus megaterium* holds two major advantages, making it a suitable and a commercially effective tool for biotechnological applications: 1) no endotoxins have been found in the cell wall, and 2) it shows low protease activity [138].

The *ker* gene (responsible for keratinase activity) from *B. licheniformis* was expressed in *B. megaterium*, using a xylose inducible promoter. The recombinant strain showed a protease activity of 29.5 U/mL, a 59-fold higher activity than the wild-type *B. megaterium* (Paper III). The feather degradation obtained after treatment with this recombinant strain, and after treatment with *B. licheniformis* and the wild-type *B. megaterium*, are presented in Figure 4.9. The recombinant *B. megaterium* strain generated a higher amount of soluble proteins than the two other strains. The final protein concentration was 0.51 mg/mL, which was significantly

higher than in the samples hydrolyzed by *B. licheniformis*, (0.25 mg/mL) or by the wild-type *B. megaterium* (0.05 mg/mL) (Paper III).



Figure 4.9. Soluble protein concentrations obtained after biological treatment of feather with different bacterial strains, and feather incubated with recombinant *B. megaterium*, *B. licheniformis* and the wild-type *B. megaterium* (Paper III)

In accordance with the feather degradation, feather hydrolyzed by the recombinant *B. megaterium* strain exhibited the highest methane yields of 0.39, 0.40, and 0.41 Nm³/kg VS after 1, 2, and 8 days of degradation, respectively, significantly higher than the methane yield obtained from untreated feather (0.18 Nm³/kg VS). Biological treatment with *B. licheniformis* increased the methane yield to 0.28, 0.35, and 0.33 Nm³/kg VS, while the treatment with the wild-type *B. megaterium* failed to significantly increase the methane yield (Paper III).

4.3.5. Enzymatic pretreatment of feather

Proteolytic enzymes are one of the most important groups of commercial enzymes [139]. Several industries are using this kind of enzymes in a purified form, including textile, leather, dairy, and detergent industries [140]. Keratinases are a particular type of proteolytic enzymes, possessing the ability to degrade insoluble keratin-rich substrates. Most of the keratinases reported to date are serine proteases, but a few are metalloproteases [141]. Keratinolytic metalloproteases contain mainly Ca^{2+} and Zn^{2+} [142]. Although keratin degradation by enzymes is a promising technology, it has some limitations and disadvantages as well. The

main disadvantage of using enzymes is the high cost of the enzyme production, mostly related to the purification steps.

4.3.6. Biogas production of enzyme treated feather

Feather keratin was hydrolyzed by a commercial enzyme, Savinase[®], a subtilisin-like protease, with enzyme concentrations of 0.53–2.6 mL/g VS feather for 0, 2, or 24 hours at 55 °C, in order to improve the biogas yield in the subsequent anaerobic digestion. According to the sCOD determination in the feather hydrolyzates, 16–40 % of the feather was solubilized after the enzymatic treatment (Figure 4.10). Enzymatic treatment under similar conditions was even more effective after an initial thermal treatment at 120 °C for 10 min, resulting in sCOD values increasing to 39–94 % of the theoretical maximum (Paper IV).



Figure 4.10. The degree of feather degradation (expressed as % sCOD of the theoretical yield) in the hydrolyzate samples after enzymatic and combined thermal and enzymatic treatments

During the following anaerobic batch digestion assays, up to 0.40 Nm³/kg VS methane yield was obtained (Figure 4.11). The enzymatic treatment resulted in the best biogas yields, between 0.32 and 0.40 Nm³ methane/kg VS. The combined treatment, *i.e.* thermal and enzymatic treatment, was less effective, with methane yields of 0.21–0.27 Nm³/kg VS. This was probably a result of undesirable compounds (e.g. ammonia) being formed. The statistical analysis revealed no interaction between the sCOD and the methane yield (Paper IV).



Figure 4.11. Methane yields obtained during the anaerobic batch digestion assays of untreated feather and pretreated feather samples, the latter comprising enzymatic and combined (*i.e.* thermal followed by enzymatic) treatments

Since Savinase[®] is a thermophilic enzyme, able to function at 55 °C, a direct enzyme feeding strategy was also investigated. In this strategy, the enzyme is added directly to the digester, which facilitates the process, making it more economically feasible by saving time, since no additional treatment step is required. The results revealed that the methane yield obtained (0.40 Nm³/kg VS) using this strategy did not differ from the yield acquired when the enzymatic treatment step was extended in time (Paper IV).

4.3.7. Comparison of the different pretreatment methods applied on feather

As the previous subsections suggest, all treatment methods used in the different studies are suitable pretreatments for improving the methane yield of feather waste. Moreover, methane yields up to $0.40 \text{ Nm}^3/\text{kg}$ VS, corresponding to 80% of the theoretical yield from proteins, was acquired, showing no relation to the kind of pretreatment applied prior to the anaerobic digestion. However, these pretreatment methods have their advantages and disadvantages. Chemical pretreatment with Ca(OH)₂ requires a temperature of 100 °C, and relative small amount of chemicals. This treatment can replace the hygienization step, which is mandatory for animal byproducts, according to the European legislation [71]. The accumulation of calcium may, however, inhibit the system since its threshold level for inhibition is around

2.5–7 g/L [14]. Moreover, calcium can precipitate in the biogas digester; hence, removal of calcium is essential before AD. The biological method requires a thermal pretreatment, followed by treatment with a recombinant strain possessing high keratinase activity. This method is environmentally friendly, since it requires no application of chemicals. Nevertheless, the process is slow and demands several days. Furthermore, the application of genetically modified organisms is strongly regulated, and not widely accepted. Enzymatic pretreatment with Savinase[®] is a fast and environmentally friendly method, but the process requires a relatively high enzyme load (0.5 mL/ g VS_{feather}). Consequently, the economic viability of this process greatly depends on the price of the enzyme.

Feather has a low C/N ratio of around 4 [143]. Hence, to ensure an optimal C/N ratio when pretreating feather to enable its utilization for biogas production, co-digestion with other substrates is recommended. The present study explored the potential of co-digesting feather and the organic fraction of municipal solid waste (OFMSW).

4.4. Co-digestion with OFMSW

Co-digestion is an anaerobic treatment of a homogenous mixture of at least two different substrates, in order to improve the efficiency and efficacy of the anaerobic digestion process [144]. It maximizes the methane production because of positive synergisms being established when balancing several parameters, such as macro- and micronutrients, C/N ratio, pH, and dry weight [145]. Co-digestion also lowers the stress of the reactors, by diluting potential inhibitors and toxic components in any of the substrates [146]. A co-digestion system is therefore often used to avoid inhibition, thus making the biogas plant more profitable [145].

4.4.1. Co-digestion of citrus wastes with the organic fraction of municipal solid waste

Three 5 L continuously stirred reactors were operated with three different substrates. One of the reactors was considered a control reactor and was fed only with OFMSW. The other two reactors were fed a mixture of untreated or steam explosion treated CWs, as well as OFMSW in the ratio of 3:7 (corresponding to VS loading). The digesters were operated at a final organic loading rate (OLR) of 3 kg VS/m³day, with a hydraulic retention time (HRT) of 21

days (Paper II). The methane production started decreasing after 15 days of operation, terminating after 26 days of operation when untreated CWs was present in the feed (Figure 4.12.).



Figure 4.12. Methane production during semi-continuous co-digestion of untreated CWs and OFMSW at OLR of 3 kg VS/m^3 day and HRT of 21 days (Paper II)

During the process, the VFAs accumulated to a value of 6 510 mg/L resulting in a pH drop to 5.5, which caused the failure of the process (Paper II). Data obtained during batch digestion of a similar substrate mixture did not suggest any inhibition, but the continuous system in the present study revealed the long-term effects, and suggested an inhibitory compound overloading the system. Mizuki *et al.* [50] reported inhibition by D-limonene at a concentration of 65 μ L/L day in a mesophilic anaerobic digestion process. The D-limonene load in our system, however, was only 40 μ L/L day. The inhibition in the present long-term study probably refers to thermophilic microbial flora having higher susceptibility to disturbance factors in the process.

4.4.2. Co-digestion of steam exploded citrus wastes with OFMSW

In another digester, steam exploded CWs were used in order to decrease the D-limonene load of the digester. The steam explosion (150 °C, 20 min) pretreatment opened up the structure of the lignocellulosic waste, removing more than 94 % of the D-limonene content. Co-digestion of the steam exploded CWs and OFMSW, in a similar mixture as reported above, was

successful, showing a methane production of 0.555 ± 0.016 Nm³/kg VS (Figure 4.13). During the continuous digestion experiment, the concentration of total VFAs remained under 2 g/L, and the pH remained stable between 7.5–7.9, indicating a stable process performance in the digester (Paper II).



Figure 4.13. Methane production during semi-continuous co-digestion of steam exploded CWs and OFMSW at OLR of 3 kg VS/m^3 day and HRT of 21 days (Paper II)

4.4.3. Co-digestion of feather with OFMSW

To my knowledge, no study has investigated the co-digestion possibilities of feather to date. However, solid poultry slaughterhouse waste (SHW) is similarly composed. Thus, this subsection summarizes the data found in the literature in relation to co-digestion of SHW with other substrates. Anaerobic digestion of slaughterhouse wastes, including feather, are difficult due to the high protein and lipid contents of this kind of waste streams [147]. Lipid degrades to long chain fatty acids (LCFAs), which in high concentrations are toxic to the acetogens and methanogens working in the digester [148, 149].

Anaerobic digestion of SHW has been reported to be possible under mesophilic conditions, but only with very low loading rates of up to 0.8 kg VS/m³ day, and with long HRTs of 50-100 days [148]. At higher OLRs or shorter HRTs, the process was overloaded or inhibited by accumulated VFAs, long-chain fatty acids (LCFAs), or ammonia.

Co-digestion may be a possible solution for utilizing this kind of wastes in anaerobic digestion, since achieving a better balance when composing a substrate mixture makes it possible to increase the OLR and/or shorten the HRT, at the same time reducing the stress conditions in the process. Salminen and Rintala [150] reported a stable co-digestion process with OLRs up to 4.6 g VS/L day and an 18-day HRT, when poultry waste was co-digested with waste from a food packing plant. Cuetos *et al.* [147] found that co-digestion of slaughterhouse waste (SHW) with OFMSW in a 1:5 mixture (based on wet weight) at a 25-day HRT and an OLR of 3.7 g VS/L day, was not successful unless a long adaptation period of 100 days was introduced. In a later work, Cuetos *et al.* [151] investigated the effect of heat and pressure pretreatment (133 °C, > 3 bar, 20 min) on biogas production from SHW. In that study, co-digestion of SHW mixed with OFMSW (in a ratio of 1:5, based on wet weight) using an HRT of 36 days and an OLR of 2.6 kg VS/m³ day was investigated. They found that co-digestion of pretreated SHW and OFMSW. They suggested that the hygienization treatment might cause formation of refractory compounds with inhibitory effects.

In the study presented here, feather was co-digested with OFMSW to avoid the process instability caused by the high protein content of feather. Feather was mixed with OFMSW to a ratio of 1:4 (based on VS) to ensure the optimal C/N ratio of 20:1. The HRT was adjusted to 25 days, and the OLR was increased stepwise from an initial value of 0.5 to a final value of 2.5 g VS/L day during the first three weeks of the operational period. Two CSTR reactor setups were investigated, both operating with a mixture of untreated feather and OFMSW, but in one of them, Savinase[®] (0.5 mL/g VS_{feather}) was added directly. The purpose of the enzyme addition was to improve the degradation of feather (Paper IV).



Figure 4.14. Methane production during semi-continuous co-digestion of feather and OFMSW, with and without the addition of Savinase[®], at an OLR of 3 kg VS/m³ day and a 21-day HRT. The figure reveals a start-up period of 21 days (Paper IV)

As disclosed in Figure 4.14, the addition of enzyme to the substrate mixture resulted in a higher methane yield, 0.485±0.021 Nm³/kg VS, than was acquired from co-digestion of feather without enzyme addition, where methane production gradually decreased after less than 50 days. These results indicate that a direct enzyme feeding strategy may be an accessible method to increase the digestion efficiency, when utilizing feather for biogas production. Co-digestion without enzyme addition produced less amount of methane due to incomplete degradation of the feathers, as was manifested by a significant amount of undigested feather being present in the digester at the end of the experiment (Paper IV). This implies that keratin is not able to degrade in anaerobic digesters, which can cause accumulation during a long-term process, resulting in various problems, e.g. a decreased effective reactor volume, and mixing problems.

5. Economics of Anaerobic Digestion

The economic viability of the installation and operation of anaerobic digesters is imperative for commercial applications. Several factors affect the feasibility of the anaerobic digestion process, including process configuration, location, size of the digester, etc. This chapter summarizes the most important factors to consider when evaluating the economics of the anaerobic digestion process:

- Capital cost
- Operating cost
- Types of feedstock
- Cost of feedstock or gate fee
- Digestate value or cost
- Electrical efficiency of combined heat and power unit (CHP)
- Value of electricity (EUR/kWh)
- Value of heat (EUR/kWh)
- Cost of upgrading

Capital cost

The capital cost depends on several factors, e.g. plant size, location, engineering, and the composition of waste. The characteristics of the incoming organic waste are important to consider, because they determine the necessary units required for preprocessing prior to digestion. Generally, a larger plant size requires less investment per production unit, because the capital cost does not increase linearly with the plant size. Capital cost can be estimated by using the "six-tenth" rule: doubling the plant size will result in an increased capital cost by 52 % [152]. According to Monnet [153], the capital cost in England of an AD plant treating waste from farms, is probably around 600 000–6 000 000 EUR for a capacity of 10 000–200 000 tons/year, while the capital cost of AD plants treating 100 000 tons of source-sorted organic fraction of municipal waste/year, is around 15 300 000 EUR. In terms of using

municipal waste, additional preprocessing steps are required, such as removal of plastic, glass, and metal, consequently increasing the capital cost [153].

Operating cost

Operating cost is related to the operation of the biogas plant, and includes the costs associated with the operating staff (salaries, insurances, etc.), transportation, licenses, price of the feedstock, and maintenance. The operating cost is in the range of 38 000–640 000 EUR/year for the AD plants mentioned above, treating 10 000–200 000 tons waste per year, while the operating cost for the AD plant operating with MSW, is close to 1.2 million EUR/year [153].

Type of feedstock

The most important issue when considering the application of anaerobic digestion systems is the feedstock. The feedstock determines energy and mass balance; it influences the reactor configuration (design, operating conditions, etc.), and even the bacterial physiology during the biological degradation process [154]. Thus, a suitable feedstock is essential for a feasible operation. Figure 5.1 shows mass and energy balance of a typical wet anaerobic digestion process.



Figure 5.1. Mass and energy balance of a wet anaerobic digestion process treating 1 000 kg of organic waste, with a TS content of 35 %, and acquiring a biogas yield of 222 m³/ton waste (modified from[155])

Cost of feedstock or gate fee

The cost or price for the feedstock shows significant variations, depending on the type of waste and the region/country where the utilization plant is located. In some countries, including England, it is customary that the operator of the AD plant charges a waste management gate fee per ton waste taken care of by the plant. In other countries, like in Germany, the waste management sites compete for certain organic waste streams [5]. In these countries, the gate fee of this kind of wastes is zero, and the biogas plants must sometimes even pay for the waste.

Digestate value or cost

Beside the methane produced, the digestate residue is another valuable product of anaerobic digestion. The digestate residue holds a high nutrient value, making it appropriate for crops fertilization [156]. The price highly depends on the chemical, biological, and physical properties of the digestate residue. A high water content of the digestate is however a disadvantage, making the transportation of this fertilizer expensive [157]. In spite of it being a valuable byproduct, the digestate residue is in most cases just given away, rather than sold on the open market.

Cost of upgrading

Biogas can be upgraded to biomethane and can then be used as vehicle fuel [158] or as an alternative for natural gas. It can be directly injected into the national grid, if the technical specifications are fulfilled [158]. CH₄ combusts very cleanly without any soot particles or other pollutants being discharged, making it a clean renewable fuel. Several existing upgrading techniques are available, including water scrubbing, pressure swing adsorption, chemical absorption as well as cryogenic and membrane separation [159]. However, the cost of upgrading depends on the amount of biogas produced and the upgrading technology used, because of its low cost. Table 5.1 summarizes the prices, and the purities acquired, using different upgrading technologies. In Sweden, around 84 % of the produced biogas is currently being upgraded to biomethane and used as vehicle fuel, or injected into the national grid of gas [160].

Technology	Price per Nm ³ of biogas (EUR)	Purity (%)
Chemical Absorption	0.28	98
High Pressure Water Scrubbing	0.15	98
Pressure Swing Adsorption	0.26	98
Cryogenic separation	0.40	91
Membrane separation	0.22	89

Table 5.1. Comparison of prices and purity acquired, using different upgrading technologies. The calculation is based on a biogas input flow of 250 Nm³/h with a 60 % CH₄ content [159]

The electrical efficiency of Combined Heat and Power units

In other European countries, the produced biogas is usually converted to heat and electricity in combined heat and power units (CHP). The produced electricity is usually directed into the public electricity net, while one part of the produced heat is used to provide energy for the process, and the remaining part can be sold for central and district heating [161]. The efficiency of the CHP unit is crucial for an economical operation. The electrical efficiency of around 34%, increasing to 41–42 % when the capacity of the CHP unit exceeds 1000 kW_{el} [162].

Value of the heat

One part of the heat generated by a CHP is used as energy supply for the process, while the remaining part can be used for the district heating system. The price usually parallels the market price level, although in some countries (like the UK), the tariff for renewable heat is proposed by the Renewable Energy Association. Currently, most of the biogas plants operating in Europe cannot sell the excess heat [162].

Value of the electricity

Most countries support the production of renewable energy; therefore, many countries have introduced a system called feed-in tariffs. The system offers a higher price for the produced electricity and a long-term contract (15–25 years), which aids financing investments in renewable energy production. In several countries, this tariff is 4–5 times higher than the market price of the electricity. The tariff depends on the size of the investment, and on the location of the investment. Table 5.2 summarizes the feed-in tariff for biomass in the European countries.

Member state	Feed-in tariff Biomass (EUR/kWh)	Member state	Feed-in tariff Biomass (EUR/kWh)
Austria	0.06-0.16	Latvia	n/a
Belgium	n/a	Lithuania	0.08
Bulgaria	0.08-0.10	Luxembourg	0.103-0.128
Cyprus	0.135	Malta	n/a
Czech Republic	0.077-0.103	Netherlands	0.115-0.177
Denmark	0.039	Poland	0.038
Estonia	0.051	Portugal	0.1-0.11
Finland	n/a	Romania	n/a
France	0.125	Slovakia	0.072-0.10
Germany	0.08-0.12	Slovenia	0.074-0.224
Greece	0.07-0.08	Spain	0.107-0.158
Hungary	n/a	Sweden	n/a
Ireland	0.072	United Kingdom	0.12
Italy	0.2-0.3		

Table 5.2. Prices paid for renewable electricity in EU, given in euro per kilowatt-hour (EUR/kWh). The data are from April 1^{st} , 2010^1

5.1. Economic evaluation of biogas production from CWs

As previously mentioned, CWs can be used for production of various products. Beside biogas production, ethanol can be generated from CWs, because of their high sugar content. However, a pretreatment of the CWs is necessary for both these processes. The economical analysis of utilizing CWs in a biorefinery is out of the scope of this thesis. Nonetheless, based on the results of the present study, Lohrasbi *et al.* [47] determined that a biorefinery concept, producing ethanol, biogas, D-limonene, and pectin, may be economically feasible with a plant capacity exceeding 400 000 tons CWs per year. In the present thesis, the utilization of CWs for biogas production was investigated with around 10 000 tons of CWs being available on a yearly basis (Paper II). The developed process included a stem explosion step prior to the anaerobic digestion, and resulted in 107 m³ CH₄ and 8.4 L D-limonene per ton CWs. In addition; the steam explosion pretreatment step can easily be connected to an existing biogas plant. The equipment cost was analyzed and estimated to 0.65 million EUR, while the operating cost of the pretreatment unit would be around 150 000 EUR/year. The block flow diagram of the developed process is presented in Figure 5.2.

¹ Europe`s Energy Portal

Website: http://www.energy.eu/



Figure 5.2. Block flow diagram of the steam explosion pre-treatment process combined with the D-limonene recovery (Paper II)

In Sweden, the price of upgraded biogas, sold as vehicle fuel, need to set below 1.38 EUR/Nm³ (12.42 SEK/Nm³) to be competitive with petrol [163]. Considering that the upgrading and the compression cost is 0.73 EUR/Nm³ [164], this process is able to generate biomethane with a minimum selling price of 0.43 EUR/Nm³, which indicates that the process would be economically feasible.

5.2. Economic evaluation of biogas production from feather

The present study furthermore developed an industrial process for utilizing feather waste in the anaerobic digestion process. This process entails a chemical pretreatment of the feather, applying $Ca(OH)_2$ at 100 °C for 1 hour. This step complies with the requirements of the EU legislation of hygienization when handling animal byproducts. The hydrolysis step is followed by addition of carbon dioxide, which results in removal of the calcium in the form of CaCO₃. For the economical evaluation, five different process sizes, ranging from 625 to 10 000 tons/year of feather, were analyzed. The model assumed that the incoming feather entails no cost or value. The estimated capital and operating costs in relation to capacity are presented in Figure 5.3.



Figure 5.3. The prediction of operating and equipment cost for the pretreatment process as a function of process capacity (Paper V)

The minimum price of the produced methane was calculated as the price making the net present value (NPV) equal to zero over 20 years, taking into account a 15 % discounted cash flow rate of return (DCFROR). The calculated minimum selling price of the upgraded methane (used as vehicle fuel) ranged between 0.21 and 1.07 EUR/Nm³, conveying that a process holding a capacity of at least 2 500 tons feather/year would be economically viable (Figure 5.4).



Figure 5.4. Minimum selling price of the upgraded biomethane as a function of process capacity

Furthermore, the feasibility of heat and electricity production from biogas was investigated. The results showed that a process capacity of 2 500 tons feather/year might be economically viable even without a gate fee (Paper V).

6. Concluding Remarks

The present thesis mainly focused on biogas production as a waste management tool for two byproducts from the food-processing industries, namely citrus wastes from the juice– processing industry and feather waste from poultry slaughterhouses. These waste streams hold a high biogas potential, but are recalcitrant to the anaerobic digestion process in different ways. CWs contain an inhibitory agent, D-limonene, exerting an antimicrobial effect while feather has a complex structure as the main obstacle. Thus, both waste types are resistible to biological degradation, making a pre-processing step necessary in order to render them suitable for biogas production.

A) The major conclusions of the citrus waste project are summarized as follows:

- D-limonene was successfully removed from citrus wastes using steam explosion as a pretreatment step, resulting in an increase in methane yield by 426 % compared to the untreated CWs.
- A biorefinery concept was developed for the utilization of CWs, resulting in multiple products, such as ethanol, methane, D-limonene, and pectin. The developed process is able to generate 40 L ethanol, 45 m³ CH₄, 9 L limonene, and 39 kg pectin per ton CWs.
- An economic study of the utilization of CWs for smaller amounts (*i.e.* 10 000 tons per year being available) was also performed. This study manifested that biogas production might be viable when integrating the developed pretreatment process in an existing biogas plant.

B) The major conclusions of the feather project are summarized as follows:

- The compact structure of feather was successfully degraded, by using chemical, enzymatic, or biological pretreatment methods.
- The methane yield of feather was doubled compared to the yield of untreated feather, and 0.40 Nm³ CH₄/kg VS was acquired after these pretreatments.
- The economic viability of an industrial process, employing a chemical pretreatment prior to the anaerobic digestion, was explored. Process capacities of at least 2 500 tons /year would be viable under the suggested conditions.

Nomenclature

AD	Anaerobic digestion
СНР	Combined heat and power
COD	Chemical oxygen demand
CSTR	Continuous stirred-tank reactor
CWs	Citrus wastes
FAO	Food and Agriculture Organization
GHGs	Greenhouse gases
GW	Global warming
HRT	Hydraulic retention time
IRR	Internal return rate
LCFAs	Long-chain fatty acids
NPV	Net present value
OFMSW	Organic fraction of municipal solid waste
PEO	Polyethylene oxide
RS ⁻	Thiolate
RSO ₂ ⁻	Sulphinic acid
sCOD	Soluble chemical oxygen demand
SHW	Slaughterhouse waste
SRT	Solid retention time
TS	Total solids
VFAs	Volatile fatty acids
VS	Volatile solids

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