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Improved anaerobic digestion of energy crops and agricultural residues

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Publication date:
2010

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

Link back to DTU Orbit

Citation (APA):
Bruni, E., & Angelidaki, I. (2010). Improved anaerobic digestion of energy crops and agricultural residues. Kgs. Lyngby, Denmark: Technical University of Denmark (DTU).

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Emiliano Bruni

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PhD Thesis
June 2010

Department of Environmental Engineering
Technical University of Denmark

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Preface

This thesis is the result of a PhD study conducted at the Department of Environmental Engineering of the Technical University of Denmark (DTU) and at the Department Process Research of Xergi A/S from December 2006 to May 2010. The study was carried out in the framework of an Industrial PhD and was supervised by Irini Angelidaki (DTU) and Anders Peter Jensen (Xergi). The following manuscripts were prepared during the study and are enclosed as appendices. In the text, they are referred to by their roman numerals.

I. Bruni, E., Jensen, A.P., Pedersen, E. S., & Angelidaki, I. (2010). Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. *Appl. Energy, 87*, 2212-2217

II. Bruni, E., Jensen, A.P., & Angelidaki, I. (2010). Steam treatment of digested biofibers for increasing biogas production. *Bioresour. Technol.* (doi: 10.1016/j.biortech.2010.04.064, in press).

III. Bruni, E., Jensen, A.P., & Angelidaki, I. (2010). Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. Submitted to *Bioresour. Technol*.

IV. Bruni, E., Boe, K., Jensen, A.P., & Angelidaki, I. (2010). Online VFA measurements in biogas processes: sampling, calibration and integration of SCADA systems. Technical note.

The papers are not included in this www-version, but can be obtained from the Library at DTU Environment
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June 2010
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Summary

This thesis deals with two strategies to increase the efficiency of biogas plants: the improvement of the quality of the substrate for biogas production and online monitoring and automatic control of the process.

Biogas processes convert organic matter into methane and carbon dioxide. Often, the effluent from the biogas plant is used as a fertilizer. In commercial-scale applications, optimization is crucial to achieve cost-effective processes. Optimization can be achieved in three ways:
- increasing the methane yield of the substrate;
- using online monitoring and automatic control to run highly-loaded biogas processes, minimizing the risk of process failures;
- with optimal plant design (type and configuration of reactors).

This PhD study focused on treatments and management of the substrate and on online monitoring. The substrates considered during this study were lignocellulosic materials (biofibers from manure and maize).

The following treatment methods were tested to increase the methane yield of biofibers from digested manure: physical treatment (milling), chemical treatment (CaO), biological treatment (partial aerobic microbial conversion and enzymatic conversion), steam treatment (without catalyst and with catalysts H_2SO_4, H_3PO_4, NaOH) and a combination of biological and steam treatments (biofibers steam-treated with catalyst were treated with laccase enzyme). The work on maize focused on management (variety and harvest time of fresh maize) and on mechanical treatment (milling) of maize silage to increase the methane production.

The treatments that resulted in the highest increases of methane yield were steam treatment at 155 °C with addition of H_2SO_4 (67% higher methane production compared to untreated biofibers) and chemical treatment with CaO (66% increase). Also steam treatment at 160 °C with H_3PO_4 or with NaOH addition resulted in increased methane yields, but only to 8% and 26%, respectively. Higher treatment temperatures (180 °C without addition of catalyst) improved the methane production by 29% compared to untreated biofibers. Enzymatic treatments did not result in higher methane yield, unless the biofibers were

previously treated with steam. Combination of steam treatment with NaOH and subsequent enzymatic treatment with laccase increased further the methane yield of the biofibers, achieving 34% higher yield compared to untreated biofibers. Physical treatment resulted in 10% higher methane yield for maize silage and biofibers from digested manure. Partial aerobic microbial conversion did not increase the methane yield. When choosing the optimal treatment the energy requirements of the treatment have to be taken into account as well as the energy gain as extra biogas production and the costs of necessary investments for equipment, downstream processes and addition of chemicals and enzymes. Treatments such as steam treatment that use thermal energy as energy input are interesting for full-scale biogas plants equipped with CHP units (combined heat and power), where the thermal energy needed may be available as waste heat.

When crops such as maize are used as the substrate, the methane energy output per hectare depends also on the crop yield and management. Thus, the efficiency of biogas processes can be increased through optimizing crop yield and management. In this study, it was found that fresh maize had the highest methane yield per hectare at late harvest. The specific methane yield per volatile solids content (m^3 CH_4 $(kg\ VS)^{-1}$) was not significantly influenced neither by the variety nor by the harvest time of fresh maize.

The work on online monitoring of the biogas process focused on online measurements of the concentration of volatile fatty acids (VFA) in the biogas digester. An online VFA analyzer was adapted for operation with pilot-scale biogas plants. A filter for automatic sampling was developed and installed at a pilot-scale plant digesting cow manure. The filter successfully allowed automatic sampling. The SCADA system (Supervision Control And Data Acquisition) of the analyzer was integrated into the SCADA of the biogas plant. Automatic sampling and online VFA measurements were tested for 14 days at a pilot-scale biogas plant and good agreement between manual and online VFA measurements was found. However, the calibration of the VFA analyzer showed strong uncertainties, indicating that further investigations of the repeatability of the measurements are needed.

Dansk resumé

Denne afhandling handler om to strategier til forøgelse af effektiviteten af biogasanlæg: Den første strategi er forbedring af kvaliteten af substratet til biogasproduktion. Den anden strategi er online overvågning og automatisk proceskontrol.

Biogasprocesser omdanner organisk stof til metan og kuldioxid. Spildevandet fra biogasanlægget anvendes ofte som gødning. I kommerciel skala er optimering af afgørende betydning for at opnå omkostningseffektive processer. Optimering kan opnås på tre måder:
- forøgelse af metanudbyttet af substratet;
- optimering af anlægsudnyttelsen ved hjælp af online overvågning og automatisk proceskontrol for at køre stærkt-loaded biogas processer og for at minimere risikoen for processen fejl;
- optimering af anlægsdesign (type og konfiguration af reaktorer).

Dette Ph.d.-studie fokuserede på forbehandlinger og håndtering af substratet og på online overvågning. De undersøgte substrater var lignocellulose-holdige biomasser(biofibre fra gylle og majs).

Følgende forbehandlingsmetoder til forøgelse af metanudbyttet fra afgassede gyllefibre blev testet: Fysisk behandling (formaling), kemisk behandling (CaO), biologisk behandling (delvis, aerob mikrobiel omdannelse og enzymatisk omdannelse), dampbehandling (uden katalysator og med katalysatorerne H_2SO_4, H_3PO_4, NaOH) og kombination af biologisk behandling med dampbehandling (fibers damp-behandlede med katalysator blev behandlet med laccase). Arbejdet med majs fokuserede på håndteringen (sort og høsttidspunkt) og mekanisk behandling (formaling) af majsensilage for at øge metanproduktionen.

De behandlinger, der resulterede i de højeste stigninger i metanudbyttet, var dampbehandling ved 155 °C med tilsætning af H_2SO_4 (67 % højere metanproduktion i forhold til ubehandlede biofibre) og kemisk behandling med CaO (66 % stigning). Også dampbehandling ved 160 °C med H_3PO_4 eller med NaOH resulterede i øgede metanudbytter, men kun med henholdsvis 8 % og 26 %, henholdsvis. Højere behandlingstemperatur (180 °C uden tilsætning af katalysator) forbedrede metanproduktionen med 29% i forhold til ubehandlede

biofibre. Enzymatisk behandling resulterede ikke i højere metanudbytte, medmindre biofibrene forinden varblevet behandlet med damp. Kombination af dampbehandlingen med NaOH og efterfølgende enzymatisk behandling med laccase øgede yderligede metanudbyttet af biofibrene og gav 34% højere udbytte i forhold til ubehandlede biofibre. Fysisk behandling resulterede i 10% højere metanudbytte for majsensilage og biofibre fra afgasset gylle. Delvis aerob mikrobiel forbehandling øgede ikke metanudbyttet. Valget af den optimale behandling skal tage højde for behandlingens energibalance, samt udgifterne til de nødvendige investeringer i udstyr, downstreamprocesser og tilsætning af kemikalier og enzymer. Dampbehandling, der bruger termisk energi som energiinput er interessant til fuldskala biogasanlæg udstyret med CHP (kombineret kraft-varmeproduktion), hvor den termiske energi ofte er til rådighed som spildvarme.

Når energiafgrøder som for eksempel majs bruges som substrat til et biogasanlæg, kan biogasprocessens samlede effektivitet øges ved at optimere hektarudbyttet og håndteringen af energiafgrøden . I denne undersøgelse blev det konstateret, at majs havde det højeste metanudbytte/hektar ved sen høst. Metanudbyttet i Nm3 CH4/ton våd vægtsteg fra 80 vedtidlig høsttil 137ved sen høst, mens det specifikke metanudbytte fra det organiske tørstof ikke var signifikant påvirket hverken af sort eller af høsttidspunkt.

Arbejdet med online overvågning af biogas processen fokuserede på online målinger af koncentrationen af flygtige fedtsyrer (VFA) i en biogasreaktor. En online VFA-analysator blev tilpasset til brug på et pilot-skala biogasanlæg, hvortil et filter til automatisk prøvetagning blev udviklet og succesfuldt installeret. Analysatorens SCADA-system (Supervision Control And Data Acquisition) blev integreret i biogasanlæggets SCADA. Der blev gennemført automatisk prøvetagning og online VFA-målinger i 14 dage på et pilot-skala biogasanlæg og god overensstemmelse mellem manuell og online VFA-målinger blev fundet. Kalibreringen af VFA-analysatoren viste dogstore usikkerheder, hvilket indikerer, at yderligere undersøgelse af målingernes reproducerbarhed er nødvendige.

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1. Background and aim of the study

Biogas is among the alternatives to fossil fuels. Its use diversifies the energy supply and reduces greenhouse gas emissions and dependence on fossil fuel markets such as oil and gas (European Commission Energy, 2010a). Also, biogas processes are a low-cost waste treatment (Verstraete et al., 2005).

Biogas processes are among the most interesting bioprocesses taking place in nature: they convert organic matter into a mixture of methane and carbon dioxide (biogas). To implement biogas processes at industrial scale and to feed them with organic waste is a sustainable waste treatment that produces renewable energy in the form of biomethane. Biogas processes involve microorganisms that are fascinating from the point of view of energy conservation and utilization (Schink, 1997). Using the minimum quantum of energy that living cells can exploit, these microorganisms convert organic matter into the end products methane and carbon dioxide.

Biogas is considered in the context of alternatives to oil and renewable fuels, together with bigger players (in terms of volume) such as bioethanol (Talebnia et al., 2009; Verstraete, 2007). Biogas is politically demanded, the new European Commission's directive on renewable energy (European Commission, 2009) sets out high targets for all Member States (European Commission Energy, 2010b), including that the EU will reach a 20% share of energy from renewable sources by 2020 and a 10% share of renewable energy. The advantage of biogas compared to other biofues in order to reach this aim is the versatility to treat a broad variety of substrates. Biogas can be considered as a low-cost waste treatment because the microorganisms involved in the process can degrade a wide range of organic substances. In fact, biogas can be produced from substrates such as manure, energy crops, industrial waste and sludge. Even the effluent from the biogas processes can be used in agriculture, as fertilizer. Biogas can be upgraded and injected into the natural gas grid or can be burnt directly at the biogas plant for co-generation of heat and electricity (Taherzadeh & Karimi, 2008). This makes biogas processes particularly interesting for applications in decentralized areas with high production of organic waste. For example, in areas with intense agriculture and animal production, biogas processes can convert the waste into heat, electricity and fertilizer. Based on these highly useful

characteristics, this study centered around the question, how to optimize the biogas process, in other words how to get even more energy out of the waste.

The use of low-cost feedstocks is crucial to obtain cost-effective biotechnologies for biogas production (Ni & Sun, 2009; Rabelo, 2009). Unfortunately, low-cost is often coupled to low biodegradability. In biogas plants digesting agricultural residues (lignocellulose), the low digestibility of the substrate causes a loss of methane production and limits the overall efficiency of the process (Jin et al., 2009). For example, agricultural residues such as straw or biofibers from manure are among the low-cost feedstocks, but they are relatively recalcitrant to anaerobic digestion and need treatment to be efficiently degraded in biogas processes (Demirbas, 2008). Optimization was demanded in this context.

The second problem that was considered is that to exploit the capacity of the biogas plant, the biogas process has to run at high load. On the other side, at increasing process load, also the risk of process failure increases. Additionally, inhibitory or toxic compounds in the substrate can cause process failure (Steyer et al., 1999). Online monitoring and automatic control were investigated to protect against process failure while maintaining the process at high load.

The aim of this PhD study was therefore to identify methods to improve the efficiency of biogas processes, focusing on optimization of management and treatments to increase the methane yield of lignocellulose and on online monitoring of concentration of VFA in the reactor. In this context, the study makes a contribution towards more efficient and thus more competitive production of renewable energies.

2. The biogas process

The microorganisms involved in the biogas process use organic material partly as energy source and partly to generate the electron acceptors. The final products of the biogas process are carbon dioxide and methane (carbon at its most oxidized and most reduced state, respectively). Only a small fraction of the energy content of the substrate is used by the microorganisms, while the rest is stored in the product methane. The energy gain for the microorganisms is very low and anaerobic processes take place only when the more energetically favorable electron acceptors such as oxygen, nitrate or sulfate have been reduced (Zehnder & Stumm, 1988). Anaerobic decomposition of organic material into methane and carbon dioxide is a complex process that involves different microbial populations, most of which do not produce methane as such, but perform a step of the whole chain of reactions. The chain of reaction steps aims at optimizing the energy yield.

The process is the result of the combined action of four groups of microorganisms: primary fermenting bacteria, secondary fermenting bacteria and two types of archae. Although the flow pattern and the formation of intermediate products are complex and depend on the microbial status and operating conditions, a simplified three-step process can give an overall overview (Figure 1). First (hydrolysis), primary fermenting bacteria hydrolyze the substrate to smaller units. Then (acidogenesis and acetogenesis), primary fermenting bacteria and secondary fermenting bacteria convert the formed soluble oligomers and monomers into acetic acid, hydrogen and carbon dioxide. With the last step (methanogenesis), the archae convert acetic acid, hydrogen and carbon dioxide into biogas (Schink, 1997).
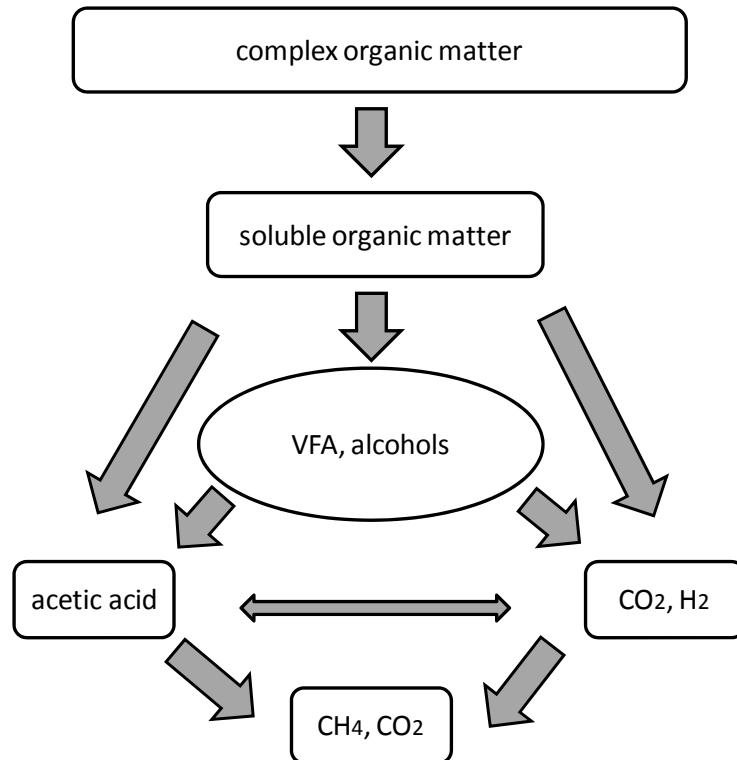
Figure 1 – Carbon flow in methanogenic environments

2.1. Hydrolysis

Hydrolysis takes place outside the microbial cells. Hydrolytic bacteria (primary fermenting bacteria) hydrolyze the substrate with extracellular enzymes (either excreted or attached to the cell surface). These bacteria are facultative anaerobes. During the hydrolysis step, polymers are hydrolyzed into soluble oligomers and monomers. The enzymes involved in this process are cellulases, hemicellulases, proteases, amylases, lipases (Taherzadeh & Karimi, 2008). Thus, a wide range of enzymes can be produced during the biogas process and as a consequence, biogas processes can hydrolyze almost all kinds of substrates. Lignin and waxes (Fernandes et al., 2009) are among the exceptions. When the microorganisms can produce the suitable enzymes, hydrolysis is a relatively fast step. However, physical contact between the enzymes and the substrate is required for hydrolysis to take place and the hydrolysis step can become rate-limiting if the substrate is hardly accessible for the enzymes (Taherzadeh & Karimi, 2008).

When the substrate is hydrolyzed, it becomes available to cell transport and can be degraded during the following steps of the biogas process.

2.2. Acidogenesis and acetogenesis

Primary fermenting bacteria absorb the products of hydrolysis and convert them into VFA, hydrogen and alcohols (acidogenesis). These microorganisms are both obligate and facultative anaerobes. In a balanced, well-functioning biogas process, primary fermentative bacteria produce mainly acetic acid, hydrogen and carbon dioxide and these can be used directly as substrates by the methanogenic microorganisms. The most energetically favorable pathway of primary fermentative bacteria is production of acetate via pyruvate with production of hydrogen. If the environmental conditions are not optimal (high partial pressure of hydrogen), this pathway is not favorable and the primary fermenting bacteria switch metabolism (branched metabolism), producing other intermediates (Klass, 1984). Shifts in the environmental conditions can be due to excess of supply of substrate, or presence of toxic compounds and cause an increase of the concentration of hydrogen. In such conditions, intermediates such as VFA longer than two carbon atoms and alcohols longer than one carbon atom are formed (Bryant, 1979; Schink, 1997). These products are more reduced than the products that would be produced under optimal conditions, however this metabolism is still yielding small amounts of energy. Methanogenic microorganisms can not use directly these reduced intermediates, therefore these products have to be further modified before they can be converted into biogas. The conversion of these products into acetic acid, hydrogen and carbon dioxide takes place during acetogenesis and is carried out by secondary fermenting bacteria. These microorganisms are obligate hydrogen-producing bacteria (linear metabolism): the substrates can be converted into the more oxidized acetic acid and carbon dioxide only by reduction of protons to hydrogen. It is not possible for these microorganisms to switch metabolisms (de Bok et al., 2004). At standard conditions, the reactions carried out by acetogenic microorganisms are not exergonic. Low partial pressures of hydrogen (lower than 10^{-5} bar) are needed for the reactions to be energetically feasible and for the hydrogen-producing microorganisms to have energy gain. The syntrophic association between the secondary fermenting bacteria and one of the two types of archae can maintain the partial pressure of hydrogen within the range suitable for energy gain and will be discussed later.

The acetic acid, hydrogen and carbon dioxide produced during acidogenesis and acetogenesis are the substrates for the methanogenesis step.

2.3. Methanogenesis

Methanogenic microorganisms are obligate anaerobic archae that convert hydrogen, acetic acid, carbon dioxide and other one-carbon compounds like methanol and formate into biogas: methane and carbon dioxide. Aceticlastic microorganisms and hydrogenotrophic microorganisms use acetate and hydrogen as substrate, respectively. Approximately 70% of the carbon flow is via aceticlastic microorganisms, even if this pathway provides much lower energy for microbial growth compared to the hydrogenotrophic one (Klass, 1984). Hydrogenotrophic microorganisms use the hydrogen produced by the secondary fermenting bacteria and reduce carbon dioxide. Because hydrogen is a substrate for hydrogenotrophic microorganisms, the partial pressure of hydrogen has to be above a minimum level (higher than 10^{-6} bar) for the reaction to be exergonic. At the same time, low partial pressure of hydrogen is needed by secondary fermenting microorganisms as described earlier in this thesis. A narrow range of hydrogen partial pressure allows the growth of both the hydrogenotrophic microorganisms and the secondary fermenting microorganisms. Because of the strict energy constrains, these populations depend on each-other (syntrophic relationship) and hydrogen has to be consumed as soon as it is produced. Close physical contact between these two types of microorganisms ensures that the partial pressure of hydrogen is within the optimal range that allows both reactions of hydrogen formation and consumption to be exergonic.

3. Improving the biogas process

In biogas plants, biology and engineering are closely connected. The efficiency of the bioprocess depends on three main factors: substrate characteristics (digestibility, nutrients, inhibitors, co-digestion), control (monitored parameters, analyzers, control algorithms), process design (biomass retention, two-phase, multi-step). During this PhD, the quality of the substrate for biogas digestion and online monitoring and automatic control have been considered and are described in the following paragraphs.

The below provides an overview on possible process design solutions. Two-phase systems separate the acidogenic and the methanogenic phases into two different reactors in series (Azbar et al., 2001). This configuration enhances the performance of the process, but on the other hand two-phase processes are more sensitive to product inhibition because of the disruption of the syntrophic relations between bacteria and archea. Process control of two-phase systems may be difficult and the effluent from the first phase to the second phase may need pH adjustments. Configurations of one-phase continuous flow stirred tank reactors (CSTR) in series (i.e. reactors where the different biomasses involved in the biogas process are present in a mixed culture) achieve higher methane yields compared to single CSTR with same total volume (Boe & Angelidaki, 2009). The advantage of CSTR in series is clear when treating substrates with low reaction kinetics, while for easily degradable substrates the performance of single-CSTR is nearly the same as that of series of CSTR. Configurations with CSTR in series allow a high degree of automation, high productivity and relatively constant treatment quality (Nielsen et al., 2003). A drawback of the CSTR is the loss of microorganisms within the effluent. To overcome the latter problem, i.e. to decouple the cells retention time from the hydraulic retention time, the microorganisms have to be retained in the reactor. This can be done by sedimentation or by allowing growth of granulated sludge or biofilm on a support material. For example, immobilized-cell configurations are used in upflow anaerobic sludge blanket reactors (UASB), where the biomass is retained while the substrate is pumped through, allowing a high organic loading rate (OLR) (Kaparaju et al., 2009). Among the drawbacks of UASB reactors is the difficulty to treat substrates containing particulate matter (Boe et al., 2009).

Also the process temperature influences the efficiency of the biogas process. Temperature does not directly affect the methane yield, but it has an indirect effect on the overall performance of the biogas plant as it influences the kinetics of the process. Thermophilic microorganisms have higher growth rates compared to mesophilic microorganisms. The overall higher microbial activity results in higher methane productivity at thermophilic conditions. Under such conditions, shorter hydraulic retention time (or smaller reactor volume) is needed compared to mesophilic conditions, which may be attractive for commercial-scale applications. The temperature also influences foaming, as foaming has lower tendency to occur at thermophilic conditions than at mesophilic conditions (Palatsi et al., 2009a). On the other hand, thermophilic conditions can result in less stable processes, for example because of ammonia inhibition or LCFA toxicity (Boe et al., 2009; Hwu & Lettinga, 1997; Palatsi et al., 2009b). The tendency to unstable processes is mainly due to the lower microbial diversity obtained at thermophilic conditions compared to mesophilic conditions.

3.1. Substrate quality for biogas production

As mentioned earlier in this thesis, hydrolysis is a relatively fast step in the whole chain of the biogas process. However, hydrolysis may become rate-limiting when the substrate is not accesible for the enzymes. In such situation, although the hydrolytic bacteria can produce the required enzymes in sufficient amounts, hydrolysis can not take place or it takes place at a low rate. This is the case of lignocellulosic substrates (crops or agricultural waste). Part of the organic content of lignocellulosic substrates is not digested in biogas processes, causing an overall productivity loss and affecting negatively the overall performance of the biogas process.

The quality of the substrate for biogas production can be optimized. Cultivation and management (for example harvest time) influence the methane yield of energy crops. Fresh maize had the highest methane yield/hectare at late harvest ($6270 \text{ m}^3 \text{ CH}_4 (10^4 \text{ m}^2)^{-1}$, Figure 2). The methane yield/wet weight (WW) increased from 80 (early harvest) to $137 \text{ m}^3 \text{ CH}_4 (\text{t WW})^{-1}$ (late harvest), while the specific methane yield/volatile solids content ($\text{m}^3 \text{ CH}_4 (\text{kg VS})^{-1}$) was not significantly influenced neither by the variety nor by the harvest time (Bruni et al., **I**, 2010).
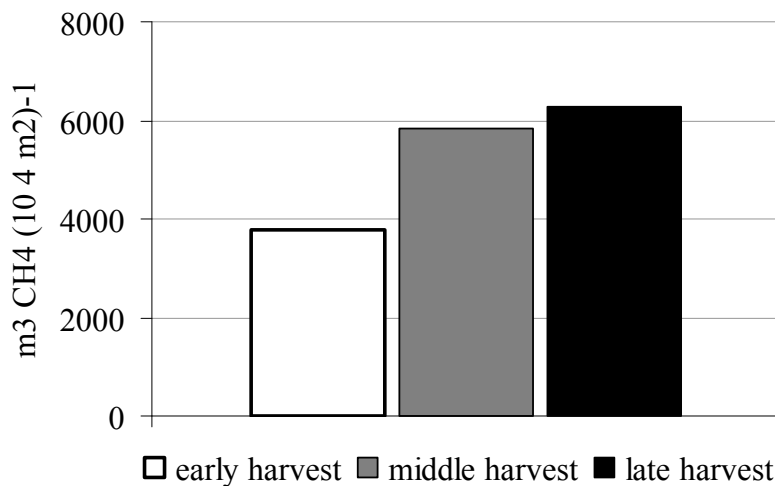
Figure 2 – Methane yield of fresh maize/hectare

The quality of the substrate can be further improved with treatments prior to anaerobic digestion. Treatments on lignocellulosic materials are made to facilitate the hydrolysis of the sugars (cellulose and hemicellulose). However, being successful in improving the methane yield of the substrate is not enough for a treatment to be considered optimal for biogas production. The degradation or loss of organic matter and the formation of inhibitors have to be avoided. Also, some treatments are capital-intensive, while other treatments are too slow or have high energy requirements. The choice of the treatments for industrial applications has to take into account technological and environmental factors such as energy balance, recycling of chemicals and downstream processes. For example, thermal liquefaction (with pyrolysis and solvolysis as the two main areas of research) are very promising methods to obtain low molecular weight liquid, gas fuel and solid residue (Liu & Zhang, 2008). On the other side, these need temperatures of 250-450 °C and pressures around 10 bar, making these treatments energy demanding and attractive mainly for substrates with high dry matter content (nutshell, cherry stones, sawdust). Treatments such as microwave radiation, ozonolysis or concentrated acid hydrolysis have been technically successful to treat lignocellulose, but they have been shown to be energetically and/or economically not feasible due to either the large energy input, or amounts of reactants and downstream processing needed (Eggeman & Elander, 2005; Sun & Cheng, 2002).

Many treatments have been suggested and tested so far and each of them has advantages and drawbacks. The treatments considered during this PhD study

have been selected among those with low energy requirements either as energy input or as downstream processes. The effect of each treatment has been evaluated according to the effect on the measured methane potential.

3.2. Process monitoring and control

High utilization of the potential of the process and minimization of process failures lead to an overall high efficiency of the biogas process (Heinzle et al., 1993). The availability of reliable process indicators and analyzers is crucial to maintain full-scale biogas processes at high load avoiding failures. For this purpose, bioprocesses are often equipped with SCADA systems.

To date, biogas processes use SCADA systems for online monitoring of parameters such as biogas production rate, pH and for automatic control of e.g. temperature and level in the reactor. Contrary to other bioprocesses like brewery industry or aerobic wastewater treatment, there is still a demand for proper monitoring and control of full-scale biogas processes (Boe et al., 2008). Applications of process control to full-scale biogas digesters treating substrates such as solid waste or manure are needed. In commercial-scale biogas plants, the control is left to the operator. Depending on the substrate, on the experience and on the information from the available online sensors, the operator adjusts the feed flow. This approach for the control presents risks. If the substrate contains inhibiting or toxic compounds, if the operator is inexperienced or too brave or too cautious, if the online sensors do not measure parameters that are relevant for process control, the biogas process will run at low efficiency or will fail. Thus, there is a need for the development of online analyzers to measure parameters that are good indicators of the biogas process and there is a need for algorithms for automatic control of the biogas process.

3.2.1. Online monitoring

Different parameters for online monitoring of biogas processes have been studied and are currently the object of research (Scherer et al., 2009). The monitored parameter should detect imbalances at an early stage and should be easy to measure online with simple and cheap equipment without knowledge on the characteristics of the influent. Biogas production, pH, VFA concentration in the biogas digester are among the parameters that have been suggested (Liu et al.,

2006; Steyer et al., 1999). For biogas processes digesting well-buffered substrates such as manure, the VFA concentration seems to be among the most suitable parameters. The concentration of VFA gives quick reactions to changes in the reactor, indicating imbalances between the populations of microorganisms involved in the biogas process. Boe et al. (2008) tested control algorithms based on online monitoring of VFA concentration using a prototype online VFA analyzer (Boe et al., 2007). A new VFA analyser based on this prototype was tested during this PhD study and was installed at pilot-scale biogas plants digesting cow manure (Bruni et al., **IV**, 2010). A filter was developed to allow automatic sampling (Figure 3).
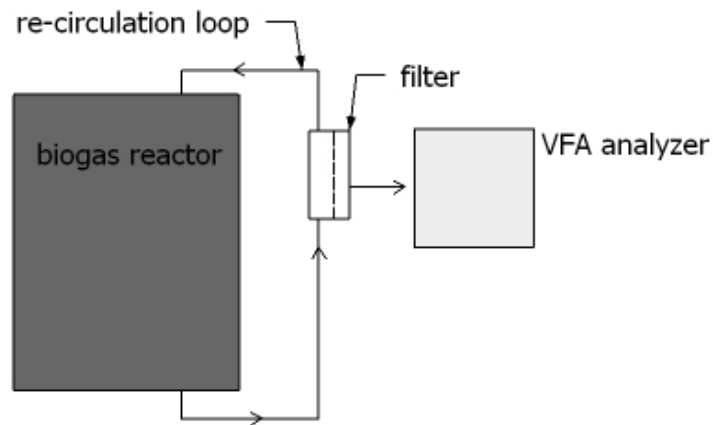

Figure 3 – Pilot-scale biogas plant: position of filter and VFA analyzer

With regular backwashing (every seven days), the filter worked well and allowed automatic sampling. On a 14-day measuring campaign, the online VFA measurements showed good agreement with the manual measurements, although the repeatability needs further investigations (Figure 4).
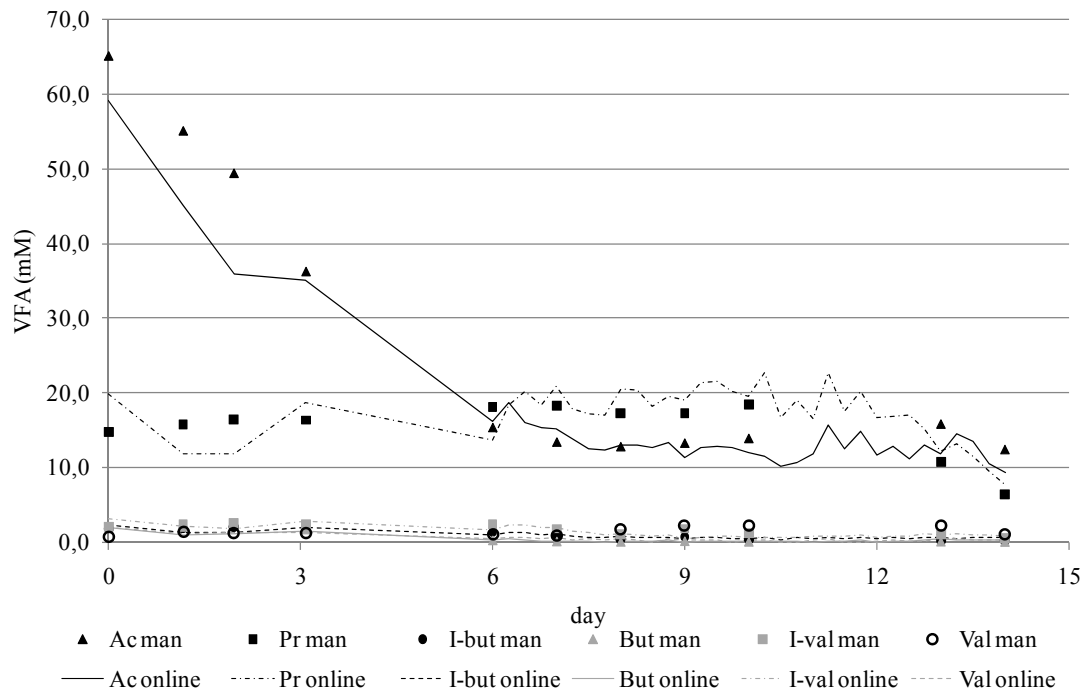
Figure 4 – VFA measurements. Online VFA analyzer (online) and manual measurements (man)

3.2.2. Automatic control

Once reliable online analyzers are available, automatic control can be implemented. During this PhD study, the SCADA of the online VFA analyzer was integrated into the already existing SCADA of the pilot-scale biogas plant (Bruni et al., **IV**, 2010). This step is required for implementation of automatic control.

Once reliable online analyzers and SCADA integration are achieved, the control strategy can be applied. Previous researchers reported that bio-chemical processes are difficult to control (Bastin & Dochain, 1993; Simeonov, 1999). The main factors contributing to the difficulty in control of bio-processes are the non-linear and dynamic behavior, the complexity of available mathematical models and the difficulty to predict model parameters. Heinzle et al. (1993) presented a review of modeling and control of anaerobic wastewater treatment processes. Control algorithms are mostly simple conventional PID type (proportional-integral-derivative). Adaptive controls seem to be the best solution for applications with bio-processes (Polihronakis et al., 1993), but these control strategies are complex and difficult to implement. Feed-forward control strategies

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require detailed knowledge on the dynamics of the process and on the influent characteristics, therefore this kind of control strategy does not seem to be feasible for bio-processes, especially for full-scale biogas plants digesting inhomogeneous waste. Feed-backward control strategies (the input is modified after the value of the output is known) can be implemented to control bio-processes. This type of control strategy is recommended for operation with the online VFA analyzer considered during this PhD study.

4. Waste lignocellulose

4.1. Waste lignocellulose as a resource

Waste lignocellulose has become a valuable resource and biogas processes are among the biotechnologies that can convert it into valuable products.

Lignocellulose is the main component of plants and it is made mainly of cellulose, hemicellulose and lignin. The carbohydrate fraction of lignocellulose (cellulose and hemicellulose) is called holocellulose. Although different types of hemicelluloses and lignins can be found in nature, in this thesis the common terms "hemicellulose" and "lignin" are often used because the distinction among the different types is not relevant for the purpose of this study. The composition of herbaceous plants and agricultural waste is summarized in Table 1.

Table 1 – cellulose, hemicellulose and lignin content in agricultural residues (Akpinar et al., 2010; Cybulska et al., 2010; Jiele et al., 2010; McKendry, 2002; Olsson, et al., 1996; Sun & Cheng, 2002)

	Cellulose (% of TS)	Hemicellulose	Lignin
Hardwood stem	35-50	25-40	15-20
Softwood stem	40-50	25-35	25-35
Grasses	25-40	25-50	10-30
Leaves	15-20	80-85	0-5
Solid cattle manure	1-5	1-3	2-6

Lignocellulose is considered a valuable resource because it is widely available, it has techno-economical advantages and an environmentally friendly character (Avérous & Le Digabel, 2006).

Lignocellulose can be utilized as substrate for fuel production (for example for biogas or ethanol production) or in biorefinery concepts, where it is treated to separate it into its three main components and to convert them into different biofuels or biomaterials (Demirbas, 2008; Lee et al., 2009; Ni & Sun, 2009; da Silva et al., 2009). Biotechnologies for optimal utilization of lignocellulose are being developed; a discussion on the best management practices can be found in Cherubini & Ulgiati (2010) or Demirbas (2009).

High-value lignocellulosic materials have been used for years as substrates in first generation biofuel production. In recent years, dedicated non-food energy crops and waste lignocellulosic substrates have come to the attention at industrial level. Waste lignocellulosic substrates include forestry and crop residues, grasses and biofibers from manure.

Bioconversion of lignocellulose poses several challenges. Aside from logistical and technical problems like harvesting, storage and mixing in the biogas reactor (Bruni et al., **I**, 2010; Gibbons & Hughes, 2009), the main challenge is to enhance the susceptibility to biodegradation of lignocellulose (Bruni et al., **II**, **III**, 2010). Knowledge about the composition and structure of lignocellulose is crucial to design effective treatments and this is described in the following paragraph.

4.2. Composition and structure of lignocellulose

Plant cells are completely surrounded (aside for some selective passages) by the cell membrane and by one or two cell walls, depending on the plant specie and on the tissue considered. The primary cell wall is the most external protection, the secondary cell wall is placed between the primary cell wall and the cell membrane (Figure 5). A layer of polysaccharides (pectin) is at the interface between walls of adjacent cells and cements them together. The primary cell wall is more flexible and thin compared to the secondary cell wall. It is composed mainly by polysaccharides, while the secondary cell wall contains higher amounts of lignin embedded in the carbohydrate polymer matrix.

Lignin is among the most resistant components in plants. Lignin is produced in different amounts in the primary and secondary cell wall, depending on the function of that tissue and not all plants produce lignin in the same amounts. The highest amount of lignin is found in the xylem, the tissue with function of transporting water and nutrients through the plant. As the plant grows, the walls of the cells are encrusted with lignin and become thicker and stiffer. Also small amounts of proteins (enzymes and structural proteins), lipids, resins, tannins and flavonoids are found in the cell walls.
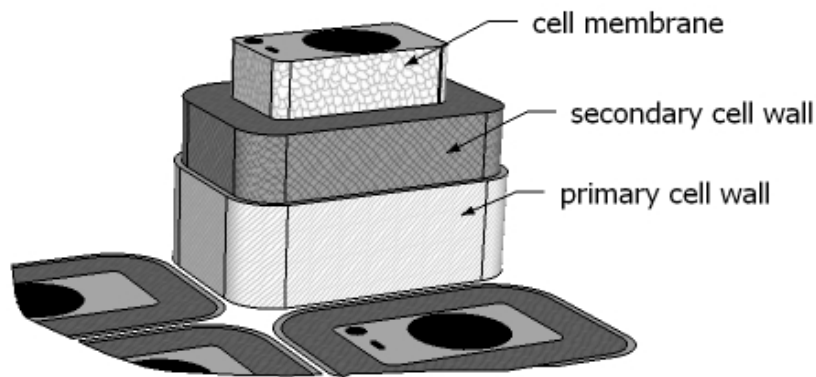
Figure 5 –Plant cell walls (adapted from Klemm et al., 2005)

4.2.1. Cellulose

Cellulose is the main component of lignocellulose. Cellulose is a polymer made of cellobiose units (two β-1,4-glycosidic bound glucose molecules) rotated by 180 degrees with respect to the neighbor molecules. Cellobiose units form chains with number of units between 100 and 14000. These chains are grouped into water-insoluble aggregates (elementary fibrils) that present crystalline regions and less ordered amorphous regions (Béguin & Aubert, 1994). Elementary fibrils are organized in microfibrils, which are embedded into a matrix of hemicellulose and lignin (Klemm et al., 2005; Ramos, 2003). Microfibrils are further organized into microfibrillar bands (Figure 6). Because of the presence of hydroxyl groups R-OH, cellulose is hydrophilic and can form hydrogen bonds. However, cellulose is water-insoluble because of the large dimensions of the molecule. Intra- and intermolecular hydrogen bonds strengthen cellulose along the direction of the chains and connect it to the network formed by hemicellulose and lignin (Saha, 2003).
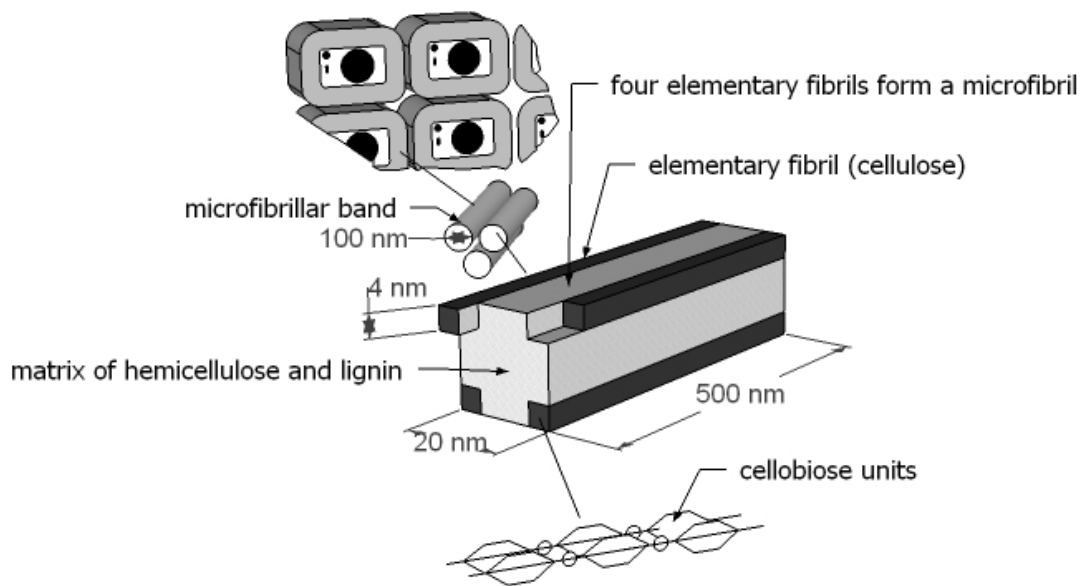
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Figure 6 – Representation of plant cell walls

4.2.2. Hemicelluloses

Hemicelluloses are not chemically homogeneous. Hemicellulose is composed mainly by pentose sugars and the basic structure is formed by 1,4-bound xylose units with different side chains. Other carbohydrates forming hemicellulose are arabinan, mannan, galactan, glucan. The degree of polymerization of hemicellulose is between 70 and 200. Depending on the plant species, hemicellulose is acetylated to different degrees: some of the -OH groups at C_2 and C_3 of the xylose units are replaced by O-acetyl groups (Sassner et al., 2008). Although hemicellulose is the weakest compound in lignocellulose, it plays a fundamental role in strengthening the structure: hemicellulose is linked to the other polysaccharides, to lignin and to proteins, forming a network.

4.2.3. Lignins

Lignins are phenolic polymers with complex three-dimensional structure. The monomeric unit is composed by an aromatic nucleus with an aliphatic chain, $(C_6-C_3)_n$. Lignin polymers are formed by syringyl, guaiacyl and p-hydroxyphenyl units that are chains formed from sinapyl alcohol, coniferyl alcohol and p-coumaryl alcohol, respectively. The composition of lignin changes depending on the plant specie and age. Guaiacyl lignin is the most abundant compound in lignins from conifers, syringyl and p-hydroxyphenyl in dicotyledons

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angiosperms, while lignins from monocotyledons angiosperms (grasses and herbaceous crops) contain the three components in similar proportions (Cultrera, 1968; Widsten & Kandelbauer, 2008a). Amorphous regions are found together with more structured regions (globules and oblong particles), resulting in an inhomogeneous structure (Novikova et al., 2002).

4.3. The need for treatments

Lignocellulose has a complex and rigid structure insoluble in water and resistant to mechanical stress and enzymatic attack. Because of the combined effects of accessible surface area, presence of lignin and crystallinity of cellulose, water molecules can not enter the lignocellulosic fibers. Lignin protects and strengthens the fibers, inhibiting the action of enzymes (Saulnier et al., 1995). Also, the crystalline structure of cellulose decreases the surface available for contact with enzymes.

Although lignocellulose is porous (600-800 m^2 of surface area per gram of substrate), the pore size of lignocellulose is approximately 5 nm because of the tight connections between the three main components cellulose, hemicellulose and lignin. Only molecules smaller than 5 nm can enter the structure. This explains why enzymes (dimensions between 5 and 18 nm depending on the shape) need long reaction times while acids (hydrated hydrogen ions of 0.4 nm diameter) can diffuse through the substrate (Grethlein & Converse, 1991; Schacht et al., 2008).

A method suitable to treat all types of lignocellulosic raw materials for production of all different types of biofuels can not be identified (Hann-Hägerdal et al., 2006; Sun & Cheng, 2002). An effective treatment should increase the porosity of the substrate making the carbohydrates more accessible for the enzymes, preserving the different fractions without degrading or loosing organic matter and limiting the formation of inhibitors. Additionally, the treatment should be inexpensive. Many treatments have been suggested and tested so far and each of them has advantages and drawbacks. The optimal conditions for the treatment depend on the characteristics desired for the product. Treatment conditions have to be optimized according to the process that will utilize the treated substrate. Because the microorganisms involved in the biogas process are able to utilize a broad variety of organic compounds (such as pentoses, hexoses,

fatty acids, proteins and lipids), the main goal of a treatment for biogas production is to increase the accessibility to the holocellulose content of the lignocellulosic material.

During this PhD study, four treatments (biological, chemical, mechanical, hydrothermal) and combinations of them have been tested on biofibers from digested manure or on maize silage. The results are summarized in Table 2 and are described more in detail in the following paragraphs.

Table 2 effects on methane yield of biofibers from digested manure

treatment		variation % of yield m^3 CH_4 $(t\ WW)^{-1\ a}$
Biological	partial aerobic	no effect
	enzymatic	no effect
Chemical	CaO	+ 66%
Mechanical	size reduction	+ 10%
Steam	no catalyst added	+ 29%
	H_2SO_4	+ 67%
	H_3PO_4	+ 8%
	NaOH	+ 26%
Combined	steam + H_3PO_4 + laccase	+ 18%
	steam + NaOH + laccase	+ 34%

[a] WW of untreated biofibers

4.4. Biological treatment

4.4.1. Enzymatic treatment

Enzymatic treatments on lignocellulosic substrates use enzymes for hydrolysis or oxidation of lignocellulose.

The enzymatic hydrolysis of holocellulose requires the action of different groups of cellulases and hemicellulases. Although the chemical specificity for β-1,4-glycosidic bonds is common for all cellulases, there are differences depending on the fiber morphology. The result is that the combined action of different cellulases with different specificities for crystalline and amorphous cellulose enhances the overall activity of the enzymes (Teeri, 1997). Similarly, because hemicelluloses are not chemically homogenous, the action of different enzymes is needed. Commercially available enzymes are mixtures of cellulases and

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hemicellulases and have been used in this study. The addition of the enzymes did not enhance the hydrolysis and the overall conversion into biogas, because improvements of the methane yield were not observed (Bruni et al., **III**, 2010). Probably, the small pores size of the substrate did not allow the enzymatic reaction to take place.

Oxidative enzymes are used for enzymatic treatment of lignin, which is an oxidation process rather than a hydrolysis. Oxidative enzymes use oxygen as the electron acceptor and can catalyze the oxidation of a wide range of substrates, including phenols (lignin). Oxidases such as laccase, lignin peroxidase and manganese peroxidase can delignify lignocellulose (Hatakka, 1994; Ohkuma, 2003). Laccases are used in industry (textile bleaching or pulp delignification) and are found in nature (polymerization in plants and depolymerization of lignin by fungi). As many other enzymes, different laccases present different specificity depending on the substrate, but all of them catalyze the oxidation of the hydroxyl group of the phenols to phenoxy radicals. These radicals can undergo further non-enzymatic transformations and the oxidative degradation products of lignin are typically carboxylic acids and aromatic carbonyl compounds. Because of their large dimensions and because of their low redox potential, laccases alone can oxidize only the easily oxidisable phenolic units at the substrate surface. Therefore, laccases are often used in combination with an electron-transferring mediator. A mediator is a small molecule that can diffuse through the structure of the substrate and oxidize lignin. The first step of the laccase-mediator system is the oxidation of the mediator by the enzyme. Then, the oxidized mediator oxidizes the substrate (Figure 7). Thus, the mediator acts as an electron shuttle between the substrate and the enzyme (Galli & Gentili, 2004; Widsten & Kandelbauer, 2008b).
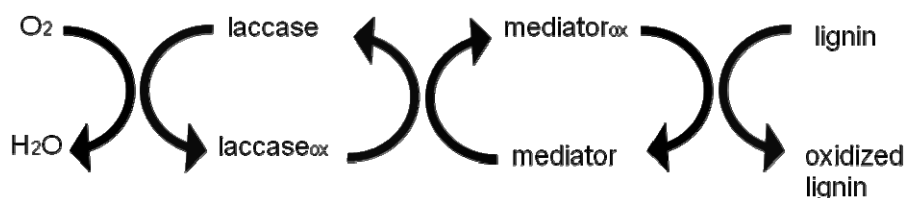

Figure 7 –Mediated oxidation of lignin by laccase (Palonen, 2004)

In this study, commercial products for laccase enzymes and mediators were used. Treatment with laccases (with or without mediator) and with cellulases and hemicellulases did not increase the methane yield of biofibers from digested

manure (Bruni et al., **III**, 2010). Methane yield improvements were obtained when enzymatic treatment was combined with steam treatment, as described later in this thesis.

4.4.2. Partial aerobic treatment

Some aerobic microorganisms such as fungi (white-, brown-, soft-rot fungi) or bacteria can selectively degrade lignin or holocellulose. The aim of partial aerobic treatments is to aerobically treat the lignocellulose for a short time to initiate decomposition of the lignocellulosic structure and increase its biodegradability, avoiding that the aerobes can achieve the oxidation of the holocellulose.

Mild environmental conditions, low energy and no chemical requirements are among the advantages of treatments with microorganisms. On the other side, these treatments have very low reaction rates (days or weeks) and in some cases microorganisms consume holocellulose. The ideal partial aerobic treatment should have high rate of lignin degradation and very low rate of carbohydrates degradation. Some microorganisms such as brown-rot fungi attack preferably cellulose and are not recommended for partial aerobic treatment for biogas production. White-rot fungi (and soft-rot fungi to a smaller extent) attack only hemicellulose and lignin, leaving cellulose available for conversion in the following biogas process. These fungi can have a secondary metabolism in response to carbon or nitrogen limitation and can produce lignin-degrading enzymes such as laccase, lignin peroxidase and manganese peroxidase (Kumar et al., 2009).

Some treatments with microorganisms succeeded in delignification, increasing the susceptibility to enzymatic hydrolysis. Treatments with microorganisms have been applied to substrates such as rice straw, office paper, agricultural waste, kitchen waste. Often, the studies were made with pure cultures of microorganisms and the effect of the treatment was evaluated from the structural changes of the components of the substrate and from the susceptibility to enzymatic hydrolysis (Dhouib et al., 2006; Kurakake et al., 2007; Schober & Trösch, 2000; Srilatha et al., 1995; Taniguchi et al., 2005). During this PhD study, the effect on methane production was used as the parameter to evaluate the partial aerobic treatment. Because for full scale applications the use of pure

cultures of aerobic microorganisms may not be possible (unless these can be cheaply cultivated at the biogas plant), only partial aerobic treatments with mixed cultures have been tested during this PhD study. Partial aerobic treatments did not increase the methane yield of the substrate (Bruni et al., **III**, 2010).

4.5. Chemical treatment

Chemical treatments include treatments with acids, bases, oxidants. Treatments with acids can hydrolyze carbohydrates, while treatments with bases and oxidants can attack also lignin (Bezzi, 1968; Sanchez & Cardona, 2008) and avoid fragmentation of the hemicellulose polymers (Taherzadeh & Karimi, 2008). This study focused on treatment with alkali (CaO). Alkaline hydrolysis decreases the degree of polymerization and acts as saponification of the intermolecular ester bonds crosslinking hemicellulose and lignins. When the structural linkages between lignin and the carbohydrates are disrupted, the porosity and the internal surface area of lignocellulose are increased. Chemical treatments with bases can convert lignin (recalcitrant to anaerobic digestion) into substrates such as VFA (Kaparaju & Felby, 2010) suitable for biogas production. Chemical treatments with sodium hydroxide (NaOH) are among those that have been investigated most. Alkaline hydrolysis with NaOH has been successfully applied to treat lignocellulosic materials such as straw or hardwood (Sun & Cheng, 2002). However, treatment with NaOH may be problematic because the effluent from biogas plants is often used as a fertilizer. The presence of NaOH in the fertilizer is not desired because sodium ions can degrade the quality of the soil. Treatment with CaO can be an attractive and low-cost alternative compared to NaOH. In this study, methane yield improvements of up to 66% were obtained treating biofibers from digested manure with CaO (Bruni et al., **III**, 2010).

4.6. Mechanical treatment

Mechanical treatment (size reduction) is a straightforward treatment for biofibers from digested manure (Hartmann et al., 2000). Increasing the accessible surface area, this treatment can improve the methane yield of the substrate. Methane yield improvements of 10-20% were registered for biofibers from manure and maize silage (Angelidaki & Ahring, 2000; Bruni et al., **I**, **III**, 2010). Mechanical treatment proved to be suitable for applications at pilot-scale and full-scale biogas plants and increased the methane yield of lignocellulosic substrates by up

to 25% (Hartmann et al., 2000) and of municipal solid waste by 14% (Ghosh et al., 2000). Even higher effects on lignocellulosic substrates like herbaceous grasses have been reported (Seppälä et al., 2009), with reductions of the required digestion time by 23-59% (Hendriks & Zeeman, 2009).

Depending on initial and final particle size and substrate properties such as moisture content, mechanical treatment can have high energy requirements (often as electricity). Because the highest methane yield increases are obtained with the smallest particle sizes, mechanical treatment may become energetically and economically unsustainable (Rabelo et al., 2009).

4.7. Steam treatment

Steam treatment applies steam at high temperature and pressure, often in combination with a catalyst. The objective is to achieve sufficient solubilization of the lignocellulose to enhance the hydrolysis. Typically, sulfuric acid H_2SO_4 (Bruni et al., **II**, 2010) is used as the catalyst, but also other acids, bases (Bruni et al., **III**, 2010) or oxidants can be used.

The treatment takes place in a pressure vessel where the substrate and catalyst are introduced (Figure 8). The desired temperature is reached introducing steam and is maintained for the duration of the treatment. After the set time, the steam is slowly released and the treated substrate is collected (Figure 9). The treated material is composed by a solid fraction and a liquid fraction (hydrolysate). The solubilization of the lignocellulose depends on treatment temperature, duration, pH, moisture content (Talebnia et al., 2009). Hemicellulose is the first component to be hydrolyzed (at around 150 °C), because of its short chains and the relatively weak hydrogen bonds with cellulose. Cellulose is affected by steam treatment to a minor extent and needs harsher treatment conditions (Allen et al., 2001; Caparrós et al., 2008; Kaparaju et al., 2009; Sassner et al., 2008). Lignin is fluidized at temperatures between 120 °C and 200 °C and then it coalesces into smaller particles that are deposited when the temperature decreases. When acids are used as the catalyst, the overall effect of steam treatment on lignin is its reallocation (Kaparaju & Felby, 2010). Oxidants or bases can react with lignin and can form fatty acids, either by reaction with the phenols or with the aliphatic chains (Passera, 1983).
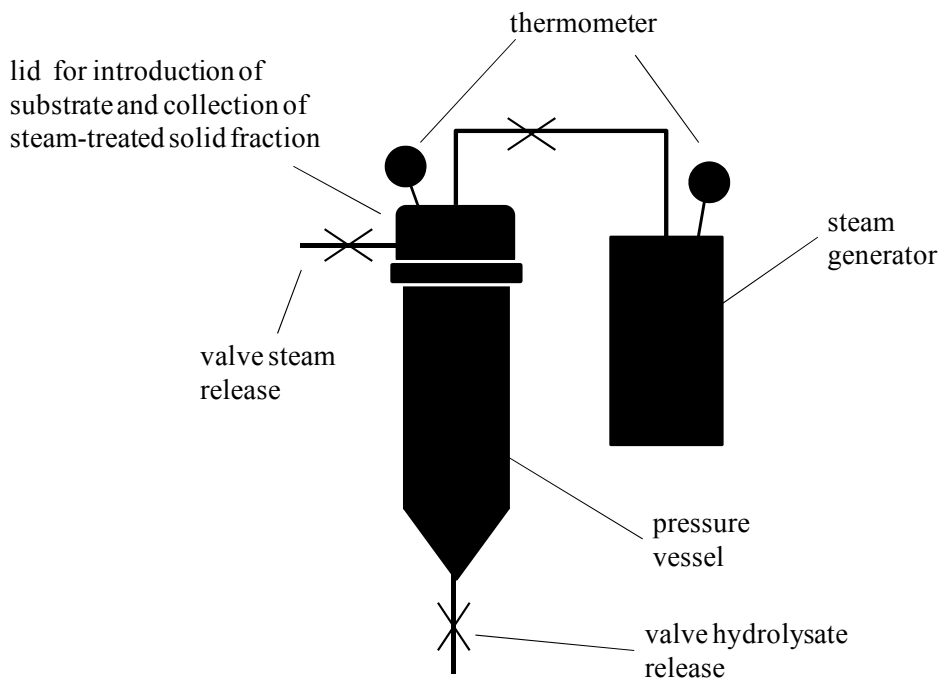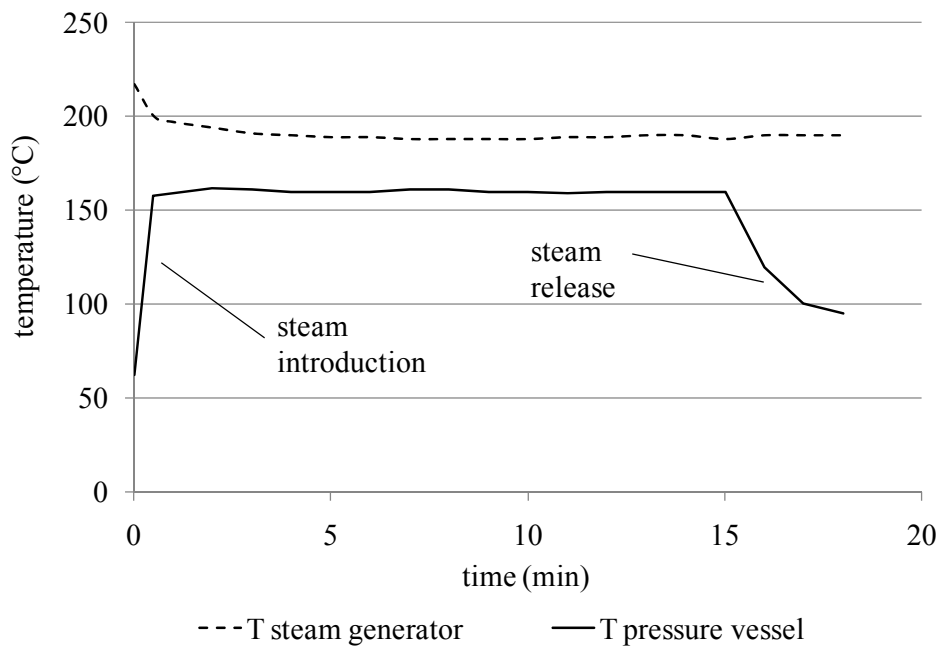
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Figure 8 – Unit for steam treatment

Figure 9 – Temperature profile during steam treatment

Steam treatment was applied to municipal solid waste to produce homogeneous pulps, to facilitate the extraction of recyclable components like metal and glass and to increase biodegradation (Glass et al., 2005). Wang et al. (2009) studied hydrothermal treatment of sorted municipal solid waste at 170 °C with addition

of NaOH for biogas production. In this PhD work, steam treatment was applied to biofibers from digested manure. It was found that optimal steam treatment conditions for increasing biogas production (67-43% increase) were 155-160 °C, with dosage of H_2SO_4 (2.1-2.3% w/w TS) (Bruni et al., **II**, 2010). Higher steam treatment temperature (180 °C) without addition of catalyst increased the methane yield by 29% (Figure 10). Long duration of pre-soaking in H_2SO_4 (24 hours) resulted in inhibitory hydrolysate, probably because of the presence of inhibitory compounds such as furfural or HMF (Figure 11). As steam treatment with H_2SO_4 proceeds, carbohydrate polymers are converted into shorter chain polymers and then into monomers. Steam treatment can further convert pentose and hexose monomer sugars into furfural and HMF (hydroxymethylfurfural), respectively. These are aromatic compounds with four carbon atoms and one oxygen atom and they are formed from the sugars by elimination of three molecules of water. Furfural and HMF are toxic for microorganisms (Bezzi, 1968) and are undesired in the hydrolysate.
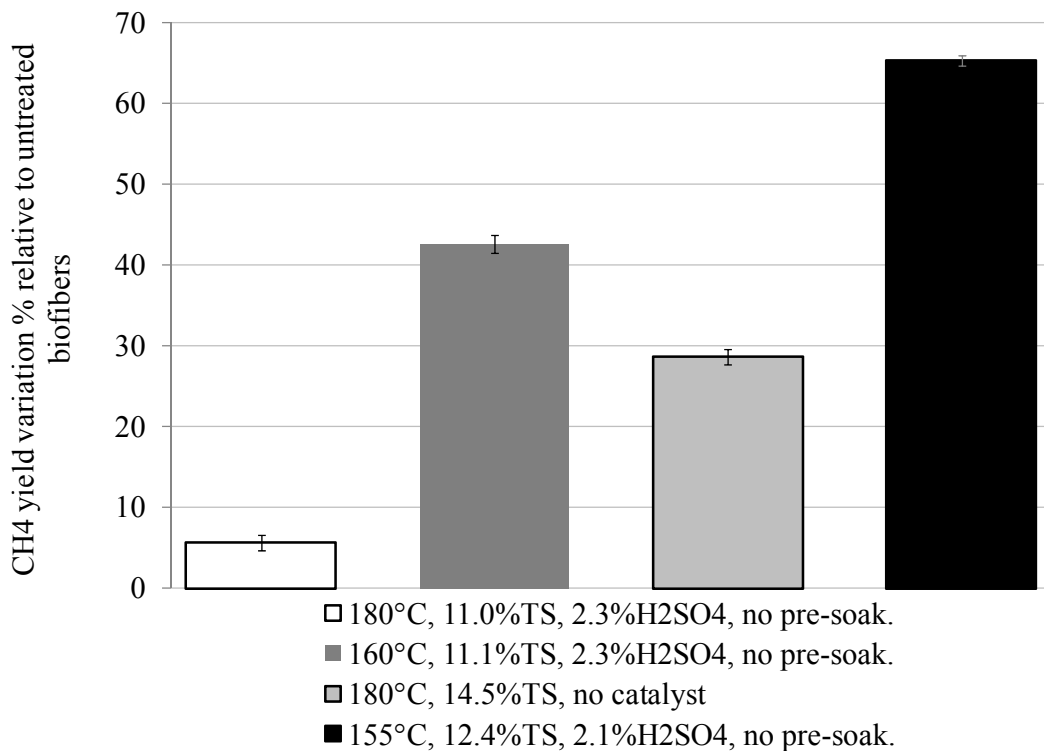

Figure 10 – Variation % of methane yield m^3 CH_4 (t WW)$^{-1}$ of the treated biofibers (mixture of solid fraction and hydrolysate) with respect to yield of untreated biofibers
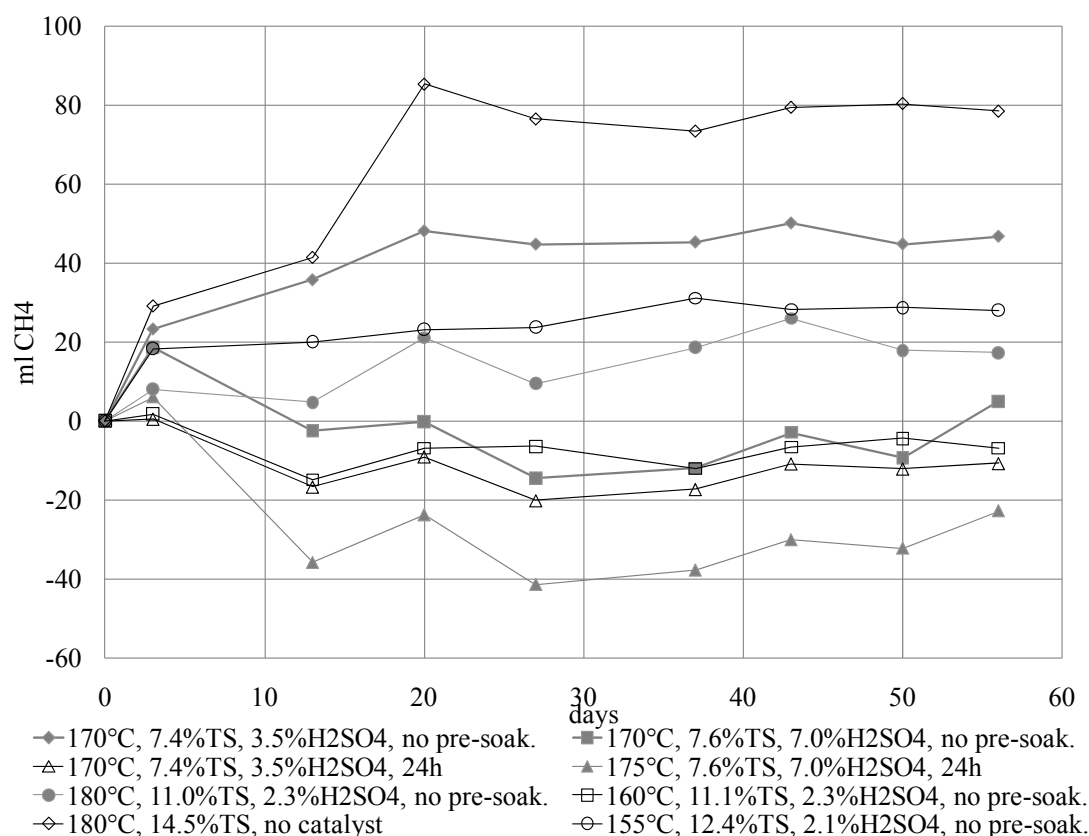
Figure 11 – Net methane production from hydrolysate

Aside from the formation of inhibitors, H_2SO_4 may have another adverse effect on the biogas process. The sulfate ions SO_4^{2-} used for steam treatment are introduced through the treated substrate into the biogas digester. The reduction of SO_4^{2-} to H_2S competes with the biogas process (hydrogenotrophic and aceticlastic microorganisms) (Zehnder & Stumm, 1988). Also, H_2S is an undesired compound in the biogas, because it can be corrosive and polluting if oxidized to SO_2 or SO_3 (Ahammad et al., 2008). Although it was calculated that the concentration of SO_4^{2-} in the steam-treated substrates was insignificant compared to the VS content of the substrate used for the biogas process (Bruni et al., **II**, 2010), the use of catalysts H_3PO_4 and NaOH was investigated (Bruni et al., **III**, 2010). The catalyst H_3PO_4 has the advantage of lower rate of side reactions and formation of inhibiting compounds compared to H_2SO_4 (Geddes, et al., 2010). Steam treatment with H_3PO_4 improved the methane yield by 8%, that was low compared to steam treatment with H_2SO_4. Probably, higher concentrations of H_3PO_4 have to be used to achieve higher methane yield improvements (Gámez et al., 2004; Geddes, et al., 2010; Um et al., 2003).

Biofibers treated with steam and catalyst NaOH resulted in 26% higher methane yield compared to untreated biofibers (Bruni et al., **III**, 2010) and had high conversion rate (Figure 12). Steam treatment with NaOH addition may have converted part of the lignin into acetic acid while steam treatment with H_3PO_4 addition may have just reallocated lignin (Kaparaju & Felby, 2010). It is reported that oxidative treatments in alkaline conditions convert carbohydrates and lignin into carboxylic acids (Schmidt & Thomsen, 1998). In this study, steam treatment with NaOH may have had a similar effect of acetic acid formation. The presence of the easily degradable compound acetic acid in the steam-treated material explains the high conversion rate of this material into methane.

Advantages of steam treatment are the low reaction time (in Bruni et al., **II, III** (2010), 15 minutes were used) and low dosage of catalyst (in Bruni et al., **II** (2010), the best results were obtained without H_2SO_4 dosage or with 2.3% H_2SO_4 w/w). Energy requirements for steam generation are among the drawbacks of steam treatment. Also, acetylated xylose units of hemicellulose may be released as acetic acid during steam treatment (Allen et al., 2001; Duff & Murray, 1996; Sassner et al., 2008). This process of autohydrolysis (Di Stefano & Ambulkar, 2006) may cause losses of volatile compounds when the steam is released at the end of the treatment. This represents a potential energy loss and has to be measured with mass balances in order to be taken into account when the overall efficiency of the treatment is calculated. However, the amount of acetic acid formed during the treatment may be negligible.
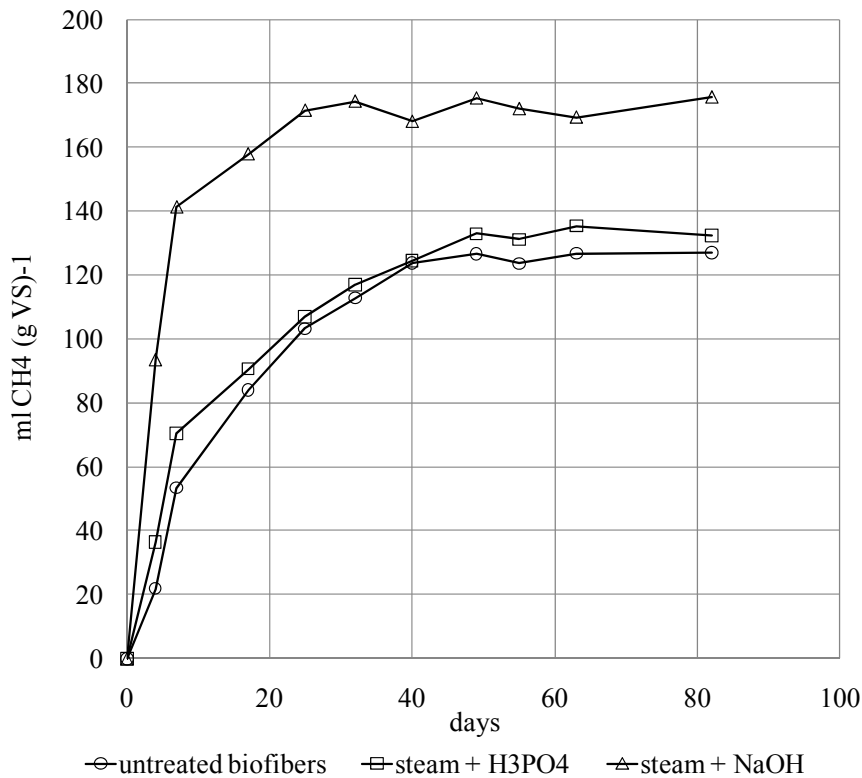
Figure 12 – Steam treatment, specific methane yield from batch tests

4.8. Combined steam and enzymatic treatment

Treatments can be applied in series to enhance the susceptibility to biodegradation and the methane yield of lignocellulose. For example, mechanical treatment is sometimes applied prior to steam or chemical treatment (Ghosh et al., 2000). During this PhD study, enzymatic treatment with laccase was applied in series after steam treatment with H_3PO_4 and steam treatment with NaOH. The overall methane yield increase of the combined treatment steam with catalyst and substequent enzymatic was higher compared to the methane yield registered for steam treatment alone (Table 2). Enzymatic treatment alone did not result in higher methane yield (Bruni et al., **III**, 2010). This suggests that the tight association of lignocellulose did not allow effective enzymatic treatment, if the porosity of the substrate was not previously increased by steam treatment (Lu et al., 2009). Treating with laccase the steam-treated material without adding mediator increased the methane yield of the biofibers. Probably, laccase oxidized lignin to an extent sufficient to improve the biodegradability, although it is reported that laccase in the absence of a mediator can only oxidize small fractions of lignin (Widsten & Kandelbauer, 2008b). Similar findings without

addition of mediator were obtained by Palonen & Viikari (2004). It is possible that the oxidizing substances (solubilized or colloidal lignin) contained in the steam-treated material acted as mediators in the enzymatic reactions (Felby et al., 1997; Grönqvist et al., 2003).

Combined steam treatments and laccase treatments (with or without mediator) may be economically not feasible for full-scale applications because of the costs of the enzymes and mediators (Maijala, et al., 2008). However, some enzymes and cheap mediators originating from plants or industrial by-products are available at prices interesting for commercial applications (Widsten & Kandelbauer, 2008b).

It would be beneficial to carry out simultaneous enzymatic hydrolysis of lignin and biogas processes: whereas the hydrolysis of lignin proceeds, cellulose and hemicellulose can be converted into methane. However, a difficulty is that the enzyme laccase and the biogas process need different conditions. In particular, lignin oxidation by laccase takes place in presence of oxygen, while oxygen is poisonous for methanogenic microorganisms.

5. Concluding remarks

The efficiency of the biogas process can be improved with treatments on the substrate or introducing online monitoring and automatic control. Different advantages have been reported for some methods, however none of the treatments tested during the PhD study was able to meet all requirements of an ideal treatment for lignocellulosic substrates. For example, the chemical treatment (66% methane yield increase) required the longest reaction time (10 days) and high CaO dosage. The steam treatment with H_2SO_4 (67% increase) or the combination of steam treatment with NaOH and enzymatic treatment (34% increase) may be capital-intensive and have high energy costs connected to the high temperature needed for steam generation. However, steam treatment is probably the most suitable for biogas production, because of the short reaction time, low dosage of catalyst and due to the possibility to use waste heat from a CHP unit as energy input for steam production. Mechanical treatment is a straightforward treatment method, but the energy input (as electric power) may become higher than the energy content of the extra methane produced.

Integration between two SCADA systems was successfully achieved for a pilot-scale biogas plant. SCADA systems of biogas plants can be modified for automatic feeding based on the setpoint from a different SCADA system.

Some of the issues raised during this PhD study need further investigations. Although the evaluation of the advantage of a treatment should take into consideration total environmental effects, this PhD study focused only on the effect on the methane yield of the substrate and on some considerations on the energy requirements. Optimization of steam treatment with NaOH and detailed energy analysis of steam treatment are required.

Before the online VFA analyzer can be used for full-scale applications, technical obstacles have to be overcome. Errors in the VFA measurements may lead to wrong calculations of the amount of substrate to be dosed with automatic feeding. This can be dangerous for the stability of the biogas process. Carry-over from previous measurements has to be minimized and safety devices have to be installed to stop automatic sampling in case of clogging. The repeatability will have to be investigated within pilot-scale and full-scale experiments.

Nevertheless, online monitoring of VFA at pilot-scale showed good agreement with manual VFA measurements, making automatic control based on online VFA measurements an interesting opportunity for future applications.

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7. Appendices

I.   Bruni, E., Jensen, A.P., Pedersen, E. S., & Angelidaki, I. (2010). Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. *Appl. Energy, 87,* 2212-2217

II.   Bruni, E., Jensen, A.P., & Angelidaki, I. (2010). Steam treatment of digested biofibers for increasing biogas production. *Bioresour. Technol.* (doi: 10.1016/j.biortech.2010.04.064, in press).

III.   Bruni, E., Jensen, A.P., & Angelidaki, I. (2010). Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. Submitted to *Bioresour. Technol.*

IV.   Bruni, E., Boe, K., Jensen, A.P., & Angelidaki, I. (2010). Online VFA measurements in biogas processes: sampling, calibration and integration of SCADA systems. Technical note.

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Water Resource Engineering, Urban Water Engineering,
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The department dates back to 1865, when Ludvig August Colding, the founder of the department, gave the first lecture on sanitary engineering as response to the cholera epidemics in Copenhagen in the late 1800s.