THESIS FOR THE DEGREE OF PHILOSOPHY

Biogas Production from Lignocelluloses:

Pretreatment, Substrate Characterization, Co-digestion, and Economic Evaluation

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Abstract

Biogas production from organic materials can be used as a renewable vehicle fuel, provide heat and generate electricity and can thereby reduce the greenhouse gas emissions. This thesis focuses on the biogas production based on lignocelluloses. There is an abundant availability of lignocelluloses, constituting 50% of the total biomass worldwide. However, the biomass recalcitrance limits the microbial degradation as well as the biogas production from these types of materials.

In the present work different pretreatment methods have been performed in order to decrease the biomass recalcitrance and improve the biogas production. Steam explosion pretreatment, together with the addition of sodium hydroxide and hydrogen peroxide, has been performed on lignocellulosic-rich paper tube residuals. The pretreatment has resulted in methane yields of up to 493 NmL/gVS, which is an increase by 107% compared with untreated material. Furthermore, the use of an organic solvent, N-methylmorpholine-N-oxide (NMMO), was evaluated as a pretreatment method for spruce (both chips and milled), rice straw, and triticale straw. The NMMO pretreatment resulted in 202, 395, 328, and 362 NmL CH_4/g carbohydrates produced of these substrates, respectively, corresponding to an increase of between 400-1,200% compared with the untreated version of the same material.

Moreover, the paper tube residuals have been co-digested with an unstable nitrogen-rich substrate mixture, mainly based on municipal solid waste. The addition of the lignocellulosic-rich paper tubes in a co-digestion process showed stabilizing effects and prevented the accumulation of volatile fatty acids with a subsequent reactor failure. Additionally, synergistic effects have been found leading to between 15-33% higher methane yields when paper tubes were added to the co-digestion process compared with the yields calculated from the methane potentials of the two substrates.

Substrate characterization analysis can be used to study the changes on the lignocellulosic components after the pretreatment, relating the changes to the performance in the anaerobic digestion. Increased accessible surface area, measured by the Simons' stain and the enzymatic adsorption methods, as well as decreased crystallinity, determined by using the Fourier Transform Infrared Spectroscopy, can all be linked to improved biogas production after pretreatment.

Finally, the NMMO pretreatment on forest residues has been financially evaluated for an industrial scale process design. The base case that was evaluated simulated a case where pretreated forest residues were co-digested with the organic fraction of municipal solid waste to obtain optimal nutritional balance for the anaerobic digestion. This process has been found to be economically feasible with an internal rate of return of 20.7%.

Keywords: anaerobic digestion, biogas, lignocellulose, pretreatment, co-digestion, substrate characterization, economic evaluation.

List of publications

This thesis is mainly based on the results from the following articles:

- I. **Teghammar, Anna**; Yngvesson, Johan; Lundin, Magnus; Taherzadeh, Mohammad, J. and Sárvári Horváth, Ilona. Pretreatment of paper tube residuals for improved biogas production. *Bioresource Technology*, 2010, 101, 1206-1212
- II. Teghammar, Anna; Karimi, Keikhosro; Sárvári Horváth, Ilona and Taherzadeh, Mohammad, J. Enhanced biogas production from rice straw, triticale straw and softwood spruce by NMMO pretreatment. *Biomass and Bioenergy*, 2012, 36, 116-120
- III. Teghammar, Anna; Chandra, Richard; Saddler, Jack, N; Taherzadeh, Mohammad, J. and Sárvári Horváth, Ilona. Substrate characteristic analysis for anaerobic digestion: A study on rice and triticale straw. *BioResources*, 2012, 7 (3), 3921-3934
- IV. Teghammar, Anna; del Pilar Castillo, Maria; Ascue, Johnny; Niklasson, Claes; Sárvári Horváth, Ilona. Improved anaerobic digestion by the addition of paper tube residuals: pretreatment, stabilizing, and synergetic effects. *Energy and Fuels*, 2013, 27 (1), 277-284
- V. **Teghammar, Anna**; Sárvári Horváth, Ilona and Taherzadeh, Mohammad, J. Technoeconomic study of NMMO pretreatment and biogas production from forest residues. Submitted manuscript.

State of Contribution

- Paper I: The author of this thesis, Anna Teghammar (AT), performed all experimental work and analysis, contributed to the idea, and was responsible for the manuscript preparation and its revision.
- Paper II: AT performed and planned all experimental work, except the pretreatment of the spruce. The author was responsible for the analysis, the manuscript preparation, and its revision.
- Paper III: AT planned and performed all experimental work and analysis, was responsible for the idea, the manuscript preparation, and its revision.
- Paper IV: AT was responsible for the major part of the experimental work, i.e., the pretreatments, the batch anaerobic digestion tests and subsequent analysis, the manuscript preparation, and its revision.
- Paper V: AT performed the planning and simulation work, the data analysis, and the manuscript preparation.

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1. INTRODUCTION

In the last few decades, increasing emission of greenhouse gases worldwide has become a major concern. With a growing world population and with increasing energy consumption coupled with higher living standards, there is a huge challenge in limiting the emissions of pollution and greenhouse gases. Moreover, the available fossil fuels are limited; consequently, with increasing prices we need alternative energy sources to replace the fossil fuels. The European Commission has set the goal that by 2020, 20% of the energy consumed should come from renewable energy sources, as well as 10% of the energy consumed within the transport sector [1]. The commercially renewable vehicle fuels available today are ethanol, biogas, biodiesel, and electricity produced from renewable energy sources. This thesis focuses on biogas. Biogas is a renewable energy source, which can be upgraded to be used as vehicle fuel, and moreover be used for electricity and heat production.

The present feedstocks for anaerobic digestion, such as the organic fraction of municipal solid waste (OFMSW), sewage sludge, and manure, are limited; thus, new renewable sources are sought after. The abundant availability of lignocelluloses worldwide together with the high carbohydrate content makes lignocelluloses a highly interesting feedstock for biofuel production. The present thesis focuses mainly on lignocellulosic residuals, such as paper tube residuals, rice and triticale straw as well as forest residues. An additional advantage of the digestion of these materials is that they do not compete with the land usage for feed or food production.

However, the microorganisms in the anaerobic digester have difficulty in digesting the lignocelluloses due to its compact and complex structure, with high lignin content. Different pretreatment methods can open up the structure and make it possible to degrade the lignin. The pretreatments result in a more easily digested feedstock for the anaerobic microorganisms.

1.1 Aim of study

The main goal of the present thesis is to study the possibilities of using lignocellulosic residuals for biogas production. The work was divided into four different parts:

- 1. Enhance the methane production from different lignocelluloses (paper tube residuals, rice straw, triticale straw, and spruce in the present thesis), with the help of different pretreatment methods (steam explosion and hydrothermal pretreatment, together with sodium hydroxide and hydrogen peroxide, as well as NMMO pretreatment in the present thesis). Results and methods are presented in chapter 4 as well as in Papers I, II and IV).
- 2. Characterize the physical changes in the lignocelluloses after pretreatment, using substrate characteristic analysis (Paper III). Results and methods are presented in chapter 4.
- 3. Study the long-term co-digestion effects of pretreated and untreated lignocelluloses together with the organic fraction of municipal solid waste (Paper IV). Results and methods are presented in chapter 3.
- 4. Evaluate the economic feasibility of a full-scale pretreatment and anaerobic digestion plant using lignocelluloses as a substrate (Paper V). Results and methods are presented in chapter 5.

1.2 Outline of the thesis

This thesis contains five chapters, summarized as follows:

- Chapter 1 introduces the thesis and the main objectives of the research work.
- Chapter 2 provides a background on lignocelluloses and its structure and discusses the biomass recalcitrance and different lignocellulosic waste fractions available.
- **Chapter 3** describes the biogas market, the anaerobic digestion process, microbial degradation of lignocelluloses, different important process parameters for the anaerobic digestion, as well as methods for determining the biogas potential (Paper IV).
- **Chapter 4** reviews different pretreatment methods as well as substrate characterization methods (Papers I-IV).
- Chapter 5 discusses the economics of the anaerobic digestion process and investigates the economic feasibility of using a developed pretreatment process prior to anaerobic digestion in a full-scale process (Paper V).

2. LIGNOCELLULOSIC MATERIALS

The availability of lignocellulosic materials is abundant worldwide. Lignocelluloses have been estimated to account for approximately 50% of the biomass in the world, and to have a yearly production of about 200 billion tons per year [2, 3]. These materials are carbohydrate rich and can be used for anaerobic digestion.

2.1 The components of lignocelluloses

Lignocelluloses consist mainly of cellulose, hemicelluloses, and lignin. These polymers have different functions and different chemistry in the lignocellulosic plants, and they are further discussed in the following sections. The compositions of the three main lignocellulose groups, softwoods, hardwoods and grasses can be found in Table 2.1.

	Softwood ¹	Hardwood ¹	Grass ²
	(spruce)	(beech)	(switchgrass)
Cellulose	44.7%	45.6%	32.2%
Hemicellulose	22.9%	25.9%	24.4%
Lignin	30.6%	23.8%	23.2%
Others	1.8%	4.7%	20.2%

Table 2.1 Composition of the three main lignocellulose groups, expressed as % of original dry matter.

¹Adopted from Jin et al [4], and from ²Esteghlalian et al [5].

2.1.1 Cellulose

Cellulose is the main component of higher plants, comprising 20-40% of the cell wall [6]. Cellulose is an unbranched polymer chain, constituted by glucose units, which are linked by β -1, 4-glycosidic

bonds [7]. The degree of polymerization can be up to 14.000 glucose units, where each glucose unit is rotated 180°, relative to the adjoining unit. The cellulose chains form tight aggregates in the form of three dimensional microfibrils. These are stabilized with hydrogen and van der Waals linkages. Each glucose unit is linked via two intra-chain hydrogen bonds and two to three inter-chain bonds. This makes the configuration tightly packed and stable. Each microfibril consists of 30-36 parallel cellulose chains [6, 8]. The half-life of crystalline cellulose at a neutral pH is 100 million years [9].

2.1.2 Hemicellulose

The polysaccharides of the cell wall that are not part of the cellulosic microfibrillar phase can instead be found in the matrix phase. The matrix phase consists of different hemicelluloses as well as pectines [6], and they are attached via hydrogen bonds to the cellulose fibers. Hemicelluloses are a group of different branched polysaccharides, which are comprised of both pentoses (e.g., xylose and arabinose) and hexoses (e.g., glucose, mannose, and galactose). The contribution and constitution of hemicelluloses varies from different plants and different cells [10]. They can be divided into (1-3,1-4)- β -D-glucans, heteroxylans, heteroglucans, heteromannans (galactoglucomannans and glucomannans) and arabino-3,6-galactans [6]. In hardwood and agricultural plants such as grasses and straw, the dominant hemicelluloses is xylan, while for softwoods, it is glucomannan [11]. Lignocelluloses consist of approximately 20-35% hemicelluloses [11], and the degree of polymerization is often around 200.

2.1.3 Pectins

Pectins or pectic polysaccharides are another group of polysaccharides that occur in the primary cell wall of, for example, grasses [6]. These polysaccharides are hydrophilic and highly diverse in nature. The major components in pectins are: galacturonic acids, followed by rhamnose, arabinose, galactose, fucose, and apiose [12].

2.1.4 Lignin

Lignin is the third most abundant substance next to cellulose and hemicellulose in nature. Lignins are regarded as complex, amorphous, branched polymers constructed of different phenylpropane units [11, 13]. The monomeric composition of lignin varies; however, most monolignols are derived from p-coumaryl alcohol, coniferyl alcohol or sinapyl alcohol [14]. Lignin can also be designed as a mixture of p-hydroxyphenyl, guaiacyl, and syringyl, based on their aromatic ring substitution pattern [13]. There are inter-linkages between lignin and hemicellulose as well as between lignin and cellulose that

keep the lignocellulosic structure together. These inter-linkages can be ester, ether, or glycosidic bonds. Lignin is extremely resistant to biodegradation due to these strong linkages [13]. One of the biggest challenges in using lignocelluloses as a feedstock for biogas production is to open up and alter the lignin structure. High lignin content in the biomass has previously been connected to lower methane yields [15, 16].

2.1.5 Extractives

Extractives are non-cell wall components, which can be found in wood, bark and foliage. They are molecules with less than 40 carbon atoms per molecule. They comprise about 1-5% of the wood and the most common extractives are resin acids, fatty acids, and sterols [17, 18]. Many of the extractives can be toxic to aquatic organisms and should be removed, for example, from the waste water of pulp and paper plants in order to avoid environmental pollution [18].

2.1.6 Inorganic additives

In the pulp and paper industry, different chemicals are used together with the wood for improved paper quality, printing properties, or lower manufacturing costs. Minerals are used as fillers or for coating. The most commonly used minerals are: calcium carbonate, kaolin clay, titanium dioxide and talc. The minerals achieve a brighter and smoother paper with higher opacity, and with the exception for titanium oxide, are cheaper than the wood pulp [19, 20]. Furthermore, the pulp and paper industry use paper bleaching chemicals, printing colors as well as different glues, for instance, in the production of paper tubes where sodium silicate and polyvinyl alcohol are used (Paper I).

2.2 Lignocellulosic structure

Higher plants consist of two types of cell walls, primary and secondary. The primary wall is the outermost wall and is usually not lignified. The primary cell wall provides mechanical strength but also flexibility during the growth of the cell. The secondary wall is constructed inside the primary cell wall when the cell is mature and fully expanded. The secondary cell wall is thicker and stronger and contains a large amount of lignin. It is often divided into three layers (S1, S2 and S3) and the cellulose fibers in each layer are sorted in different directions compared to the other layers (Figure 2.1). The secondary cell wall accounts for most of the carbohydrates in biomass. The space between the cells, called the middle lamella, is often also lignified [6].



Figure 2.1 Three dimensional sketch of a tracheid (xylem cell), copyrighted by the American Society of Plant Biologists and reprinted with permission [21].

2.2.1 Biomass Recalcitrance

The complicated lignocellulosic structure within the cell walls is a way for the plants to prevent the microbial and enzymatic degradation. One of the main purposes of the construction of the lignocellulosic structure is to hinder the microbial degradation of the material. This attribute is defined as the biomass recalcitrance [22]. The degree of recalcitrance in the biomass is determined by the access of the enzymes to the polysaccharides. Plants have several different ways to provide protection against microbial degradation. The first protection layer in plants is called epidermis, this is in trees represented by the bark, while in grasses this first protection layer consist of cells with thick cell walls [22]. The second protection layer is the structure and organization of the cell walls as well as the vascular tissues [23]. The third and probably the main reason for the biomass recalcitrance is the molecular structure within the cell walls [24]. In the microfibrils, the cellulose fibers are ordered in a crystalline manner. The microfibrils are further surrounded by a matrix of different polymers, such as lignin, hemicelluloses, and pectins [22]. These polysaccharides are connected to each other via covalent and non-covalent bonds that together form a 3D structure. These cellulosic microfibrils are connected to the matrix polymers, and the different polymers in the gel matrix are connected to each

other. This crosslinking between different polymers in the cell wall has a major contribution to the lignocellulosic biomass recalcitrance [6].

The accessibility of the substrate to the enzymes and microbes are dependent not only on the interacting bonds but also on the lignin content and the cellulosic crystallinity. It is also dependent on the accessible surface area of the material, which has to be high enough in order to make the substrate available for the microorganisms [25, 26].

2.3 Lignocellulosic materials

Lignocellulosic materials available for anaerobic digestion can be divided into cultivated feedstocks, where the plant is directly dedicated for energy production, as well as into lignocelluloses that are produced as residuals. The residuals have the advantage that they do not compete with land usage for food and feed production. The two different types of feedstocks are further discussed in the following chapters.

2.3.1 Energy crops and forest production

Lignocellulosic-rich energy crops are cultivated with the direct goal of using the biomass of the plants for energy production. Plants used as energy crops in Europe include, for example reed canary grass, willow, poplar, and miscanthus [27]. The cultivation of trees in the forests can also be used for anaerobic digestion. The forests constitute high volumes of biomass. Approximately, 420×10^9 tons forest biomass is available around the world, of which more than 40% is available in South America [28]. However, the use of wood for energy production competes with other applications of the wood, which makes the use of wood for energy production an economical issue.

2.3.2 Forest residues

The forest residuals include, for example, tops and branches, needles, bark, roots, fuel wood, logging residues, sawdust, as well as stems and trees from clearing and thinning [29]. During logging, about 60% of the biomass from the harvested tree is left in the forest. Sawing the logs produces an additional 8-10% waste, while squaring the logs in the forest, produces an additional 30-50% waste of the biomass left after logging. Additionally, 45-55% of a log is wasted at the sawmill [28]. In Sweden, the waste from the forest is calculated to have an energy potential of between 49 to 59 TWh/year [29, 30]. Today, the residues from the forest industry are used as chips for the production of pulp, particleboard,

fibreboard, or used as fuel [28]. The highest potential for energy production, where the forest residues are not used today, lies in the tops and branches as well as roots/stumps, where a potential of 20 TWh/year can be withdrawn from the forest [29]. There is a high abundance of forest residues; however, they have a high fraction of lignin and are consequently hard to digest in the anaerobic digester.

2.3.3 Agricultural residues

The lignocellulosic residuals from the agriculture consist mainly of different grain and oilseed straws, where rice and wheat straw are the two largest waste fractions. The world rice straw production is approximately 730 million tons/year, distributed over Asia, America, Africa, and Europe [31]. At the same time, 530 million tons of wheat straw was produced worldwide in 2011. Both straws are often burned on cropland today, causing environmental and health problems [31, 32], while some are removed for cooking, recycling, or other uses. The biomass of the straw is the same size as the produced grain, with a factor of 0.8-1.5 kernel over straw, depending on the crop type [30]. In Sweden straw is produced mainly from wheat, rye, oat, barley and triticale, as well as oil-plants [30].

To some extent, straw can be useful for the improvement of the soil structure and therefore can partly be left on the agricultural land. In addition, straw is often used as a bedding material mixed with manure within the animal cultivation. As a consequence, the available straw for energy production is about 30% lower than the total existing amount. This also includes the case when straw is used together with manure [30]. The high availability of straw in the manure can classify the manure as a lignocellulosic feedstock. In Sweden, a future potential from agricultural residuals, including manure is calculated as being 10.8 TWh/year with improved collection techniques, with about 6.6 TWh if the manure is excluded [30].

Together with straw and manure, rice husks have a great potential for anaerobic digestion, since they are already collected at the rice plant. Other important agricultural residues are, for example, sugarcane fibers (bagasse), corn stover, coconut husk and shell, groundnut shell, and groundnut straw [33]. Agricultural residues have the advantage that in most cases they are easier to degrade in comparison with forest residues. This is due to the lower lignin content, as well as the dimensions of the straw being relatively small, which results in a material that is more easily accessible for the microbial enzymes.

2.3.4 Lignocellulosic industrial and municipal residues

The main lignocellulosic waste streams from industries originate from the pulp and paper industry. Example of these streams are waste paper products, fiber waste, sulphite liquor, sludge, and other solids from the pulp and paper mills [34]. In 2009, about 390 million tons of paper was produced worldwide [35], whereof 54% was recycled in 2011 [36]. The paper fiber can be recycled about 5-7 times before the fibers get too short for further use as a structural component in the paper industry [37]. The remaining short fibers can be used as fillers or for energy production. The biogas potential from the pulp- and paper industry sludge is calculated as being 171 GWh/year in Sweden [30]. During the paper production process, the lignocellulosic raw material is physically and chemically treated, which can make the paper product easier to digest, compared with the original wood.

Lignocellulosic residuals can further be found in the food industry. For instance, about 110 million tons of citrus crops are produced per year [38], out of which about 33% of the oranges, mandarins, grapefruits, and lemons are used in the juice production industry [39]. During the production of juice, about 50-60% remains as waste, and the total waste production per year is estimated to be between 15 and 25 million tons [40].

Other lignocellulosic waste streams come from the municipalities. These include paper waste, such as newspaper, cardboard, and office paper, yard waste, such as grass and leaves [41]; and unused furniture [42]. Moreover, a large part of the municipal solid waste is food waste, where lignocelluloses can be found in peels, stems, and leaves, etc. from fruits, flowers, and vegetables. A study in the U.S. found that the lignocellulosic content of food waste was 55.4% cellulose, 7.2% hemicelluloses, and 11.4% lignin [43].

3. ANAEROBIC DIGESTION

The production of biogas from residuals is in many ways an optimum treatment. The gas can be used for electricity and heat production, or can be upgraded for use as vehicle fuel. Furthermore, the waste product from the anaerobic digestion of "clean" substrates, such as; manure, municipal solid waste, and plant residues can be used as a fertilizer on agricultural land [44]. In this way, nutrients that originate from the plants are recycled back to nature. The digestate residue used as a fertilizer has been found to give the same or improved crop production, compared with when commercial fertilizer is used [45].

Biogas can be produced from biological materials; today, it is mainly produced from: sewage sludge, the organic fraction of municipal solid waste (OFMSW), agricultural residues, energy crops, and industrial biological food waste [46].

3.1 The biogas market

In terms of numbers, the largest fraction of biogas reactors existing in the world today is household digesters. It is assumed that there are more than 30 million household digesters in China and 3.8 million in India, as well as 200,000 in Nepal and 60,000 in Bangladesh [47, 48]. In the African countries, the biogas technology is not yet developed; however, a few small scale digesters are in operation [49]. Farm scale digesters in Europe and America are larger in size, compared with the household digesters in the developing countries. In 2010, 162 farm scale digesters were in operation in the United States, and 17 in Canada. Germany had more than 4,000 farm scale anaerobic digesters in 2011, while there were 350 in Austria, 72 in Switzerland, 65 in the United Kingdom, 35 in Denmark and 12 in Sweden [50, 51].

In 2010, 126.5 TWh biogas was produced in Europe (Figure 3.1) [52]. Biogas produced from landfills is the main activity in the United Kingdom, Italy, France, and Spain, while co-digestion plants, municipal solid waste plants, and farm scale plants are the most common in Germany, the Netherlands, Czech Republic, Austria, Belgium, Denmark, Luxemburg, and many eastern European countries [52]. The total production of biogas from landfills was 26% in 2010, 10% from sewage

sludge, and the remaining 64% originated from co-digestion plants, municipal solid waste plants, and farm scale plants. The main use of the biogas produced in Europe is its utilization for the combined heat and electricity production. In 2010, the electricity production from biogas was 35.9 TWh. The upgrading to methane, however, is mainly performed on a small scale. In 2010, 177 plants were identified in Europe, out of which 128 injected the gas produced into the gas grid [52].



Figure 3.1 Biogas production per country in Europe 2010, expressed in Gwh/year [52].

Sweden had 233 biogas plants operating in 2011, which together produced 1,473 GWh of biogas [53]. The most common plants were wastewater treatment plants (WWTP); however, the co-digestion, farm scale, and industrial plants are evolving the most rapidly. The total production of biogas in Sweden can be seen in Figure 3.2. Landfills have been forbidden as a waste management treatment of organic waste in Sweden since 2002 [54]; however, gas is still collected from them. Sweden is the only

country in Europe with the highest fraction of biogas upgraded to biomethane [55]. In 2011, 50% of all gas produced was used in vehicles [53].



Figure 3.2 Total production of biogas from various origins, in Sweden 2011, adopted from Hellberg et al [53].

3.2 The biogas process

Biogas is formed naturally in different natural environments, such as swamps, the rumen of ruminants, rice fields, landfills, and other anaerobic environments. In anaerobic digestion, organic carbon is degraded in the absence of oxygen, into the most reduced state (methane) and the most oxidized state (carbon dioxide). Trace gases such as hydrogen sulfide, nitrogen, ammonia, and hydrogen are also formed in the same process [46].

The process can be divided into four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 3.3); in each individual phase, different groups of facultative or obligatory anaerobic microorganisms work together [56]. The microorganisms use their substrate for a source of energy as well as a carbon source for growth [44].



Figure 3.3 Sketch of the microbial anaerobic degradation steps [44].

3.2.1 Hydrolysis

Hydrolysis is the first step in the anaerobic digestion. During this phase, undissolved compounds, such as polysaccharides, proteins, and fats get degraded into their monomers, such as sugars, amino acids, and fatty acids [57]. This is performed by extracellular hydrolytic enzymes, which use water to cut the covalent bonds in the polymers. The hydrolytic enzymes include cellulases, hemicellulases, amylases, lipases, and proteases [57]. Many cellulose-degrading organisms have their enzymes in exoenzyme-complexes, called cellulosomes. These complexes are attached to the cellular wall and simultaneously they attach to the substrate for a more effective degradation [58]. The hydrolysis of complicated structures, like lignocelluloses, can require weeks, and the degradation is often not complete [56]. As such, the hydrolysis is the rate-limiting step, while the methanogenesis is considered the rate-limiting step for readily available substrates [44, 59].

3.2.2 Acidogenesis

In the second phase, the monomers produced in the hydrolysis phase are further degraded by fermentative bacteria into short-chain organic acids, with one to five carbons (valeric acid, butyric acid, propionic acid, acetic acid, and formic acid), alcohols, hydrogen, ammonia, and carbon dioxide. In a stable process, with low partial pressure of hydrogen, the main products formed by the fermentative bacteria are acetate, carbon dioxide, and hydrogen. When the partial pressure of hydrogen is high, more intermediates such as volatile fatty acids and alcohols are formed [44, 60].

3.2.3 Acetogenesis

Some of the degradation products from the acidogenesis phase can be directly used by the methanogens. However, the fatty acids longer than two carbon atoms, alcohols longer than one carbon atom, and branched chained and aromatic fatty acids are degraded further into acetic acid, hydrogen, and carbon dioxide in the acetogenic phase. The acetogenic microorganisms are obligatory H_2 producers, and for the degradation to proceed, thermodynamically, the acetogenic microorganisms need low partial hydrogen pressure [60]. Therefore, the acetogens are found in symbiotic relationship with hydrogen consuming methanogens, keeping the partial hydrogen pressure low enough for the growth of the acetogenic microorganisms. When the concentration of hydrogen is too high, butyric, caprionic, propionic, and valeric acids and ethanol concentrations are increased which in turn are toxic for the methanogens. In parallel with the formation of acetic acid from short chain organic acids, homoacetogenic microorganisms reduce hydrogen and carbon dioxide into acetic acid [44, 56].

3.2.4 Methanogenesis

The last step in the anaerobic digestion is the methanogenesis. The methanogenic microorganisms, work under strictly anaerobic conditions. The methanogens, which belongs to the group archaea, differ from the other organisms in the anaerobic reactor, which are bacteria. Archaea are more sensitive compared with the bacteria, regarding environmental stresses in the reactor, such as pH, or toxic compounds such as heavy metals or different toxic organic materials [61, 62].

The methanogens mainly use acetate, carbon dioxide, and hydrogen, but also methylamines, alcohols, and formate for the production of methane [62]. About 70% of the methane production arises from the acetate, and about 30% of the methane arises from hydrogen and carbon dioxide [56, 63]. The methanogens have the longest generation times (2-25 days) of the microorganisms in the reactor, which makes this step the most time-limiting step for easily hydrolyzed materials [44].

3.3 Microbial degradation of lignocelluloses

The microorganisms hydrolyze the cellulose by extracting extra-cellular enzymes. These extra-cellular cellulases hydrolyze the β -1,4-glycosidic bonds in the cellulose. Most microorganisms are unable to transport insoluble material such as cellulose through the cell membrane. Instead, the soluble sugars produced by the degradation of cellulose are transported into the cell and metabolized [9]. Endocellulases cleave along the cellulose chain randomly, exocellulases degrade the bonds at the non-reducing ends to produce cellobiose, while cellobiases or beta-glucosidases degrade cellobiose units into two different sugar molecules [58].

Cellulose can be degraded in nature by some aerobic microorganisms, the most studied is the fungi *Trichoderma reesei*, which has been used for industrial production and extraction of cellulases [9]. In the rumen and large intestine of herbivorous mammals, anaerobic bacteria degrade cellulose and hemicellulose. These microorganisms produce short chain fatty acids that are absorbed and used as energy by the mammals [64]. In the anaerobic digester, as well as in the sewage sludge or compost piles, the same group of microorganisms that perform cellulolytic and hemicellulolytic activities are present. The difference from the rumen is that the short chain fatty acids are further degraded by other groups of microorganisms into methane and carbon dioxide in the anaerobic digester.

The rate-limiting step of the degradation of cellulose is not the cleavage of the β -1,4-glycosidic bonds by cellulase, but the cleavage of the intra-chain hydrogen bonds. These intra-chain hydrogen bonds hold the crystalline structure together. The cleavage of the intra-chain linkages has to be performed prior to the attachment of the β -1,4-glycosidic bond cleaving enzymes [58, 65].

3.3.1 Cellulosomes

While aerobic microorganisms that degrade cellulose extract copious quantities of cellulolytic enzymes, the anaerobic microorganisms are more limited in the quantity of the production of enzymes. The anaerobic microorganisms gain much less energy per degraded sugar compared with the aerobic system, and therefore have a need for a more energy efficient system. Anaerobes have adopted an alternative strategy for the degradation of lignocelluloses, which is the organization of the enzymes in cellulosomes [58, 66, 67].



Figure 3.4 Cellulosome complex, adopted from Shoham et al [68] and reprinted with permission.

The cellulosome comprises both structural and enzymatic components. The cellulosome has a noncatalytic part, scaffolding, which enables various catalytic enzymes to attach to the cellulosome [67]. This is performed via the cohesion-dockerin system, where the scaffolding contains different cohesins and the catalytic enzymes (hemicellulases, pectinases, and cellulases) are attached to different dockerins (Figure 3.4) [66]. The cohesins and dockerins are complementary to each other. Moreover there are cellulose-binding domains in the enzymes, called carbohydrate-binding module (CBM) that are attached to the substrate. In addition, there are anchoring parts of the scaffolding that attach to the microorganism. This means that there is a close proximity of the microbial cell to the polysaccharide. The availability of many different enzymes, attached closely together in one cellulosome, offers an effective degradation of the recalcitrant lignocellulosic substrate. The organization of different cellulolytic enzymes into the cellulosome is regarded as the most efficient microbial cellulose degrading system studied so far [67]. Studies have found that the rate-limiting step is the separation of different cellulose chains from each other via the cleavage of the intra-chain hydrogen bonds. Once this is performed the cellulosome complex can interact with both the individual chains as well as with the remains of the crystalline cellulose [58].

3.4 Process parameters

The biogas process can be affected by different factors, for example, the type of feedstock that are used, as well as different operational variables, such as temperature, pH, alkalinity, organic loading rate, hydraulic retention time, and different existing inhibitors.

3.4.1 Substrate and nutrients

Biogas can be produced from different biological materials. Substrates used today in anaerobic digesters, include sewage sludge, waste water, the organic fraction of municipal solid waste (OFMSW), different industrial food streams, slaughter house waste, manure and energy crops [69]. In order to achieve a sufficient growth of the microorganisms and to enhance biogas production, an appropriate nutrient solution is needed. The microorganisms require an energy source for their activity, which in the biogas process are chemical compounds such as proteins, fats, or carbohydrates. Furthermore, they require an electron acceptor, mainly CO₂ for the anaerobic digester. The energy source is oxidized, while electrons/protons are transferred through different intermediates and in the end to the electron acceptor, where energy is produced [44]. The nutrients needed for growth are the macronutrients, such as carbon, nitrogen, hydrogen, phosphorus, potassium and sulfur [70], and micronutrients such as cobalt, copper, iron, molybdenum, nickel, selenium, tungsten, and zinc, as well as vitamins [70]. The type of the substrate directly determines the biogas yield, that is, the digestion of fats results in a higher methane yield compared with proteins and carbohydrates. However, the substrate is often measured in total volatile solids (VS) or in chemical oxygen demand (COD), which determines the fraction of organic material, and is easily performed in the lab environment.

Besides the organic content of the substrate, the carbon/nitrogen ratio is regarded as an important factor for the biogas process. The C/N ratio should be between 10 and 30, and with an optimum ratio of between 25 and 30 [62, 71] for the digester to work at its full potential. At lower C/N ratios there is a risk of ammonia inhibition, the methanogens being the most sensitive ones. Consequently, this can lead to an accumulation of volatile fatty acids leading to decreased pH and a reactor failure. Higher ratios may lead to lower methane yields as there will be a deficiency of nitrogen available for the cell growth [72].

Various enzymes in the anaerobic digester use metal ions for their function. Therefore, several different trace metals are needed. The addition of selected trace metals allows for the possibility to stimulate the process and thereby increase the organic loading rate, depending on which substrate is used [73, 74]. A complex and varied substrate source is preferable for a better nutritional balance

within the process. The composition, however, of substrates should not vary over time to a great extent, since the microorganisms need time to adapt to the new environment [44].

3.4.2 Temperature

The different microorganisms have different temperature intervals, where their growth is optimal. Three temperature ranges are defined, i.e., the psycrophilic range, where the growth optima is around 10°C, the mesophilic range with an optima at around 37°C; and the thermophilic range with an optimum at above 50°C [75]. Large scale anaerobic digesters in Europe are run at mesophilic or thermophilic conditions, while psycrophilic temperatures can be used for small scale digesters without heating [44]. At thermophilic temperatures compared with mesophilic, the microorganisms are known to have a 25-50% higher activity, resulting in higher methane productivity. However, the microorganisms at thermophilic conditions are more sensitive to disturbances in temperature or to different toxic compounds [76]. On the other hand, mesophilic systems are more robust but with a lower reaction rate [44]. Generally, there are fewer organisms in the thermophilic temperature compared with the mesophilic [77], which probably means that the diversity of mesophilic microorganisms can help to stabilize the process.

3.4.3 pH and alkalinity

The microorganisms in the anaerobic digester are sensitive to the pH and have different pH optima. The methanogens have a pH optima of between 6.5-8.0, while the acetogens work with a pH between 5.0-8.5. Anaerobic digesters are preferably run at a pH range of 7.0-8.5 [78]; outside this neutral range the process can face process imbalances [44].

To keep a neutral and stable pH in the reactor, it is important to have a high and stable alkalinity. Alkalinity is a measure of the amount of basic compounds in the reactor. The higher the alkalinity, the higher the buffer capacity and the more possible it is to achieve stable pH. The alkalinity is based mainly on carbonate (CO_3^{2-}) in equilibrium with dissolved CO_2 . However, substrates rich in proteins liberate ammonia when degraded, which also contributes to the alkalinity [44, 56].

3.4.4 Organic loading rate and hydraulic retention time

The amount of substrate added per reactor volume and time is defined as the organic loading rate (OLR). Processes in their start-up periods need a lower OLR, while mature and well functioning processes can handle a higher OLR. Thermophilic processes can handle 4-5 kg VS/m³/day, while

mesophilic processes normally work at 2-3 kg VS/m³/day [44]. An overloading of the system, where easily degradable substrates are added at too high ORL, often results in an inhibition caused by VFA accumulation [79]. Hydraulic retention time (HRT) is defined as the time it takes to change all the material in the reactor. Normally the loading rate is higher than the conversion of substrate into methane and carbon dioxide, which means that not all the material is degraded. The mass of substrate is higher than the mass of residues, since a part of the substrate has gasified. The residues are made of the organic material that is not degraded, but also inorganic and inert material, as well as biomass from the microorganisms and salts and water. The HRT of anaerobic digesters is often 10-25 days or longer [44]. Slowly degraded materials, like cellulose-containing materials, often need a longer HRT than easily degraded materials such as dissolved sugars. Generally, a longer HRT is needed when the ORL is high, in order to avoid a too low degradation.

3.4.5 Toxic/inhibiting compounds

There are many compounds that could act as inhibitors in the anaerobic digester. In general methanogens are regarded as the most sensitive group among the microorganisms present in the digester. The toxic compounds can originate in the substrate or arise as a product from one of the degradation steps in the digester. The microorganisms can, in some cases, adapt to the system where the microorganisms that are tolerant to the toxic compounds grow, and in later phases of the digester, they can dominate in the reactor [44].

The most common inhibitor for the anaerobic process is ammonia. The ammonia concentration originates from the degradation of proteins or urea, or by the soluble ammonia in the influent. The non-ionized form of ammonia is regarded to be the most toxic form, since it can diffuse through the cell wall and cause a proton imbalance as well as potassium deficiency [61]. The solubility and thereby, the toxicity of ammonia is influenced by pH and temperature. The level of toxic concentration further depends on the buffer capacity of the system and on the adaptation of the microorganisms [72, 80].

An inhibition of ammonia on the methanogens leads to the accumulation of volatile fatty acids (VFA). VFAs are produced by the acetogens and consumed by the methanogens, and if the inhibition of methanogens occurs, there would be a buildup of VFAs. The accumulation of VFA leads to a pH drop wherein the whole digester could stop working [80]. Volatile fatty acids can be both in dissociated (H+ and R-) and in undissociated form. This is dependent on the pH, where the higher concentrations of undissociated VFAs are found at a lower pH. The undissociated form of VFAs has an inhibiting effect, since they are able to diffuse through the cell wall [69]. The accumulation of VFA can function as a process indicator of the performance of the anaerobic digester [81, 82]. A study of a nitrogen-rich

substrate digested in continuous reactors, at low hydraulic retention times, was performed in this work (Paper IV), and an accumulation of volatile fatty acids was found. This was followed by an inhibition and reactor failure. The inhibition in this case is thought to be an effect of a too low C/N ratio, combined with the presence of the inhibitory compound limonene [83] in the substrate and a higher stress on the system as a result of the low HRT applied. In order to avoid ammonia inhibition, carbon-rich waste streams should be added to the process in order to reach a good C/N ratio. A higher C/N ratio can stabilize the process and thus, avoid ammonia inhibition [80]. This effect can be found in Paper IV, where the addition of paper tube residuals increased the C/N ratio and avoided the reactor breakdown.

Other inhibiting compounds are: cations at high concentrations – such as Na^{2+} , K^+ , Ca^{2+} , and Mg^{2+} [84], alternative electron acceptors such as SO_4^{2-} and NO_3^{-} , phenolic compounds, cyanides, heavy metals, detergents, hydrogen sulphides, long chained fatty acids, antibiotics, etc [61].

3.5 Methods for determining the biogas potential

The biogas potential is often defined as the volume biogas production per gram VS substrate. This potential can be determined by different methods, including both theoretical as well as experimental methods.

3.5.1 Theoretical methods

The theoretical or stoichiometric production of methane and carbon dioxide in anaerobic digestion can be calculated according to Buswell's formula [85]. In this case, the elementary composition of the substrate must be known, and the production of methane and carbon dioxide can be calculated by using the following formula [85]:

$$C_a H_b O_c + \left(a - \frac{b}{4} - \frac{c}{2}\right) H_2 O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4}\right) C H_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4}\right) C O_2$$

This calculation is performed with the assumption that all energy goes to the production of methane and that the energy consumed for the growth of the microorganism can be neglected.

Furthermore, if the elemental composition of the substrate is unknown, a component analysis can be used to predict the methane potential of a substrate. This presumes that the concentration of carbohydrates, proteins, and fats are known. In Table 3.1, a theoretical composition estimation of the

above-mentioned components can be found, as well as the calculated theoretical yield from the respective groups according to Buswell's formula [86].

Table 3.1 Theoretical methane	potential according	to Buswell	's formula
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	Chemical formula	NmL CH ₄ /gVS
Carbohydrates	$(C_6H_{10}O_5)_n$	415
Fats	$C_{57}H_{104}O_6$	1014
Proteins ¹	$C_5H_7NO_2$	496

¹The nitrogen is converted into ammonia, according to Angelidaki et al [86].

3.5.2 Experimental methods

The theoretical calculated methane potential is often higher than the measured produced methane. The biodegradability as well as the eventual production of inhibitors can in many cases limit the potential degradation. In order to get a more realistic picture of the biogas production from a specific substrate, methods based on experiments should be used. These biodegradation tests can be performed in small scale batch experiments up to pilot scale continuous reactors. In the present study, two different lab scale reactors were used. Biomethane potential (BMP) tests were used to determine the methane potential in a batch process (Papers I, II, and IV), and semi-continuous stirred tank reactors (Paper IV) were used to evaluate the long-term effects of the co-digestion processes.

Biomethane potential test

The biomethane potential test (BMP) is easy to perform and can be used both for the methane potential analysis and for kinetic measurements. A certain amount of organic material is added together with an inoculum from an anaerobic digester, in an anaerobic environment, in small-scale batch flasks. The flasks are put in a thermostat and are left for up to 50 days in order to let the organic material be totally degraded by the microorganisms. During the incubation period the methane and carbon dioxide production are measured by taking samples with at specific time intervals, which allows for the determination of the degradation rate as well as studies of different phases in the degradation process. The accumulated final methane production is regarded as the methane potential of the specific substrate.

The BMP test has the advantage that it can be performed on a small-scale and that many tests can be performed in parallel. This enables the method to be used in the comparison of different materials with each other. However, the method has some limitations, specifically; the test is performed on a small-scale and in addition, the accumulation of inhibiting compounds cannot be studied. The method

determines the total accumulated methane production from when the material is left in the reactor until it is fully degraded.

The method for the BMP tests performed in Papers I, II, and IV is as suggested by Hansen et al [87]. Glass bottles of 118 mL or 2.1 L were used (Figure 3.5), sealed with a rubber septum. The VS-loading was 0.5 to 1%, with the ratio of VS-inoculum /VS-substrate at 1 to 2. The inoculum was taken from an industrial scale anaerobic digester. The reactors were flushed with a gas mixture of 80% nitrogen and 20% carbon dioxide in order to enable the anaerobic environment, and the reactors were left in thermophilic conditions at 55°C. The produced gas was measured regularly by a pressure tight gas syringe in order to determine the gas composition and the produced volume. The concentration of methane and carbon dioxide was analyzed by a gas chromatograph.



Figure 3.5 BMP test reactors, adopted from Angelidaki et al [88], and reprinted with permission.

Semi-continuous CSTR tests

To study the long-term effects of an anaerobic digestion process, semi-continuous stirred tank reactors (CSTRs) can be used. With the co-digestion of different substrate streams, the effects of mixing different substrates can be studied, as well as the effect of different operation parameters and the possible accumulation of different toxic compounds. The CSTR offers a bridge between the BMP test and the full scale industrial plant. CSTR has the advantage of being practical and simple to operate

[89], and they have been successfully applied to municipal solid waste, manure, fruit, and vegetable waste [72, 90, 91]. The reactors used in Paper IV were CSTR-reactors of 10 L, which were fed once a day. They were run at anaerobic thermophilic conditions (55°C). During these experiments, the effect of the addition of treated and untreated paper tube residuals in a co-digestion, with different substrate mixtures obtained from an industrial scale anaerobic digester, was studied. A hydraulic retention time of 20-25 days was used with organic loading rates (ORL) from 1.3 to 2.0 g VS/L/day. The reactors were run for 3 HRT after a stable process was reached, and analyses were performed on gas production and composition, as well as on VFA, ammonia, and pH levels in the effluent. The configurations of these reactors are presented in Figure 3.6.



Figure 3.6 CSTR reactors used for the semi-continuous lab experiments.

3.6 Co-digestion

Continuous co-digestion experiments were performed in this study to provide information on the longterm effects, such as eventual inhibition during the digestion of a lignocellulosic substrate. Furthermore, co-digestion is often beneficial, since it supplies the system with more nutrients, leading to a better balance in the C/N ratio and the pH; furthermore, it results in the dilution of the concentration of eventual inhibitors [44, 90]. The co-digestion is often used in industrial anaerobic digesters, in order to improve the stability of the process and increase the economic value [44]. Moreover, co-digestion has the possibility of maximizing the methane production due to positive synergistic effects [72, 91, 92], which can further increase the economic value.

3.6.1 Co-digestion of buffer tank substrate and paper tubes

Co-digestion of paper tube residuals (UP) together with a nitrogen rich-buffer tank substrate (BTS), used in an industrial scale co-digestion plant, has been performed in the present study (Paper IV). In addition to the high nitrogen content, the BTS had a high content of limonene, due to the high presence of citrus peel in the mixture. The anaerobic digestion was performed in continuously stirred tank reactors with a working volume of 5L, and the reactors were run for 3 HRTs after a stable process was reached. The organic loading rate was 1.5 gVS/L/day from BTS and 0.5 gVS/L/day from paper tubes, with a VS ratio of 3:1; the BTS over paper tubes for operation set 1. The loading rate for operation set 2 was 1.3 gVS/L/day from BTS, and 0.7 gVS/L/day from paper tubes, and with a VS ratio of 2:1. The hydraulic retention time was decreased from 25 days for operational set 1 to 20 days for operational set 2.





The addition of paper tubes to the unstable BTS mixture increased the methane production from 354 NmL CH_4/g VS for BTS only (Figure 3.7) to 444 NmL CH_4/g VS for BTS digested together with untreated paper tubes (UP). At lower hydraulic retention times and higher stress (operational set 2 compared with set 1), the reactor with BTS only collapsed, while the reactor with added paper tube

residuals reached 482 NmL CH_4/g VS. The addition of paper tube residuals prevented the accumulation of volatile fatty acids, which are toxic to the methanogens [80], and avoided the subsequent reactor failure (Paper IV).

Besides the stabilizing effect of the addition of paper tubes, synergistic effects could be found in the co-digestion of BTS and paper tubes. The co-digestion of untreated paper tubes together with BTS had a 33% increase in methane production compared with the estimated theoretical methane yield (ETMY), calculated from the methane potentials obtained when BTS and UP were digested one by one (Paper IV). The C/N ratio increased after the addition of paper tubes for all reactors, from a C/N ratio of about 6 for BTS to a ratio of 8 to 10, depending on the mixing proportion in the different co-digestion processes, which are close to the optimum [93]. Both the stabilizing and synergistic effects attained during the co-digestion of paper tubes with BTS, makes paper tubes an interesting substrate in industrial co-digestion processes.

4. LIGNOCELLULOSES: PRETREATMENT AND DIGESTION

The complex structure of the lignocelluloses, with the high crystallinity and lignin content, makes the material difficult to digest naturally for the microorganisms in the anaerobic digester. This slow or incomplete digestion results in low biogas yield. In order to decrease the biomass recalcitrance, and thereby increase the biogas yield, different pretreatment methods can be used [14]. This is important for a financially viable process, where otherwise economically impossible processes can be turned into economically favorable [94]. Pretreatments can have effects on the physicochemical properties of a substrate, such as molecular size, cellulose crystallinity, surface accessibility, pore size distribution and particle sizes [14]. Some pretreatments have an impact on the chemical composition of the substrate, where lignin and/or hemicelluloses, to some extent, get soluble, while others have no effect on the chemical composition of the substrate [95].

4.1 Pretreatment methods

Pretreatment methods can be divided into physical, thermal, chemical (with acids, alkalis or organic solvents), and biological methods (enzymatic or microbial) [96]. The topic of pretreatment on lignocelluloses has been studied substantially, especially focusing on pretreatment prior to ethanol production. In many ways the pretreatments of lignocelluloses, prior to anaerobic digestion, have the same objectives compared to pretreatments prior to ethanol production, with the exceptions that the pretreatments can be less extensive since the anaerobic microorganisms are, to some extent, able to degrade both crystalline cellulose structures as well as hemicelluloses by themselves. The degradation of the lignin-polysaccharide linkages, together with a general opening of the structure are two of the main goals of the pretreatments prior to anaerobic digestion [14].

From a broad perspective, the requirements of the pretreatment are to: (1) improve the accessibility of the enzymes to the cellulose and hemicelluloses and thereby the degradability, (2) avoid degradation or loss of the carbohydrates, (3) avoid the production of potential inhibitors, (4) be cost and energy

efficient, and (5) have as low impact as possible on the environment [94]. Several reviews of different pretreatment methods have been published previously [14, 94-97].

4.1.1 Physical pretreatment

Size reduction

The idea behind size reduction is to reduce the particle size of the substrate [14]. This results in a larger specific surface area of the biomass as well as a decreased degree of polymerization [98]. Most pretreatments require a size reduction of the collected feedstock to a different extent, prior to the subsequent pretreatment [95]. With some, more easily degraded lignocellulosic-rich material, the size reduction or milling can function as the only pretreatment method. The size reduction of rice straw has been shown to increase the methane production; however the best methane results were obtained with a combination of milling and other pretreatment methods [99]. Milling with different municipal solid waste fractions has been successful with increasing methane yields by 5-25% [98, 100]. Milled spruce was found to give six times higher methane yield compared with spruce chips (Paper II), whereas the best pretreatment with N-methylmorpholine-N-oxide (NMMO) resulted in a 200% higher methane yield when the spruce was milled compared with spruce chips. The drawback of extensive milling methods, however, is the high energy consumption [25] and therefore, the high cost of the method, which makes it inappropriate in many cases [95].

Ultrasound

Ultrasound is a mechanical method that disintegrates and destructs the biomass, for instance, sludge particle from waste-water treatment. The efficiency of the ultrasound pretreatment is affected by the frequency, the time, the energy level, and the characteristics of the sludge. The treatment acts so that the microbial cell structure is disrupted and the internal cellular material is extracted from the cells. Ultrasonication is used at full-scale sewage sludge plants, where an increase of 50% in biogas production has been found [101]. A combined pretreatment of alkaline and ultrasound on thickened pulp mill waste activated sludge did not improve the accumulated methane yield; however, the initial digestion rate was substantially improved [102].

Irradiation

In irradiation, high-energy electron beams are used as a pretreatment method, such as gamma rays or microwave energy. The methods can disrupt the cell wall, decrease the cellulosic crystallinity, and

increase the accessible surface area [94, 95]. Cell lysis of sewage sludge as a result of irradiation pretreatment has resulted in a biogas production increase of 22% [103].

4.1.2 Chemical pretreatments

Acidic pretreatments

Acidic pretreatments are among the most studied pretreatment methods for lignocelluloses. Dilute or strong acid pretreatments with, for example sulphuric acid, hydrochloric acid, or nitric acid have been performed at elevated temperatures. The combination with other methods, such as steam explosion has been performed [104]. At stronger acid concentrations, both lignin and hemicelluses get solubilized, however, the recovery of acids is required. At dilute acid concentrations, lignin is not solubilized but redistributed, on the other hand, neutralization prior to anaerobic digestion is substantial in order to achieve the targeted neutral pH [14, 16, 105]. Studies have been performed on newspaper, which was treated with nitric and acetic acid, and resulted in 80% lignin removal [16] and rice straw has been pretreated with acetic and propionic acid which enhanced the methane production by 36% compared with that of untreated rice straw [106].

Alkaline pretreatments

Alkaline pretreatments are effective in the removal of lignin, while still maintaining the cellulose concentration at a high level. Pretreatment with alkalis can cause swelling of the fibers which results in a bigger accessible surface area: it can decrease the crystallinity and degrade the linkages between lignin and carbohydrates as well as cause a disruption of the lignin structure [6, 95, 107, 108]. Alkaline pretreatment studies have been performed on wheat straw which resulted in an increase of the methane production by 100% [109]. Newspaper has been treated with alkaline subcritical water, resulting in a methane increase of 36-45% [110]. Rice straw has been pretreated with sodium hydroxide 4-10%, with a following methane increase of 3-58% [111], and with a combination of 4% sodium hydroxide and a hydrothermal pretreatment the methane production increased with up to 112% [112]. The methane production from municipal solid waste after pretreatment with calcium hydroxide improved by 172% [113]. The cost of the catalyst is dependent on the amount used in the pretreatment, as well as on the purchase price where, for example, lime is cheaper than sodium hydroxide, together with the cost of recovery and reuse [95].

Oxidative pretreatments

In oxidative pretreatment, an oxidizing compound such as hydrogen peroxide or peracetic acid is suspended in water and added to the biomass. The objectives are a partial degradation of hemicelluloses and a delignification of the biomass [14]. In the wet oxidation method, there is an addition of O_2 into a pretreatment reactor at temperatures up to 200°C and pressures up to 1.5MPa

[95]. A previous research study demonstrated that the addition of hydrogen peroxide was most effective at a pH above 10; no delignification was found below this pH [114]. This alkaline peroxide method was also successfully performed on wheat straw [115]. In this study, a combined pretreatment of thermal or steam explosion, sodium hydroxide, and hydrogen peroxide was performed as presented in Paper I and as it is mentioned in section 4.1.3 and 4.1.4.

4.1.3 Thermal pretreatments

Liquid hot water has been used as a pretreatment method on lignocelluloses in the pulp industries for several decades [94]. Thermal pretreatments, both without pressure and under high pressure, have shown to solubilize both hemicellulose and lignin [94, 116]. A major advantage is that in strict thermal pretreatments, there is no need for the addition of chemicals [94]. A drawback with pretreatments at high temperatures, however, is the possible production of phenolic compounds as well as furfural and hydroxymethylfurfural (HMF), which are degradation products of lignin as well as sugars, respectively [14, 117]. The phenolic compound can be toxic to the microorganisms in the anaerobic digester, depending on the concentration [118], while they can tolerate furfural and HMF to a higher extent. It was reported that hydrothermal pretreatment of rice straw resulted in 222% higher methane yield in a pretreatment study [112].

Thermal pretreatments were performed in the present study, combined with the addition of sodium hydroxide and hydrogen peroxide in order to improve the anaerobic digestibility of paper tube residues (Paper I). The addition of sodium hydroxide has previously proved to have good lignin removal properties while still maintaining the cellulose concentration [94]. Furthermore, the addition of hydrogen peroxide at a high pH, i.e., around 11.5, has been showed to dissolve half of the lignin and most of the hemicellulose [114]. Therefore, the pretreatments in this study were performed with the addition of sodium hydroxide as well as hydrogen peroxide in the concentrations of 0-2% using sealed 150 ml stainless steel tubular reactors, heated in an oil bath at 190-220°C, for 30 min.


Figure 4.1 Accumulated methane productions from thermal pretreatments, expressed in NmL/gVS. All analyses were performed in triplicates, and the standard deviations can be found in Paper I.

The results showed that the methane production could be improved by 21% (269 Nml/gVS) compared with that of untreated paper tubes (222 Nml/gVS), after the thermal pretreatment with 2% NaOH added at 190°C (Figure 4.1). Thermal pretreatments at the higher temperature, 220°C, indicated inhibition generated by different compounds revealed at this higher temperature, while pretreatment at 190°C with 2% H₂O₂ had no effect compared with the untreated material (Paper I).

4.1.4 Rapid decompression pretreatments

Steam explosion

In steam explosion, chipped or coarsely shredded lignocellulosic feedstocks are in contact with highpressure steam in a pressure vessel. The residence time can vary between 30 s to 30 min, at a temperature of between 120-260°C, and pressure of between 5-20 bar. At the end of the pretreatment time, the material is released into a flash tank, with atmospheric pressure, which causes an instant decompression and deconstruction of the feedstock (Figure 4.2). The release of organic acids during the steam explosion process, which originates from the acetyl groups of the hemicelluloses, can act as a catalyst during the process. These acids catalyze different hydrolysis reactions in hemicellulose and lignin, which get partly degraded or solubilized [95]. The addition of acids or alkalis to the steam explosion process can further improve the pretreatment process. A steam explosion pretreatment study on wheat straw resulted in 20% higher methane yield compared with untreated wheat straw [119]. Biofibers from digested manure reached 67% increase in methane production compared with untreated, after steam explosion [120]. On bulrush, a methane yield of 205 mL/g degradable VS was reached after steam explosion pretreatment [121], and on salix 240 mL/g VS [122]. Steam explosion requires less energy input compared with milling for the same size reduction; furthermore, the method has been regarded as one of the most cost effective [96].

The steam explosion on paper tubes prior to anaerobic digestion has been investigated in the present study and the results are presented in Papers I and IV. The steam explosion unit used was a 10L reaction chamber with high pressure steam (60 bar) provided by a power plant, entering together with the feed into the unit. After the pretreatment was carried out, the biomass was flashed into an atmospheric pressure expansion tank. The pretreatments in the present study were performed between 190-220°C, for 10-30 min with or without the addition of sodium hydroxide and hydrogen peroxide.



Figure 4.2 Schematic figure of the steam explosion unit, adopted from Forgács [38].



Figures 4.3A and 4.3B Accumulated methane production from steam explosion pretreated paper tubes, expressed in NmL/gVS. All analyses were performed in triplicates, and the standard deviations can be found in Paper I.

The results of the accumulated methane productions obtained during the following anaerobic batch digestion assays can be found in Figures 4.3A and 4.3B. The treatments with the addition of 2% NaOH at 190°C for 10 or 30 minutes increased the methane production with 70% (403 NmL/gVS or 405 NmL/gVS, respectively), compared with that of the untreated paper tubes, which produced 238 NmL/gVS. The treatment at 220°C, with addition of both 2% NaOH and 2% H₂O₂ for 10 min, resulted in the highest methane volume of 493 NmL/gVS, 107% higher production compared with that of the untreated feedstock. The steam explosion pretreatments without the addition of NaOH had no effect, or negatively affected the methane production from the paper tube residuals (Figure 4.3B). Analysis of variance (ANOVA) was performed on the steam explosion pretreatment, with 5% significance value, and showed that the effect of the addition of NaOH was a significant factor for the enhancement in methane production obtained after the treatment (p-value 0.003).

Additional steam explosion pretreatments were performed in the present study in order to study the effects of lower concentrations of NaOH added in the pretreatment process. They were all performed on the same paper tubes as raw material, at 190°C, for 10 min, and the effects were evaluated by batch digestion assays (Figure 4.4). The accumulated methane yields obtained after treatments with NaOH in the range of 0-2% demonstrates the trend of higher NaOH concentration resulting in higher methane yields. This was verified by one-way ANOVA analysis, defining the concentration level of NaOH as a significant factor between 0 and 2% (p-value 0.048, with 5% significance level), affecting the accumulated methane yields (Paper IV).



Figure 4.4 Accumulated methane productions after steam explosion with low NaOH concentrations. All analyses were performed in triplicates, and the standard deviations can be found in Paper IV.

A co-digestion study of steam exploded paper tube residuals together with a nitrogen rich buffer tank substrate (BTS) is presented in Paper IV. The BTS used in the present pretreatment study is more stable compared with the BTS used in the study presented in section 3.6.1. The anaerobic digestion was performed in continuously stirred tank reactors with a working volume of 5L, with a HRT of 25 days and a VS ratio of 3:1, BTS over paper tubes. The reactors were run for 3 HRTs after a stable process condition was reached. The organic loading rates were 1.3 g VS/L/day of BTS and 0.4 g VS/L/day of pretreated or untreated paper tubes.

A co-digestion study of steam exploded paper tube residuals together with a nitrogen rich buffer tank substrate (BTS) is presented in Paper IV. The BTS used in the present pretreatment study is more stable compared with the BTS used in the study presented in section 3.6.1. The anaerobic digestion was performed in continuously stirred tank reactors with a working volume of 5L, with a HRT of 25 days and a VS ratio of 3:1, BTS over paper tubes. The reactors were run for 3 HRTs after a stable

process condition was reached. The organic loading rates were 1.3 g VS/L/day of BTS and 0.4 g VS/L/day of pretreated or untreated paper tubes.

The addition of the paper tubes in the co-digestion of BTS increased the methane production from 470 NmL CH_4/g VS for BTS only, to 498 NmL CH_4/g VS when BTS was co-digested with untreated paper tubes, and furthermore to 521 NmL CH_4/g VS when BTS was co-digested with steam exploded paper tubes at 190°C, for 10 min, with 2% NaOH (Figure 4.5). Synergistic effects were found, since the methane production of the co-digestion per gram volatile solid was higher than the estimated theoretical yield (ETMY), calculated from the yields obtained when digesting BTS and untreated paper tubes one by one. The co-digestion of untreated paper tubes together with BTS had a 19% increase in methane production compared with the ETMY, while the co-digestion of pretreated paper tubes together with BTS had a 15% increase in the methane production compared with the ETMY (Paper IV).



Figure 4.5 Accumulated methane production expressed in normal milliliter per gram volatile solids, from the co-digestion of BTS and untreated paper tubes (UP) as well as co-digestion with pretreated paper tubes (PP).

Ammonia fiber expansion (AFEX)

The AFEX process is similar to the steam explosion process, where the feedstock is in contact with anhydrous liquid ammonia at temperatures of 60-100°C, and pressures of 10-20 bars with a residence

time of around 5-30 minutes. After the pretreatment, the pressure is released, causing a deconstruction of the biomass. This causes swelling and physical deconstruction of the material, and partial solubilization of the lignin [95]. The carbohydrate content remains intact after the AFEX process [94]. AFEX has been successfully performed on various lignocelluloses with low lignin content: however, the process was not as effective on lignocelluloses with a high lignin content [96]. The complexity and cost of the recovery of the ammonia after the pretreatment are drawbacks of the AFEX pretreatment method [95].

4.1.5 Organic solvent pretreatments

Organic solvent processes are used for the disruption of the intra-molecular bonds in order to facilitate the enzymatic degradation of the lignocelluloses. The solvent must be recovered and reused to a large extent, which can be performed in different extraction and filtration processes, in addition, no inhibitors should be left together with the feedstock in order to avoid inhibition in the anaerobic digestion process. The cost of the process depends on the recovering technique, for example evaporation and condensation, as well as on the price of the solvent [94, 95]. The pretreatment with the organic solvent NMMO (N-methylmorpholine-N-oxide) has been successful in the pretreatment of lignocelluloses where it can improve the methane yields substantially (Paper II). This solvent degrades the intra-molecular hydrogen linkages as well as van der Waals linkages, which opens up the lignocellulosic structure and decreases its crystallinity [123]. NMMO pretreatments are run at relatively low temperatures, and the solvent can be recovered by more than 98% without chemical derivatization and with no production of toxic waste pollutants. NMMO is used commercially as a solvent in the fiber making industry known as the Lyocell process [124, 125]. NMMO pretreatment has been previously performed on the straw fraction of cattle and horse manure with the following increased methane yields of 53 and 51%, respectively [126], as well as on high-crystalline cellulose [127] and on oil palm empty fruit bunch [128].

The use of the organic solvent N-methylmorpholine N-oxide (NMMO) as a pretreatment method has been performed in this study on spruce (both milled and as chips), rice straw, and triticale straw, and the results are presented in Paper II. All three feedstocks are lignin-rich lignocelluloses, which the microorganisms have difficulty in degrading during the anaerobic digestion process. The pretreatments were performed in 85% NMMO-water mixture, at 130°C, for 1, 3, or 15 h. The digestions of untreated chips (10 mm) and milled (< 1 mm) spruce, rice straw, and triticale straw resulted in 17, 106, 45, and 53 Nml CH₄/g carbohydrates, respectively, as an accumulated methane yield (Figures 4.6 and 4.7). However, the pretreatments have improved these methane yields by up to 400-1,200% depending on the substrate treated and the treatment conditions. The best digestion results of the pretreated chips and milled spruce, rice straw, and triticale straw were 202, 395, 328, and 362 Nml CH₄/g carbohydrates,

respectively, which correspond to 49, 95, 79, and 87% of the theoretical yield of 415 Nml CH_4/g carbohydrates [86]. Rice straw, however, showed an inhibition pattern, where longer treatments resulted in lower methane yields together with lower initial digestion rates (Paper II).



Figure 4.6 Accumulated methane productions after six weeks of anaerobic digestion for spruce, both milled and as chips. All values are expressed as normalized ml methane per gram carbohydrate (CH). The analyses were performed in triplicates, and the standard deviations can be found in Paper III.



Figure 4.7 Accumulated methane productions after six weeks of anaerobic digestion for rice and triticale straw. All values are expressed as normalized ml methane per gram carbohydrate (CH). The analyses were performed in triplicates, and the standard deviations can be found in Paper III.

4.1.6 Biological pretreatments

Fungi

Biological pretreatments have mainly been performed using lignin degrading fungi. A group of basidiomycetes, called white-rot fungi are known to initially degrade lignin, while leaving most of the hemicellulose and cellulose unchanged. These fungi excrete ligninolytic enzymes, such as lignin peroxidase, manganese peroxidase, and laccase [129]. The biomass is inoculated at room temperature together with the fungi and is left for several weeks during pretreatment. The advantages of these kinds of biological pretreatment methods include low energy consumption, and not needing any chemicals. There are, however, drawbacks, such as that some of the cellulose and hemicelluloses is degraded together with the lignin, during the long incubation time [129]. Rice straw was pretreated with white-rot as well as brown-rot fungi and the methane production increased by 46 and 31%, respectively, after the treatment [130]. Phenolic compounds were removed in another study on olive mill wastewater by white-rot fungus, with improved anaerobic digestion as a result [131].

Enzymes

Only a few studies have been performed with the use of ligninolytic enzymes as a pretreatment method prior to anaerobic digestion. Laccase, manganese peroxidase, and lignin peroxidase, extracted from white-rot fungi have been used. These ligninolytic enzymes are too big in size in order to work directly on the lignin; instead, they employ a low molecular weight reactive compound, termed mediators, which attack and degrade the lignin [42]. The pretreatment of laccase, together with steam explosion had a positive effect on the following anaerobic digestion of digested biofibers [132]. However, no improvement was found when the biofibers were pretreated with laccase exclusively.

4.2 Pretreatment and characterization of lignocelluloses

In order to understand the changes in the substrate after pretreatment, substrate characteristic analysis can be performed. The alterations in the lignocellulose after pretreatment will affect the subsequent anaerobic digestion, and studying these modifications by different characterization methods could predict the outcome of a biomethane potential test.

As previously stated in section 2.9, the pretreatment of lignocelluloses affects the chemical composition, such as degradation of lignin, hemicelluloses and intra-linkage bonds. There are, however, many physical changes in the substrates, which cannot be directly correlated to the chemical composition. The affected physical properties are altered surface area, decrease in crystallinity, and change in pore size distribution. These attributes of the pretreated lignocelluloses affect the interaction with the hydrolyzing enzymes and enzymatic complexes [133], and are important in the understanding of the lignocellulosic structural changes after pretreatment.

Studies have previously been performed in order to correlate substrate characteristic changes and free enzyme systems [133-135] after pretreatment. Increased surface area [135, 136] and decreased crystallinity have been studied. Compared with investigations with the free enzyme systems, only a few studies have been performed prior to anaerobic digestion. The specific surface area was measured after milling as a pretreatment prior to biogas production on different organic materials [137]. In the present study, five different substrate characterization analyses were performed (Paper III), which are described in the following subchapters. Each of these analyses indicates that the accessible surface area of the straw material studied was increased as a result of the pretreatment.

4.2.1 Simons' Stain

Simons' Stain is a method that measures the pore size of the substrate by applying two different dyes: direct orange and direct blue. The two dyes have different particle sizes and different cellulosic affinity. The blue dye has a well-defined chemical formula and a diameter of 1 nm, while the orange dye has molecules with diameters of 5 to 36 nm [138]. This can be compared to the size of a typical bacterial cellulase of approximately 4 to 16 nm [137], which means that the enzyme can penetrate the substrate at pores where the orange dye can. Moreover, the orange dye has a higher affinity for the cellulosic hydroxyl groups than the blue dye [138, 139]. Different amounts of the orange and blue dye can be absorbed into the substrate, depending on the pore sizes of the substrates. The small pores are populated with the blue dye and the large pores with the orange dye. The increased adsorbed orange dye after the pretreatment indicates an increase in the biodegradability of the substrate, which in turn will result in an increased biogas production. A good correlation has previously been found between the improved enzymatic hydrolysis of free enzymatic systems and increased surface area [134]. This actual study is the first one correlating results from Simons' Stain analysis and biogas production (Paper III).

Rice and triticale straw, pretreated with NMMO as well as paper tube residuals pretreated with steam explosion together with the addition of 0-2% NaOH, were analyzed with Simons' Stain (Figure 4.8). Increased adsorption of both total and orange dye, suggested increased surface area after longer and stronger pretreatments. The same trend could be obtained during the BMP tests (Papers II and IV), where stronger pretreatments, resulted in increased methane productions.



Figure 4.8 Adsorbed dye, expressed as mg dye/g material, from Simons' Stain analysis on triticale (Trit) and rice (Rice) straw, untreated and after NMMO pretreatment for 1 to 15 h, as well as on paper tube residuals (Paper), untreated and after steam explosion treatment with the addition of 0-2% NaOH. The gray dotted bars show the orange dye adsorption and the black bars show the blue dye adsorption.

4.2.2 Water Retention Value

The water retention value (WRV) is an analysis that shows the ability of a substrate to swell. Since water adsorption occurs mainly in amorphous regions of cellulose [140], WRV can be an indicator of the changes of crystalline regions to more amorphous regions after pretreatment. Moreover, the WRV depends on the pore sizes and the surface area. The WRV results in this study of rice straw and triticale straw could be associated with the results obtained by Simons' stain (SS) analyses, however, with less sensitivity and being less informative than SS (Paper III).

4.2.3 Crystallinity

The increase in biodegradability can partly be explained by a decrease in the crystallinity of the cellulose fibers. This characteristic was measured by Fourier transform infrared spectroscopy (FTIR), and the lateral order index (LOI) at 1422/898 cm⁻¹ was also determined [127, 141, 142]. The LOI

provides an indication of the transition from the natural cellulose I to the cellulose polymorph II and/or amorphous cellulose. The pretreatment with NMMO on rice and triticale straw resulted in the absorption band at 1422 cm⁻¹ representing cellulose I being decreased after the pretreatment, while the band at 898 cm⁻¹, representing cellulose II and/or amorphous cellulose was increased (Paper III, Figures 4.9A and B). Since the cellulose binding module (CBM) of the cellulosome complex bind more effectively to amorphous than to crystalline cellulose [58], the decrease of the crystallinity is of the main importance, as this could increase the binding of the cellulosome complex to the lignocellulosic substrate, thereby, increasing the anaerobic degradability. Cellulose crystallinity can also be measured by X-ray [143] or preferably X-ray diffraction [144], due to the overlap of crystalline peaks in X-ray measurements on lignocelluloses (not performed in the present study).



Figure 4.9A: FTIR spectra from rice straw showing the peak pattern for cellulose LOI crystallinity index. (a) – untreated rice straw, (b) – 1 h NMMO pretreatment, (c) – 3 h pretreatment, and (d) – 15 h NMMO pretreated rice straw.



Figure 4.9B: FTIR spectra from triticale straw showing peak pattern for cellulose LOI crystallinity index. (a) – untreated triticale straw, (b) – 1 h NMMO pretreatment, (c) – 3 h pretreatment, and (d) – 15 h NMMO pretreated triticale straw.

4.2.4 Enzymatic Adsorption

The enzymatic adsorption method gives an indication of how easily accessible the lignocellulosic substrate is to the enzymes. The method measures the amount of cellulase that adsorbs onto the material after a defined time [145, 146]. The investigations presented in Paper III showed that there is a link between the measured accessible surface area (by e.g., Simon's stain analyses) and the enzymatic adsorption. The total binding of the cellulases was increased after the pretreatment, which can be explained by an increase in the accessible surface area on the substrates. This is in line with the results presented by Piccolo et al, [146] where surface area, enzymatic adsorption and enzymatic hydrolysis rate interactions were studied. Furthermore, Kumar et al, [145] found a correlation between increased enzymatic adsorption and glucan hydrolysis prior to ethanol production.

4.2.5 ToF-SIMS

A new technology for structural investigations is Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). ToF-SIMS measures the chemical composition on the surface not deeper than 1 nm on the actual material [147]. A primary ion beam ionizes the sample, followed by the release of secondary

ions, which are analyzed by a high-resolution mass spectrophotometer. Only the first layer of molecules is influenced by the beam. By this method, the chemical changes on the surface can be studied, caused by the pretreatments, such as the ratios of lignin, hemicelluloses, and cellulose. The selected ion masses representing lignin (m/z 137 and 151) and cellulose (m/z 127 and 145) were adopted from [148, 149].

The results in Paper III and in Figure 4.10 show that the ratio of cellulose over lignin increases after longer pretreatment with NMMO on triticale straw. In Figure 4.11, similar results are found, with increased ratio of total cellulose over total lignin, coupled with higher concentrations of sodium hydroxide in steam explosion pretreatment on paper tube residuals. ToF-SIMS is, however, a relatively expensive and time-consuming analysis method, which makes its use in lab environments limited.



Figure 4.10 Relative ion intensity from ToF-SIMS analyses on triticale straw after NMMOpretreatment. Four different peaks were analyzed, m/z 127 and 145 for cellulose, 137 and 151 for lignin. Trit unt – untreated triticale straw, Trit 1 h, Trit 3 h, and Trit 15 h – triticale straw after 1, 3, and 15 h treatment. The error bars show \pm one relative standard deviation.



Figure 4.11 Relative ion intensity from ToF-SIMS analyses on paper tube residuals after steam explosion pretreatment. Four different peaks were analyzed, m/z 127 and 145 for cellulose, 137 and 151 for lignin. Paper unt - untreated paper tubes, paper 0, 1, or 2% - paper tubes with 0-2% addition of sodium hydroxide in the steam explosion pretreatment. The error bars show \pm one relative standard deviation.

According to the results obtained, Simons' Stain, and the enzymatic adsorption method for the determination of accessible surface area, as well as the determination of cellulosic crystallinity measured by FTIR were found to be the most promising methods. These analyzed characterizations can be linked with improved biogas production (Paper III), and could in the future be alternatives to the time-consuming biomethane potential (BMP) test, in order to predict the methane potential of pretreated and untreated lignocelluloses.

5. ECONOMICAL FEASIBILITY OF THE DIGESTION OF LIGNOCELLULOSES

5.1 Economical parameters

Potential economic profit is one of the most important driving forces for an investment in an anaerobic digestion plant run on lignocelluloses. It is also critical for the plant to be run successfully for a long period. The most important parameters that affect the potential economic profit are: (1) The investment costs, which depend on the type, the size and the location of the plant, etc., (2) the operating, transport and maintenance costs, (3) the cost of biomass feedstock, as well as the availability, and (4) the product revenues [150]. Moreover, the anaerobic digestion of pretreated lignocelluloses is a new, not yet commercialized technique, which will increase the risk of the investment. The above-mentioned parameters are further discussed in this chapter.

5.1.1 Capital cost

The capital cost or fixed capital investment costs depend on several different factors, such as the size and type of plant, the type of pretreatment unit, the location, and engineering. For co-digestion processes, different additional utilities might be needed for the organic fraction of municipal solid waste, such as fractionation, size reduction, hygienization, etc. It is noteworthy that the size of the plant does not linearly affect the capital cost of the plant. The larger the utilities, the cheaper per unit biomass treated. According to Turton et al, [151], there is a six-tenth rule that states that doubling the plant size will result in an increase of capital costs by 52%.

5.1.2 Operation cost

The operating costs are, for example, the price of the feedstock, salaries, insurances, taxes, energy, power consumption, and maintenance. These costs are either direct, which means that they are dependent on the amount of feedstock used, how many workers the plant has, etc, or indirect, which

means that they are independent regardless of whether the plant is in operation or not. Indirect costs are insurances, taxes, salaries, etc [151].

5.1.3 Type and availability of feedstock

The specific type of lignocellulosic feedstock used within the process affects the economy of the process. The location of the plant compared to the location of where the biomass is produced can have large effects on the transportation costs. Biomass is presumably produced at many different sites, and therefore, needs a good collection and transportation system. The demand for the feedstock by other industries will also affect the price of the feedstock. Biomass regarded as waste with no other potential use will have a lower price than biomass that can be used effectively by other industries, for example, incineration for heat and electricity production.

5.1.4 Digestate value or cost

The digestate from anaerobic digestion can be used as a fertilizer on crop-land, due to the high nutrient content, especially nitrogen and phosphorus. There are different certification systems that regulate the composition of the digestate prior to its use as fertilizer [53]. The digestate from the digestion of lignocelluloses, however, are rich in lignin due to the limited degradation of lignin in the anaerobic digester. The high lignin content makes the digestate energy-rich and suitable for incineration after dewatering and/or drying processes. Furthermore, this value of the digestate can contribute to the economy of the anaerobic digestion of lignocellulosic feedstocks.

5.1.5 Upgrading and injection into the gas grid

The upgrading of the biogas to 95-98% methane can be performed by many different techniques. Typical examples are chemical absorption, water scrubbing, pressure swing adsorption, cryogenic separation and membrane separation [152]. The choice of the upgrading technique depends on the composition of the biogas produced in the digester as well as on the amount. The most widespread technique both globally and in Sweden, is the water scrubbing technique [152, 153]. This technique is based on the higher solubility of the carbon dioxide in water, compared to that of methane, which makes the separation possible. There should also be a removal of hydrogen sulphide, water and other contaminants in the upgrading step. Fifty percent of the biogas produced in Sweden today is upgraded to methane, with an increasing trend for every year [53]. Today the upgraded biomethane produced from anaerobic digestion is mixed with natural gas in the gas grid system in Sweden. The two gases have the same content but different origin, with the exception that upgraded biogas is often

complemented with an addition of propane in order to have the same energy value per volume as natural gas [154]. Injecting the upgraded methane directly into the gas grid, offers the advantages of being cost effective, having good distribution and transport possibilities and having a buffering capacity already provided in the gas grid [150].

5.1.6 Combined heat and power production

The produced biogas can also be used for the combined production of heat and power. This is the most common use of produced biogas in, for example, Germany and Denmark. The electricity production has a typical efficiency of 20-35%, while the combined heat and power efficiency can be 80-90% [150]. In Sweden, 38% of the produced biogas is used as heat and 3% for electricity production [53]. The produced electricity can be sold to the public electricity net, while the produced heat is often used as heating within the process, but can also be sold as district heating.

5.2 Economic evaluation of biogas production from forest residues

Process description

The pretreatment with NMMO on lignocelluloses has shown to have a major effect on the methane production during anaerobic digestion. An economic evaluation of the pretreatment process has been performed in order to evaluate the economical feasibility on an industrial scale (Paper V). Forest residues which are an abundant lignocellulosic-rich waste stream in Sweden, were used as feedstock in the simulation study, having a plant capacity of 100,000 tons treated forest residues co-digested with 200,000 tons of the organic fraction of municipal solid waste (OFMSW) per year. The process includes the NMMO pretreatment at 90°C for 12h, with a filtration, evaporation, and recirculation of the NMMO and washing water. The forest residues are further co-digested with the organic fraction of municipal solid waste (OFMSW) in a thermophilic anaerobic digester in order to reach an optimal C/N ratio of 20 to 30. The biogas is upgraded with the water scrubber technique, and the lignin-rich digestate is dewatered and sold to incineration plants. The calculations are based on lab scale BMP test, where NMMO pretreatment at 90°C for 15h results in 0.137 Nm³ CH₄ per kg total solids at thermophilic conditions [155]. SuperPro Designer[®] 8.0 (Intelligen, Inc., NJ, USA) was used for the simulation of the main steps of the process. The block flow diagram of the process can be found in Figure 5.1.



Figure 5.1 Block flow diagram of NMMO pretreatment of forest residues followed by the codigestion together with the organic fraction of municipal solid waste (OFMSW).

Section and sensitivity analysis

The fixed capital investment for the base case (Figure 5.2) was divided into five different sections: (1) the NMMO pretreatment, (2) the filtration and evaporation, (3) the anaerobic digestion, (4) the upgrading of the biogas, as well as (5) the dewatering of the digestate residue. The biggest capital investments are connected to the anaerobic digesters as well as the evaporators. The estimated annual operating costs can be found in Figure 5.2, where it is evident that the materials represent the major part of the cost, where the organic solvent NMMO stands for about 80% of the material costs.



Figure 5.2 FCI, Fixed capital investment per section, including equipment prices, installation, instrumentation, electricity, piping, insulation, engineering and construction, contractor's fee and contingency. Auxiliary facilities, yard improvements and buildings are excluded in the FCI table. Annual operating costs for the base case divided into different cost items are presented in the second table.

Furthermore, scenarios with 50% more or less water volume during the washing step in the NMMO pretreatment was evaluated, as was scenarios with 20% higher or lower price of the methane and of the forest residues. The results are expressed as the internal rate of return (IRR) calculated according to Dolan et al [156], and can be found in Figure 5.3. The IRR was calculated when the net present value was set to zero, with a project lifetime of 20 years, and the project was regarded as financially viable when the IRR was 15% or higher.



Figure 5.3 Result of cash flow analysis. IRR of 50% increased or decreased water consumption during washing, and of 20% increased or decreased price of methane and forest residues, compared with the base case.



Figure 5.4 Production cost per kilogram methane produced for plant capacities between 25 to 400 thousand tons forest residues per year.

The base case scenario with 100,000 tons forest residues per year was further compared with plant capacities of 25, 50, 200, and 400 thousand tons forest residues per year. The production cost per kg methane for the five different plant capacities can be found in Figure 5.4. The cash flow analysis reveals, that plant sizes treating and digesting 50,000 tons forest residues or more, are economically feasible with an IRR of 15% or more (Paper V).

The anaerobic digestion of NMMO pretreatment in co-digestion with OFMSW is regarded as a financially viable process with an IRR over 15%. The biggest improvements of the process can be performed with a higher recycling rate of NMMO, as well as decreased water consumption during the washing step. However, in order to achieve a financially viable process of the digestion of forest residues itself, the methane prices need to increase substantially, together with the above-mentioned improvements.

6. CONCLUDING REMARKS

This thesis focuses on the anaerobic digestion of lignocelluloses. The abundance of lignocelluloses worldwide, together with the increasing demand for green energy, and the high biogas potential of these materials, open up great possibilities for lignocellulose biogas production. The biomass recalcitrance, i.e. the compact structure of the lignocellulose, the high lignin content and the high cellulosic crystallinity, however, limit the microbial degradation. Thus the preprocessing steps are of great importance. A suitable pretreatment can open up the structure and subsequently improve the biogas production.

The major conclusions from the present work can be summarized as follows:

- Paper tube residuals were successfully pretreated with thermal and steam explosion pretreatment, combined with NaOH addition, resulting in up to 21 and 70 % increase in methane production, respectively.
- Spruce chips, milled spruce, as well as rice and triticale straw were successfully pretreated with NMMO resulting in increased biogas potential between 400-1,200%.
- Increased accessible surface area and decreased crystallinity can be linked to improved biogas production. Substrate characterization methods can be alternatives to the BMP test in order to predict the methane potential of pretreated and untreated lignocelluloses.
- The co-digestion of paper tube residuals with a nitrogen-rich buffer tank substrate resulted in a stabilized process, thus avoiding a reactor failure. Furthermore, synergistic effects in the co-digestion were found.
- The co-digestion of NMMO pretreated forest residues together with OFMSW resulted in an IRR over 15% and thereby a financially viable process. Technical improvements, such as the recycling of the NMMO, as well as the amount of water used in the washing section are considered as being the most important steps in order to improve the economic feasibility of the process.

7. FUTURE STUDIES

The production of biogas from lignocellulosic rich residuals is at the present moment in its preliminary stage and much more research needs to be performed in order to make it possible to use lignocelluloses for biogas production on an economically feasible, larger scale in the future. The following subjects and areas are interesting and challenging for future investigations:

- Several pretreatment studies have been performed on different lignocellulosic materials, however, it still remains to evaluate which kinds of pretreatments are best suited for which kinds of feedstocks, followed by the optimization of these pretreatment conditions. We need to find suitable pretreatment methods, which are not only economically feasible on a full scale process, but also effective, energy efficient and use as little chemicals as possible.
- So far, most of the pretreatment methods and conditions studied have been evaluated by anaerobic batch digestion assays to determine the methane potential achieved after the pretreatment. Very little experimental work has been done to investigate the long-term effects in a continuous co-digestion process where lignocelluloses are one of the substrates utilized. This step is important to be able to investigate the eventual inhibiting as well as synergistic effects. Furthermore, pilot scale studies, including the pretreatment and anaerobic digestion steps, are of great importance toward the commercialization of these kinds of processes.
- There is still very little known about the specific changes in the structure that will affect the microbial degradation of lignocelluloses. Limited research has been performed on the substrate characteristics that are the most important for high degradation rates, as well as how they can be measured. If we are able to find answers to these basic questions, it will be easier in the future to construct a pretreatment method that works in the most effective way.
- Another interesting area for further investigations is how substrate composition, pretreatment methods and operational parameters during anaerobic digestion affect the microbial consortia working in the digester. Only a few studies have been performed on the relationships between pretreated lignocelluloses as a feedstock and the microbial constitution; thus more knowledge is needed.
- The cellulosomes, or the cellulose degrading enzyme complex, is another fascinating area, where there is still much to learn. Do the microorganisms adopt the design of the cellulosomes of the substrate they meet? Is it possible to genetically modify these microorganisms to produce cellulosomes, with an optimum structure and composition of enzymes in order to achieve the best degradation?

NOMENCLATURE

AFEX	Ammonia fiber explosion
ANOVA	Analysis of variance
ВМР	Biomethane potential test
BTS	Buffer tank substrate
СВМ	Cellulose binding module
СНР	Combined heat and power plant
COD	Chemical oxygen demand
CSTR	Continuously stirred tank reactor
ETMY	Estimated theoretical methane yield
FTIR	Fourier transform infrared spectroscopy
GLM	General linear model
HMF	Hydroxymethylfurfural
HRT	Hydraulic retention time
IRR	Internal rate of return
LOI	Lateral order index
NMMO	N-methylmorpholine N-oxide
OFMSW	Organic fraction of municipal solid waste
ORL	Organic loading rate
ToF-SIMS	Time of flight secondary ion mass spectroscopy
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids
WRV	Water retention value
WWTP	Waste water treatment plant

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