Different Pretreatments to Enhance Biogas Production

-A comparison of thermal, chemical and ultrasonic methods

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Summary

Fossil energy sources are the most used energy supply in the world today, however the increased prices of oil and increased awareness of climate change will trigger the increasing use of renewable energy, such as biogas.

The objective of this study is (1) to investigate how much pretreatment processes can influence the result in biogas production, (2) to compare which pretreatment is the optimal option in the balance of economical and environmental considerations and (3) to find out the application of pretreatment in large-scale biogas production in the future.

It is hard to identify the most suitable pretreatment for all types of lignocellulosic materials (Hann-Hägerdal et al., 2006). The effective pretreatment should have three qualities: (1) increase the porosity of the substrate which makes the carbohydrates more accessible for enzymes, (2) preserving the different fractions without losing or degrading organic matters and (3) limiting the formation of inhibitors. Furthermore, the pretreatment should take economic issues into consideration. Each pretreatment has advantages and drawbacks. The optimal operation depends on the characteristics of the materials. The main purpose of pretreatment for biogas production is to increase the accessibility to the hemicellulose content of the lignocellulosic material.

Inoculum which is based on cow manure, crop residues and fruit and vegetable waste, was used in the experiments and was collected from Plönninge biogas plant outside Halmstad. Substrates such as sugar beets, maize and straw were collected in Halmstad, Ensiled lay and stored under mesophilic temperature. In this study, all of the substrates were chopped into small pieces.

Different pretreatments have different effect on different substrates with different mixing ratio of inoculum and substrates.

From this study, it can be concluded that, chemical pretreatment is not suitable for carbohydrate-rich and easily degradable substrate. Although all the thermal pretreatments had positive effect on methane yield especially with straw which increased methane yield by 54%. The difference between thermal pretreatment and reference was not significant. Although ultrasonic pretreatments had a positive effect on the methane yield of most substrates with the highest obtained with sugar beets/sugar beet leaves and maize which increased by 43% and 41% respectively. The difference between ultrasonic pretreatment and reference was not significant.

However, chemical pretreatments increased methane yield the most with sugar beets/sugar beet leaves and straw by 68% and 102% respectively which is an amazing increase. Considering of full-scale applications, chemical pretreatment is a good option with highest increased methane yield and low-cost operation.
Abstract

Europe Commission emphasizes the 2020 target that the share of renewable energy should reach 20% and the share of renewable energy fuel should increase by 10%.

In Sweden, according to Environmental Objectives Portal three actions are underway to achieve the goal of Reduced Climate Impact. (1) At least 50% of Sweden's energy consumption should come from renewable sources by 2020. (2) Efficiency of energy use should increase by 20%.(3) Vehicles and boats will not depend on fossil energy in 2030. There is no doubt that renewable energy resources are needed urgently.

The objective of this study is (1) to investigate how much pretreatment processes can influence the result in biogas production, (2) to compare which pretreatment is the optimal option in the balance of economical and environmental considerations and (3) to find out the application of pretreatment in large-scale biogas production in the future.

In the group of different substrates, only ensiled lay and straw showed significant difference among the pretreatments with p-values lower than 0.05.

Chemical pretreatments increased the most methane yield with sugar beets/ sugar beet leaves and straw by 68% and 102% respectively while it had a negative effect on the methane yield of ensiled ley by 39%. All the thermal pretreatments had positive effect on methane yield especially with straw which increased methane yield by 54%. Ultrasonic pretreatments had positive effect on the methane yield of most substrates with the highest obtained with sugar beets/sugar beet leaves and maize which increased methane yield by 43% and 41% respectively.

Key words: biogas, anaerobic digestion, chemical pretreatment, thermal pretreatment, ultrasonic pretreatment
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1. Introduction

1.1. Background

Fossil energy sources are the most used energy supply in the world today, however the increased prices of oil and increased awareness of climate change will trigger the increasing use of renewable energy, such as biogas (Khanal, 2008).

According to Shell International, from the years 1990 to 2100, energy consumption will increase at least by 7 times compared to now. In the meantime, the IPCC (International Panel on Climate Change) has predicted energy consumption will increase by 3 times during this period. With the high demand for energy, renewable energy sources will cover 30% of the primary energy consumption globally in 2050. In 2075, the anticipated use renewable energy will go up to 50% and it will increase continuously to 2100. This situation has made biogas more valuable. According to IPCC in 2050 biomass will produce 5000/TWh. In 2075 this will increase to 75000/TWh and it will keep increasing to 89000/TWh in 2100.

In Europe, EC (Europe Commission, 2011) emphasizes the 2020 target that the share of renewable energy should reach 20% and the share of renewable energy fuel should increase by 10%.

In Sweden, according to Environmental Objectives Portal, three actions are underway to achieve the goal of Reduced Climate Impact. (1) At least 50% of Sweden's energy consumption should come from renewable sources by 2020. (2) Efficiency of energy use should increase by 20%. (3) Vehicles and boats will not depend on fossil energy in 2030. There is no doubt that renewable energy resources are needed urgently.

Biogas, produced during anaerobic digestion, is mainly composed of methane and carbon dioxide and seems to be an alternative choice to traditional energy (Khanal, 2008). Typically, it contains 60-65% methane which is flammable. With the technology of biogas utilization improving, it becomes one of the most widely used waste/residues-to energy technologies (Khanal, 2008).

Traditionally, biogas has been used as fuel to support the process temperatures in anaerobic digesters. Another alternative use is that the gas is burned in an engine generator of combustion to produce electricity in biogas plants. Biogas has also been used as fuel for cooking, lightning and vehicles (Khanal, 2008). In Sweden, biogas for vehicle fuel is considered as the best alternative to traditional fuels (U.S department of ENERGY).

If at a commercial scale of producing biogas, the complex physical and chemical structure of the lignocellulosic substrates cannot be completely biodegraded in
anaerobic digesters (Rafique, 2010). To solve the problem of increasing the potential for biogas for used in the digestion process, some pretreatments can be operated. (Bruni, 2010a). Rafique (2010) reported that thermo-chemical pretreatment have a great impact on biogas production with a maximum enhancement of 78% for biogas and 60% for methane. Thermal pretreatment also have effect on biogas production with a maximum enhancement of 28% for biogas and 25% for methane. This indicates that pretreatment of substrates urgently needs further investigation.

1.2. Purpose and limitations

The objective of this study is:

- To investigate how much pretreatment processes can influence the result in biogas production
- To compare which pretreatment is the optimal option in the balance of economical and environmental considerations.
- To find out the application of pretreatment in large-scale biogas production in the future.

Low-cost feedstocks are always used to achieve cost-effective biotechnologies for biogas production. (Ni&Sun, 2009; Rabelo, 2009) However, low-cost feedstocks are usually accompanied with low biodegradability. Thus, a loss of methane production and the limitation of the whole efficiency of the anaerobic process will be caused by the low biodegradability of substrates of agriculture residuals (lignocellulose) in the biogas plant. (Jin et al., 2009). For instance, agriculture residuals such as straw and manure, are low-cost feedstocks. However, their character of low digestibility makes them relatively resistant to the anaerobic processes. (Hendriks, 2009) Pretreatments can solve the problem of low digestibility of substrates and make them degrade efficiently in biogas processes. (Demirbas, 2008)

The optimization of pretreatments needs further research. Due to different characteristics, different substrates prefer different pretreatments. Sometimes, pretreatments make no difference to the biogas production of some substrates and the energy demand of pretreatments decreases the energy efficiency of the biogas process. However, if optimal pretreatment is applied, biogas products will increase significantly. To master the optimization action is urgently needed.

Since the experiment was performed in a laboratory-scale environment, translating this into large-scale biogas plants increase the difficulties. However, this research may still open a door for further research in this area.
1.3. Benefits of Biogas Production

Biogas production, except for its use as renewable energy source, has many other benefits.

In many countries, farmers have to give up their occupation because of their land no longer produces enough yield from conventional agricultural production. Biogas production is subsidized in many countries which give an additional income to the farmers. There is a tendency for wider unused agricultural areas and of farms becoming large-scale industries, which will change the landscape. Biogas production with small-scale farm production could maintain the structure of the landscape. Energy can be generated from the unneeded biomasses which can save the natural resources. Compared anaerobic degradation metabolism products to aerobic ones, organic acid and methane contain higher energy than low-energy compounds CO₂ and H₂O, which serve other organisms as nutrients or energy as twenty times as much as the energy lost to air. Biogas plant can reduce landfill area and protect groundwater quality. Due to anaerobic processes, organic matters can be reduced down to 4% which reduce landfill area and protect the groundwater. Furthermore, because the reduction of biomass is significant, reuse of the residue from biogas process, such as fertilizers, can cut down the expenditure of organic wastes. If co-substrates are used in biogas plants, mineral fertilizers can be replaced by residue. The advantages are cutting down spends. They can reach the cycle of nutrients and reduce nitrate leaching. Methane and nitrous oxide emissions (N₂O) are reduced when residue and manure are digested instead of being spread on the field or stored. The digested residue also produce is less odorous. This process also supports the Kyoto agreement of climatic protection by achieving CO₂-neutral production of energy. It can reduce the fees for the management of waste water and avoid the connection of sewers, especially in rural areas. Also, there is a significant reduction has been monitored of pathogenic germs in the digested residue after anaerobic process. It can minimize the spread of weed seeds by eliminate by them in liquid manure. After the fermentation process, liquid manure becomes more highly liquid which is much easier for soil to absorb (Steinhauser, 2008).

1.4. Anaerobic Process

Through series reactions in the anaerobic process by different groups of bacteria, insoluble and complex organic compounds are degraded to soluble and simple organic compounds. As complex compounds are degraded to simple compounds, they are passing through an anaerobic food chain (Figure 1.4.1). (Gerardi 2003)

Methane fermentation is always the last step in the anaerobic food chain. In such
processes, insoluble and complex organic compounds are degraded to methane, carbon dioxide and minerals. During the degradation of organic compounds, some of the carbon dioxide is produced which is reduced to methane form. All the compounds must be degraded to simple organic and inorganic compounds for methane-forming bacteria to use. For example, formates, methanol, methylamine, acetate, inorganic hydrogen gas and carbon dioxide are formed. (Khanal, 2008)

Methane is the most simple organic compound at the end of the anaerobic food chain. To have success in the food chain, methane-forming bacteria have the most critical influence on the final step. (Bruni, 2010a)

Methane-forming bacteria cannot use organic compounds like butyrate and propionate directly as substrates if they are not converted to acetate. During the anaerobic processes, syntrophic relationships exist between bacteria. At least two kinds of bacteria are involved in the relationships and the action of one organism is dependent on the activity of another organism. An example of this is the syntrophic relationship between hydrogen-producing bacteria and hydrogen-consuming bacteria. Acetate is the most commonly used substrate by methane-forming bacteria which may be degraded in the absence of sulfate. In the presence of sulfate, acetate cannot split into methane and carbon dioxide. (Deublein, 2008)

The process is the achievement of four groups of microorganisms’ combined action: primary fermenting bacteria, secondary fermenting bacteria and two types of archae. The anaerobic decomposition of organic matters will finally turn into biogas (methane and carbon dioxide), typically divided into three steps. Firstly (hydrolysis), substrate is hydrolyzed to smaller units by primary fermenting bacteria. Then acidogenesis and acetogenesis, the formed soluble oligomers and monomers are converted into acetic acid, hydrogen and carbon dioxide by primary fermenting bacteria and secondary fermenting bacteria. The last step (methanogenesis), acetic acid, hydrogen and carbon dioxide are converted into biogas by the archae (see Figure 1.4.1). (Deublein, 2008)

For the optimal work of the decomposition process, the dependence of these three steps should work equally well and providing the next step with the substrate as required. For example, if hydrolysis is inhibited, the substrate to the second and third step will be limited and there is a reduction in methane production as a result (Gerardi 2003).

1.4.1. Hydrolysis

Hydrolysis step happens outside the microbial cells. With extracellular enzymes, either secreted or attached to the surface of cell, primary fermenting bacteria hydrolyzes the substrate. These bacteria are facultative anaerobic microorganisms. During the hydrolysis step, soluble oligomers and monomers are hydrolyzed from polymers with the enzymes (cellulases, hemicellulases, proteases, amylases and
lipases) (Taherzadeh & Karimi, 2008). Thus, during the biogas process a mass of enzymes can be produced and as a result, almost all kinds of substrates can be hydrolyzed during the process except lignin and waxes (Fernandes et al., 2009). The hydrolysis process is a relatively rapid step after suitable enzymes produced from microorganisms. However, if the substrate is hardly accessible for enzymes. The hydrolysis step will be limited for the reason that physical contact between the substrate and enzymes is needed for hydrolysis to happen (Taherzadeh & Karimi, 2008). After the substrate is hydrolyzed, it is available for transport into the cell and can be further degraded through the following steps of biogas processes.

In the first step of hydrolysis, during the entering of water, chemical bonds of carbohydrates, proteins and fats are hydrolyzed to organic substances by bacteria. Before hydrolyzed carbohydrates, proteins and fat molecules are insoluble in water and are too big for the microorganisms to be able to take them into the cell and use them as nutrition. Carbohydrates are divided into simple sugars, proteins into amino acids and fats into fatty acids. The substrate composition determines the rate of hydrolysis. Complex carbohydrate such as cellulose and hemicellulose are broken down more slowly than simple one, for example proteins. (Gerardi 2003)

1.4.2. Acidogenesis and acetogenesis

The products of hydrolysis are absorbed by primary fermenting bacteria and converted into VFA (volatile fatty acid), hydrogen and alcohols. In a well-functioning process acetic acid, carbon dioxide and hydrogen can be used as substrate by methanogenic microorganisms directly. The most positive way of primary fermentative bacteria is the production of acetate via pyruvate with production of hydrogen. If the conditions are not optimal, this pathway is not profitable and other intermediates will be produced by the metabolism switch of the primary fermenting bacteria (Klass, 1984). Due to the excessive supply of substrates or presence of toxic compounds, an increase of hydrogen concentration will show up with the changes in the environmental conditions. In such conditions, intermediates as alcohols longer than one carbon atom and VFA longer than two carbon atoms are formed. (Bryant, 1979; Schink, 1997). These reduced intermediates cannot be used directly by methanogenic microorganisms, therefore these compounds need to be further modified in order to be able to be converted into biogas. Acetogenesis occurs through the conversion of these products into acetic acid, carbon dioxide and hydrogen by secondary fermenting bacteria. In standard conditions, there is no energy produced through the reactions by acetogenic microorganisms. The reaction requires for low partial pressures of hydrogen (lower than $10^{-5}$ bar) to gain energy. The syntrophic association between the secondary fermenting bacteria and one of the two types of archae can keep the partial pressure of hydrogen within the suitable range.
During acidogenesis and acetogenesis, the acetic acid, carbon dioxide and hydrogen is produced as the substrates for the next step of anaerobic process. (Bruni, 2010a)

The products of decomposition are carbon dioxide, hydrogen, alcohols, organic acids and some organic compounds containing nitrogen and sulfur (Gerardi 2003). The acids formed are balanced between its charged form and its uncharged form. Thus, the acid dissociation constant (pKa) for each acid and the prevailing pH determine the present form. As most of the acid has higher pH than pKa in its charged form and lower pH than pKa in its uncharged form. To a biogas plant, anionic acetate is more interesting because it can be used directly as substrate by methane. Since pKa of acetic acid is 4.76 and biogas processes often have pH ≥ 7, acetic acid mainly present as its anion acetate. During the acidogenesis and acetogenesis process, some other products can also be used as substrate for methane, however indirectly. (Jarvis & Schnur, 2009).

1.4.3. Methanogenesis

The last step of anaerobic process is methanogenesis which is carried out by methanogens. The substrates used in the most part are acetate, carbon dioxide and hydrogen which are formed during the previous step. Other possible compounds which may indirectly serve as substrates for methane production include: formats, methylamines and some alcohols. Furthermore, acetate are divided in two parts; one is used to form carbon dioxide and the other is used to form methane (Liu & Whitman 2008).

Hydrogen, acetic acid, carbon dioxide and other one-carbon compounds like methanol and formate are converted into biogas by methanogenic microorganisms. Acetate and hydrogen are used as substrates by aceticlastic microorganisms and hydrogenotrophic microorganisms respectively. Approximately 70% of the carbon flow through aceticlastic microorganisms, even if much lower energy is provided from this pathway compared to the hydrogenotrophic microorganisms (Klass, 1984). The hydrogen produced by the secondary fermenting bacteria is used as substrate for hydrogenotrophic microorganisms. The partial pressure of hydrogen should be above 10^-6 bar, the minimum level for the reaction to be exergonic. Secondary fermenting microorganisms require low partial pressure as described earlier in this thesis. The range of hydrogen partial pressure is narrow to allow the growth of both the hydrogenotrophic microorganisms and the secondary fermenting microorganisms. The syntrophic relationship exists between these two types of microorganisms because of the rigid energy constrains and hydrogen must be consumed as soon as possible after it is produced. The partial pressure of hydrogen is ensured within the optimal range which allows both reactions and consumption to be exergonic by the close physical contact between aceticlastic microorganisms and hydrogenotrophic microorganisms.
The concentrations of acetate and ammonia and the activity of methanogens are the factors that influence this process. In addition, temperature and retention time in the digester are also important aspects. (Bruni, 2010)

![Diagram of biogas production]

Figure 1.4.1 The steps of biogas production. The final organic compound produced in the anaerobic food is methane. This compound is the most reduced form of carbon. (modified from Bruni, 2010a and Gerardi, 2003)

1.5. Process Parameters

Within all the biological processes, keeping the constancy of the living conditions is important. A change in temperature or substrates or substrate concentration can result a shutdown of biogas production. The microbial metabolism process depends on many parameters. A mass of parameters should be taken into consideration and be controlled for an optimum fermenting process. (Beublein, 2008)
1.5.1. pH

The optimal pH range can be divided into two groups, 5.5-6.5 for acidogens and 7.8-8.2 for methanogens. It is important to adjust the pH-value in the optimal range because anaerobic performance is affected by a slight pH changes away from the optimum. For the combined cultures pH ranges from 6.8 to 7.4 will be the ideal. (Beublein, 2008)

In the low pH environment, the activity of methanogens will be reduced, result in the accumulation of acetic acid and hydrogen. With higher partial pressure of hydrogen, propionic acid-degrading bacteria will be inhibited which causes the accumulation of VFA, which slows down the production of acetic acid making the pH drop further. Finally the biogas process fails. (Khanal, 2008)

1.5.2. Temperature

Temperature is one of the most important factors influencing the anaerobic process especially in methane production. Compared to the operating temperature, the variation in temperature has much more influence the methane-forming bacteria. Furthermore, it affects not only the methane-forming bacteria but also volatile acid-forming bacteria (Gerardi, 2003). Maintaining the optimal digester temperature is the most important problem during anaerobic process. (Beublein, 2008)

1.5.3. Nutrients (C/N ratio)

The C/N ratio of the substrate should be within the range of 16:1-25:1. Due to the fact that not much biomass is developed with the anaerobic process, the need for nutrients is very low. Just as too low C/N ratio causes an increase in ammonia production and an inhibition of methane production, too high a C/N ratio causes negative influence in protein formation and a decline in the energy and structural metabolism of the microorganisms. It is necessary to keep a balanced composition of C/N ratio. (Beublein, 2008)

1.5.4. Inhibitors

The concentration of the inhibitors, the composition of the substrate and the adaptation of the bacteria to the inhibitor are all matters that influence the decision of the inhibition process. Commonly inhibitors include oxygen, sulfur compounds, organic acids, nitrate, ammonium and ammonia, heavy metals. (Beublein, 2008)
1.5.5. Hydrogen Partial Pressure

An undisturbed process between the hydrogen producing acetogenic bacteria and hydrogen consuming-methanogenics is quite narrow. A well-balanced hydrogen concentration is required during the process, because methanogenics need enough hydrogen for methane production while the hydrogen partial pressure should be low enough to prevent the acetogenic bacteria from surrounding too much hydrogen and consequently stop the hydrogen production. The optimal hydrogen partial pressure depends on the species of bacteria and substrates (Deublein and Steinhauser, 2008).

1.5.6. Type of Substrate

During anaerobic process, substrates play an important role which determines the rate of the anaerobic degradation. The metabolism will shut down by the microorganisms if the important component of a substrate runs out. Therefore, it is always important to feed possibly lacking substance like carbohydrates, fat, proteins, mineral substance as well as the substrate. (Beublein, 2008)

Intermediate products of the decomposition of substrates can also inhibit degradation. For example, the degradation of fats will increase the concentration of fatty acids, which limits further degradation. (Beublein, 2008)

1.5.7. Specific Surface of Material

The material surface should be as big as possible to support a biochemical reaction. The material surface is associated with the square of particle size. It is recommened that comminution of biomass can increase the surface of material. Bigger specific surface leads to higher biogas yield though a relationship is not linear. (Beublein, 2008)

1.5.8. Disintegration

Disintegration is the destruction of the cell structure, sometimes even of the cell walls with higher energy impact. It is hard to define if disintegration is suitable for normal biomass fermentation plant for its plenty of advantages and backwards. Today, it is mainly used for swage gas production. (Beublein, 2008)
1.5.9. Cultivation, Mixing and Volume load

To avoid the failure of start-up phase of the plant, a careful but intensive mixing of the reactor is chosen. (Beublein, 2008)

The volume load depends on the residence time, the organic dry matter in the substrate and the temperature. If the substrate contains more than 12% solids, gas production is impaired. However, a too low load causes economically loose. (Beublein, 2008)

1.6. The composition and structure of lignocellulose

Plant cells are totally covered by the cell membrane and one or two cell walls depends on the different type of plants. The primary cell wall is the most external protection while the secondary cell wall is in the middle of primary cell wall and the cell membrane. Between walls of continuous cells there is a layer of polysaccharides, mainly pectin, to bond cells together. Compared to the secondary cell wall the primary cell wall is more flexible because of the different composition. The primary cell wall is composed mainly by polysaccharides while there is much lignin embedded in the carbohydrate polymer matrix in the secondary cell wall.

Lignocellulose consists of mainly three types of polymers, cellulose, hemicellulose, and lignin, which are related to each other (Hendriks, 2009).

1.6.1. Cellulose

The main component of lignocellulose is cellulose which exists of d-glucose subunits, linked by β-1,4-glycosidic bonds and rotated by 180 degrees with respect to the neighbor molecules. The structure of cellulose in a plant consists of two parts, the crystalline region and the amorphous region. The cellulose strains are grouped into water-insoluble aggregates so called cellulose fibrils or cellulose bundles which are mostly independent and weakly bound through hydrogen bonding (Laureano-Perez, 2005). After elementary fibrils organized in microfibrils, they are embedded into a matrix of hemicellulose and lignin (Klemm et al., 2005; Ramos, 2003). Furthermore, microfibrils are organized into microfibrillar bands. Cellulose is hydrophic and can form hydrogen bonds with the presence of hydroxyl groups. However, cellulose is insoluble in water for its character of large dimensions of the molecule. Cellulose is strengthened by its intramolecular and intermolecular hydrogen bonds along the direction of the chains and connected to the hemicellulose and lignin network(Saha, 2003).
1.6.2. Hemicellulose

Hemicellulose is a complex carbohydrate structure composed by pentose sugars, hexoses and sugar acid and the basic structure is formed by 1,4-bound xylose units with different side chains. Xylan is the dominant component of hemicellulose from hardwood and agricultural plants, like grasses and straw while glucomannan is found in softwood (Fengel and Wegener, 1984).

The molecular weight of hemicellulose is lower than cellulose and its short lateral chains which consist of different sugars are easy to be hydrolyzed (Fengel and Wegener, 1984). Hemicellulose is acetylated to different degrees depending on the different plant species (Sassner et al., 2008). Hemicellulose is the weakest compound in lignocellulose, however, it is a foundation in strengthening the structure by serving as a connection between lignin and cellulose fibers and gives the whole cellulose-hemicellulose-lignin network more intensity.

The solubility of hemicellulose is increased by the temperature. Because of unknown melting points, the solubility of higher molecular polymers is unpredictable (Gray, 2003). According to Bobleter (1994), the solubilization of hemicellulose compounds in water starts around 180° C under neutral conditions. However, parts of the hemicellulose are already solubilized from 150° C according to Garote (1999). The solubilization of lignocellulose compounds also depends on moisture content and pH (Fengel, 1984).

In an acid or alkaline environment, xylan of hemicellulose can be easily extracted while glucomannan can only be extracted in a stronger alkaline environment than xylan which makes xylan the most easily extractable among all components of hemicellulose. (Balaban, 1999; Lawther 1996b)

Among cellulose, hemicellulose and lignin, hemicelluloses are much more thermal-chemically sensitive (Levan, 1990).

1.6.3. Lignin

Lignin is present in the cellular wall, one of the most plentiful polymers in nature. It is an amorphous heteropolymer composed by syringyl, guaiacyl and p-hydroxyphenyl units that are formed from sinapyl, coniferyl and p-coumaryl alcohol, respectively. Different species and ages of plant have different composition of lignin. Conifers have rich compounds of Guaiacyl in lignin, dicotyledons angiosperms have rich syringyl and p-hydroxyphenyl while monocotyledons angiosperms have three similar proportions in lignins. (Cultrera, 1968; Widsten & Kandelbauer, 2008a). Inhomogeneous structures are found in lignins because amorphous regions are
grouped together with more structured regions (Novikova et al., 2002). The main purpose of lignin is to support the plant’s structure against microbial attack and oxidative stress. The degradation of lignin is very tough for its amorphous heteropolymer character of being non-water soluble and inactive (Fengel and Wegener, 1984).

Just like hemicellulose, lignin normally starts to dissolve into water around 180°C under neutral conditions (Bobleter, 1994). The solubility of the lignin in neutral, acid or alkaline environments depends on the composition of the lignin (Grabber, 2005).

1.7. Different Pretreatments

Pretreatment of substrates can increase biogas production and volatile solids and solubilisation of substrates which make it more accessible to enzymes. (Tanaka et al., 1997). They are particularly useful in the digestion of lignocellulosic materials as they contain high cellulose or lignin level. Pretreatment can disrupt these recalcitrant polymers chemically, thermally or physically. The addition of pretreatment can enhance the biogas rate or reduce the time of startup, however, the additional cost must be considered to be balanced against resultant improvements in efficiency (Alastair J. Ward, 2008).

Lignocellulose is a tough material which has a complex and rigid structure resistant to mechanical stress and enzymatic attack, insoluble in water. Water molecules cannot enter the lignocellulosic fiber because of the combination of accessible surface area, presence of lignin and crystallinity of cellulose. The fibers are protected and strengthened by lignin which is inhibiting to the action of enzymes (Saulnier et al., 1995). Furthermore, the crystalline structure of cellulose decrease the availability of surface contact to enzymes. (Hendriks, 2009)

It is hard to identify the most suitable pretreatment for all types of lignocellulosic materials (Hann-Hägerdal et al., 2006). The effective pretreatment should have three qualities: (1) increase the porosity of the substrate which makes the carbohydrates more accessible for enzymes, (2) preserving the different fractions without losing or degrading organic matters and (3) limiting the formation of inhibitors. Furthermore, the pretreatment should take economic issues into consideration. Each pretreatment has advantages and drawbacks. The optimal operation depends on the characteristics of the materials. The main purpose of pretreatment for biogas production is to increase the accessibility to the hemicellulose content of the lignocellulosic material. (Hendriks, 2009)

Chemical, thermal and ultrasound pretreatments are found to have a great effect on lignocellulose materials with cheap costs and they are easily access.

According to Bruni (2010b) highest methane yield increase is obtained through the
chemical treatment that resulted in 66% higher methane production compared to untreated biofibers.

In González-Fernández’s (2008) study the best pretreatment was thermal application which increases the methane production by 35%.

According to Carrère (2010) the experiment carried out in Singapore showed the methane production increased by 45%, with an energy ratio of 2.5 by ultrasonic pretreatment.

It is interesting to compare these three most commonly used pretreatment.

1.7.1. Thermal pretreatment

During thermal pretreatment, lignocellulose is heated. At above 150-180°C, parts of the lignocellulose will start to solubilize. First the hemicelluloses followed by lignin (Bobleter, 1994).

Due to thermal hydrolysis, thermal pretreatment is effective at increasing methane production. Thermal pretreatment also showed enhancement with maximum enhancement at 100 °C having 28% biogas and 25% methane increase (Rashad Rafique 2010).

Two dominant components of hemicelluloses are xylan and glucomannan and they are thermally stable. An exothermal reaction of hemicellulose starts above 180 °C. (Beall 1970). A part of hemicellulose is hydrolyzed and forms acids during thermal processes. These acids are catalyzed during the further hydrolysis of the hemicellulose (Gregg, 1996). The catalyzing effect of in situ formed acids plays an important role in the solubilization of hemicellulose (Wyman, 2003). Thermal pretreatment of 160 °C causes the solubilization of not only hemicellulose but also of lignin. The produced compounds from the solubilization of lignin are very reactive and in many cases inhibit the bacteria (Liu, 2003). Most reports showed an optimal temperature from 160 °C to 180 °C within 30 to 60 minutes. According to Dohanyos (2009), a thermal pretreatment at 170 °C only need 60 s. On the other hand, a thermal pretreatment at 70 °C may last several days. However, thermal pretreatments with temperatures above 150 °C showed an increase in solubilisation but no increase in methane production. Furthermore, higher than 170-190 °C causes a decrease in biodegradability which is called a Maillard reaction. Carbohydrates and amino acids formed melaniodins, which are hardly degraded. Thermal pretreatments can also enhance hydrolysis rates and reduce HRT days. (González-Fernández, 2008)

According to Rafique (2010), thermal pretreatment showed enhancement in the temperature range 50-100 °C, with maximum enhancement at 100 °C, having 28% biogas and 25% methane increases.
1.7.2. Chemical pretreatment

Chemical pretreatments include acid pretreatment, alkaline pretreatment and oxidative pretreatment. When treated with acid, carbohydrates can be hydrolyzed. With alkaline and oxidative pretreatment, lignin can also be attacked (Bezzi, 1968; Sanchez & Cardona, 2008) and the fragmentation of hemicellulose polymers can be avoided (Taherzadeh & Karimi, 2008). The first reactions during alkaline pretreatment are solvation and saponification which decreases the degree of polymerization and disrupt the intermolecular ester bonds crosslinking hemicellulose and lignins. This makes the substrate more accessible for enzymes and bacteria. When the disruption of the structural linkages between lignin and carbohydrates happens, this causes an increase in the internal surface area of lignocellulose. Alkaline pretreatment can convert lignin into substrate suitable for biogas production such as VFA (Kaparaju and Felby, 2010). Peeling of end-groups, alkaline hydrolysis and degradation and decomposition of dissolved polysaccharides occurs at strong alkali concentrations dissolution. This causes a loss of polysaccharides (Fengel and Wegener, 1984). For later conversions, this peeling is actually an advantage, because lower molecular compounds are formed and the loss of carbon and risk on degradation, in form of carbon dioxide, increases. An important aspect is that the biomass on itself consumes some of the alkali. After alkaline consumption by biomass, the concentration of residual alkali is left over for the reaction (Gossett et al., 1982)

Compared to NaOH, treatment with CaO is an attractive and low-cost alternative (Bruni et al., 2010b). Also according to Gossett (1982), lime works better than sodium hydroxide.

According to Bruni’s study 2010b, methane yield improvements of up to 66% were obtained treating biofibers from digested manure with CaO.

Alkali pretreatments, however, are not without problems. In continuous reactors fed with alkaline-treated sample, due to toxic compounds generated during the saponification reaction, there is a fall in acetate and glucose degradation, 5% and 50%, respectively (Mouneimne et al., 2003). (Alastair J. Ward 2008)

1.7.3. Ultrasonic Pretreatment

Ultrasonic pretreatment is one kind of mechanical pretreatment which can increase the specific surface area, reducing the degree of polymerization. All the factors increase the total hydrolysis yield of the lignocellulose. However, the high energy requirements require some kind of mechanical pretreatment not economically feasible. e.g. milling. (Khanal, 2008)
During ultrasonic pretreatment, a high alternating voltage is generated by ultrasonic energy which causes cavitation beyond the human audio range. Due to an internal negative pressure formed by ultrasonic waves, the formation of small bubbles of gas takes place during the cell disruption process. The cell membrane is destructed by the stunning pressure and temperature caused by ultrasonic energy. The longer the ultrasonic pretreatment operates, the smaller the quantity of accumulated mud bacteria which normal size is around 100µm. (Carrère, 2010)

Ultrasonic pretreatment is commonly used to disrupt the cell structure and floc matrix. According to Kim (2003) methane production was found to increase by 34% for ultrasonic-treated sludge when compared to untreated one. Two key mechanisms associated with ultrasonic pretreatment are cavitation and chemical reaction. Cavitation occurs at low frequencies and chemical reaction due to the formation of OH•, HO2•, H• radicals at high frequencies. The sonication of substrate causes disintegration of sludge floc and dissolution of microorganisms, according to the treatment time and power, equating to the energy required. (González-Fernández, 2008)

A transducer containing a piezoelectric substance that converts high-frequency electric current into vibrating ultrasonic waves is an ultrasonic waves producer (Khanal, 2008). The main components of a typical ultrasound system are:

(1) a transducer converts electrical energy into ultrasonic waves (Khanal, 2008).
(2) a booster that increases the wave amplitude by acting as a mechanical amplifier (Khanal, 2008).
(3) a sonotrode of horn which delivers the ultrasonic waves to the sludge (Khanal, 2008).

1.7.4. Biological pretreatment

Biological pretreatment includes two processes: aerobic and anaerobic. These are composed of excess sludge destruction in-process and biological pretreatment prior to anaerobic digestion (Carrère, 2010). The objective of biological pretreatment is to enhance the hydrolysis process in an additional stage prior to the main digestion process.

The enzyme can catalyze biological reactions. Almost all the enzymes we know of proteins with six basic classes: oxidoreductases, transferases, hydrolysases, lyases, isomerases and ligases. Enzymatic lysis is an enzyme catalyzed-reaction produced by the cracking of the compounds of the cell wall.

Bioly® E, an industrial process accompanied with the aerated sludge process, is becoming a commercial industry by Ondeo-Degremont (Suez). Thermophilic reactors
where enzymes are produced are used for thickened sludge.

1.7.5. Combined pretreatments

To obtain further enhancements of biogas production, a combined pretreatment, with at least two pretreatments, is suggested. Some studies have found that the synergies of mixed and matched pretreatments might optimize the overall outcome. In several studies, the combination of chemical and thermal pretreatment is found to have a great effect on increasing biogas production.

However, the use of combined pretreatment will potentially increase the complexity of process operation and require higher input economically. (EPSRC)

2. Materials and methods

2.1. Collection of materials

Inoculum which is based on cow manure, crop residues and fruit and vegetable waste, bacteria was used in the experiments and was collected from Plönninge biogas plant outside Halmstad, used to digest energy crops and other substrates. Substrates such as sugar beets, maize and straw were collected in Halmstad, Ensiled lay and stored under mesophilic temperature. In this study, all of the substrates were chopped into small pieces.

2.2. Reactor design

There are thirty-two 1000 ml glass bottles with rubber stoppers, which act as digester. The bottles are settled in a thermostat where the temperature can be observed nearly 37° C. (Fig.2.2.1) There is a hole in each rubber cap attached with a tube. The tube then goes from the bottle to another cap which is connected to a U-tube containing water at a specified level. All U-tubes are calibrated before the test is started. As gas is produced in the bottle, this increases the water column in the U-tube which finally reaches the infrared photo-electrode. Then a "bubbling" is recorded by a counter. After each bubbling, water returns to the starting level again. A counter records date, time, temperature, air pressure and the volume of gas produced.
2.3. Operation of pretreatments

Thermal pretreatment: the substrates were heated at 70 °C for 1 hour

Ultrasound pretreatment: the substrates were put into an ultrasound machine (type J.P. Selecta Ultrasons 110 W) for 3 minutes

Chemical pretreatment: This study is focusing on alkaline pretreatment with CaO, CaO was put into the substrates for 20 days in a sealed box

2.4. Analysis

Analysis of dry matter in manure was done by taking some of the material and putting it in an aluminum container. Each sample had a replicate. Empty containers were weighed first, then weighed with materials (approximate 40g). The containers were settled in a furnace at temperatures 105 °C for 24 hours. After that they were weighed again. After this operation, containers were put in another furnace with 550°C for 3 hours and weighed again. Total solids (TS) and volatile solids (VS) of contents can be calculated with the formula 1.1

Measuring the pH of the manure was performed with a pH meter.

The material was analysed in an elemental analysis to determine the content of carbon and total N. The analysis was performed with a machine of mark Flash EA 1112 Series NC Soil Analayzer. The material was crushed with a mortar and then 15-20 mg was weighed in small tin molds. The molds were placed in the machine.
The biogas composition was determined using a gas chromatograph (VARIAN CP-3800). A 0.2 µl of gas sample was injected into the chromatograph with a column temperature of 30° C. Helium was used as the carrier gas. The sample gas concentration was compared to a standard mixed gas consisting of 20% methane and 0.15 % carbon dioxide or pure methane. The gas chromatograph is calibrated with high concentrations of methane (50, 75 and 100%)

All experiments were conducted at the University of Halmstad.

2.5. Statistical Analysis

ANOVA test was performed with software SPSS 16.0 to see the statistical significant differences between the pretreatments with different substrates. The statistical significance level was selected at p-value < 0.05

2.6. Process

The first experiment was performed with 25 bottles during 25 days and gas production per day was monitored. Bottle 4, 8, 12, 16, 20, 25 served as references during the trial. Sugar beets and sugar beet leaves were the substrates mixed with inoculum at certain ratios. Thermal, ultrasound and chemical pretreatments were applied in the experiment. The ratios of inoculum and the substrates, their weights, pretreatments are shown in table 2.5.1.
Table 2.5.1. Description of digester contents and pretreatments of experiment 1

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<th>ratio</th>
<th>Inoculum (g)</th>
<th>Sugar Beets (g)</th>
<th>Sugar beet leaves (g)</th>
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<th>Ultrasound pretreatment</th>
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<td>35</td>
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</tr>
</tbody>
</table>

The second experiment was performed with 13 bottles during 25 days and gas production per day was monitored. Bottle 4, 8, 12, 13 served as references during the trial. A mixture of straw and sugar beet leaves with 74% of straw and 26% of sugar beet leaves, are the substrate mixed with inoculum at certain ratios. Thermal, ultrasound and chemical pretreatments were applied in the experiment. The ratios of inoculum and the substrates, their weights, pretreatments are shown in table 2.5.2.
Table 2.5.2. Description of digester contents and pretreatments of experiment 2

<table>
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<tr>
<th>Ratio</th>
<th>Inoculum (g)</th>
<th>Straw/Sugar (g)</th>
<th>Beet leaves (g)</th>
<th>Thermal Pretreatment</th>
<th>Ultrasound Pretreatment</th>
<th>Chemical Pretreatment</th>
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</table>

The third experiment was performed with 25 bottles during 25 days and gas production per day was monitored. Bottle 4, 8, 12, 13, 17, 18, 19, 25 served as references during the trial. Maize, ensiled ley were the substrates mixed with inoculum at certain ratios. Thermal, ultrasound and chemical pretreatments were applied in the experiment. The ratios of inoculum and the substrates, their weights, pretreatments are shown in table 2.5.3.
### Table 2.5.3. Description of digester contents and pretreatments of experiment 3

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<th>Maize (g)</th>
<th>Ensiled ley (g)</th>
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The fourth experiment was performed with 14 bottles during 25 days and gas production per day was monitored. Bottle 4, 8, 12, 13, 14 served as references during the trial. Corn was the substrates mixed with inoculum and water at certain ratios. Thermal, ultrasound and chemical pretreatments were applied in the experiment. The ratios of inoculum and the substrate, their weights, pretreatments are shown in table 2.5.4.
Table 2.5.4. Description of digester contents and pretreatments of experiment 4

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<td>90/10</td>
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<td>90/10</td>
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<td>525</td>
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<td>√</td>
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<td>85/15(ref.)</td>
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<td>26</td>
<td>525</td>
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</tr>
</tbody>
</table>

2.7. Calculation

\[
TS\%\text{(total solids)} = \frac{A - B}{C - B} \\
VS\%\text{(volatile solids)} = \frac{(B + A) - (B + D)}{(B + A)} - B \quad (2.6.1)
\]

A = weight of dried sample after 24 hours at 105 °C
B = weight of empty container,
C = weight of wet sample,
D = weight of burnt sample after 3 hours at 550 °C;

The average methane content was calculated after day 10 to the end of the experiments

\[
\text{Biogas yield} = \frac{A}{B \times C \times D} \text{ ml(gVS)}^{-1} \quad (2.6.2)
\]

A = total biogas production
B = the amount of substrates
C = TS of substrate
D = VS of substrate

Methane yield = biogas yield \times \text{ average methane content} \text{ ml(gVS)}^{-1} \quad (2.6.3)
3. Results

The experiments are presented separately with the following tables and figures.

3.1. Effect of different pretreatments on sugar beets during experiment 1

The bottle with a 85/15 percentage of inoculum and sugar beets with chemical pretreatment obtained the highest cumulative biogas production at 7562ml during 25 days in experiment 1. During the first 5 days, most bottles showed a great increase in biogas production except the bottles of 90/10 percentage and of inoculum and 95/5 percentage of inoculum and sugar beets with chemical pretreatment. However, the biogas production of both of them and the bottle with a 85/15 percentage of inoculum and sugar beets with chemical pretreatment increased rapidly during day9-18. After day18, it seems that all the bottles stopped digesting with slight increase on biogas production. (Fig.3.1.1)

![Cumulative biogas production of different pretreated sugar beets with different percentage of inoculum and substrate (T=Thermal, U=Ultrasonic, C=Chemical)](image)

The dry matter content of sugar beets was 22% of ww (wet weight) with 98% volatile solids. The variations of pH after digestion were in a range of 5.1-8.1. All the bottles with an 85/15 percentage of inoculum and sugar beets were inhibited with the result of low pH value except the one with chemical pretreatment. pH values decreased with an increase of the sugar beets added. The highest methane yield was obtained in the bottle with a 95/5 percentage of inoculum and sugar beets with ultrasonic pretreatment.
pretreatment operated at value $707 \text{ ml(gVS)}^1$. The C/N ratio tested after digestion varies from 8.6 to 11.8. (Tab.5)

Tab.3.1.1 Summary of parameters after digestion in experiment 1 with sugar beets

<table>
<thead>
<tr>
<th>Sugar beets percentage</th>
<th>pretreatment</th>
<th>pH</th>
<th>TS%</th>
<th>VS%</th>
<th>C/N</th>
<th>CH 4 vol-%</th>
<th>Biogas yield ml(gVS)$^1$</th>
<th>Methane yield ml(gVS)$^1$</th>
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</thead>
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<td>36</td>
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</tbody>
</table>

The highest methane production is obtained in the bottle with an 85/15 percentage of inoculum and sugar beets with chemical pretreatment. Whist all the other bottles with this mixed ratio were inhibited. In the bottles with a 95/5 percentage of inoculum and sugar beets, the pretreatments did not give any contribution to the methane production. Among the bottles with a 90/10 percentage of inoculum and sugar beets, the bottles without pretreatment gave almost no methane production while thermal, ultrasonic and chemical pretreatments enhance the methane production in this percentage. (Fig. 3.1.2)

![Fig.3.1.2 Methane production of different pretreated sugar beets with different percentage of inoculum and substrate (T=Thermal, U=Ultrasonic, C=Chemical)](image)

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3.2. Effect of different pretreatments on sugar beets and sugar beet leaves during experiment 1

The bottle with an 80/5/15 percentage of inoculum, sugar beets and sugar beet leaves with chemical pretreatment obtained the highest cumulative biogas production at 8057ml during 25 days in experiment 1.

All the bottles with chemical pretreatments showed a clear increase from day 1 to day 14. Biogas production of all the bottles with an 80/5/15 percentage of inoculum, sugar beets and sugar beet leaves increased between days 14-21. The rest of the bottles were inhibited with almost no biogas production. (Fig.3.2.1)

![Cumulative biogas production of different pretreated sugar beets and sugar beet leaves with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)](image)

Fig.3.2.1 Cumulative biogas production of different pretreated sugar beets and sugar beet leaves with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)

The dry matter content of sugar beets was 22% of ww with 98% volatile solids and sugar beet leaves was 13% of ww with 84% volatile solids. The variations of pH after digestion were in a range of 4.2-8. All the bottles with 80/10/10 and 80/15/5 percentages of inoculum, sugar beets and sugar beet leaves were inhibited with the results of low pH values except the ones with chemical pretreatments. The highest methane yield was obtained in the bottle with an 80/5/15 percentage of inoculum, sugar beets and sugar beet leaves with chemical pretreatment at 611 ml(gVS)\(^{-1}\). The C/N ratio tested after digestion varies from 9.1 to 11.4. (Tab.3.2.1)
### Tab.3.2.1 Summary of parameters after digestion in experiment 1 with sugar beets and sugar beet leaves

<table>
<thead>
<tr>
<th>Sugar beet/sugar beet leaves percentage</th>
<th>pretreatment</th>
<th>pH</th>
<th>TS%</th>
<th>VS%</th>
<th>C/N</th>
<th>CH₄ vol-%</th>
<th>Biogas yield ml(gVS)⁻¹</th>
<th>Methane yield ml(gVS)⁻¹</th>
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</thead>
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<tr>
<td>80/5/15</td>
<td>Thermal</td>
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<td>3.1</td>
<td>67</td>
<td>10.6</td>
<td>38.5</td>
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<td>43</td>
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<td>262</td>
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<td>2</td>
<td>57</td>
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<td>67</td>
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<td>36.5</td>
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<td>63</td>
<td>8.9</td>
<td>4</td>
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<tr>
<td>Sugar beets</td>
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<td>—</td>
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<td>98</td>
<td>36</td>
<td>—</td>
<td>—</td>
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</tr>
<tr>
<td>Sugar beet leaves</td>
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<td>—</td>
<td>13</td>
<td>84</td>
<td>11.8</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

The highest methane production was obtained in the bottle with an 80/5/15 percentage of inoculum, sugar beets and sugar beet leaves with chemical pretreatment that increased methane production by 262% while the methane production increased by thermal and chemical pretreatments by 14% and 55% respectively. With other ratios of inoculum and substrates, only the bottles with chemical pretreatment gave a high amount of methane production, whilst the other bottles with the same ratio were inhibited. (Fig 3.2.2)

**Fig.3.2.2** Methane production of different pretreated sugar beets and sugar beet leaves with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)
3.3. Effect of different pretreatments on the mixture of straw and sugar beet leaves with ratio 74:26 during experiment 2

The bottle with an 85/15 percentage of inoculum and the mixture of straw and sugar beet leaves with thermal pretreatment obtained the highest cumulative biogas production at 14251 ml during 25 days in experiment 2.

All the bottles showed the same trend of cumulative biogas production with a steady increase from the beginning to the end except the bottles with an 85/15 percentage of inoculum and the mixture of straw and sugar beet leaves with chemical pretreatment and reference started to increase after day 5. However, the bottles of 95/5 percentage of inoculum and the mixture of straw and sugar beet leaves with thermal, chemical and reference gave a relatively lower biogas production. (Fig. 3.3.1)

![Cumulative biogas production of different pretreated mixture of straw and sugar beet leaves with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)](image)

Fig. 3.3.1 Cumulative biogas production of different pretreated mixture of straw and sugar beet leaves with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)

The dry matter content of the mixture of straw and sugar beet leaves was 27.4% of ww with 91% volatile solids. The variations of pH after digestion were in a range of 7.4-7.9. The highest methane yield was obtained in the bottle with a 95/5 percentage of inoculum and the mixture of straw and sugar beet leaves with ultrasonic pretreatment at 572 ml(gVS)$^{-1}$. The C/N ratio tested after digestion varies from 10 to 13. (Tab. 3.3.1)
Tab.3.3.1 Summary of parameters after digestion in experiment 2 with mixture of straw and sugar beet leaves

<table>
<thead>
<tr>
<th>Straw/sugar beet leaves percentage</th>
<th>Pretreatment</th>
<th>pH</th>
<th>T5%</th>
<th>VS%</th>
<th>C/N</th>
<th>CH₄ vol-%</th>
<th>Biogas yield (ml(gVS)⁻¹)</th>
<th>Methane yield (ml(gVS)⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>95/5</td>
<td>Thermal</td>
<td>7.9</td>
<td>2.6</td>
<td>62</td>
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<td>276</td>
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<tr>
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<td>2.8</td>
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<td>8.2</td>
<td>28.7</td>
<td>—</td>
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<td>—</td>
<td>—</td>
<td>27.4</td>
<td>91</td>
<td>36</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

The highest methane production is obtained in the bottle with an 85/15 percentage of inoculum and mixture of straw and sugar beet leaves with thermal pretreatment. The difference of methane productions between pretreatments and references were not significant except the bottle with a 95/5 percentage of inoculum and a mixture of straw and sugar beet leaves with ultrasonic pretreatment increased methane production by 72%. (Fig.3.3.2)
3.4. Effect of different pretreatments on maize during experiment 3

The bottle with an 85/15 percentage of inoculum and maize with chemical pretreatment obtained the highest cumulative biogas production at 25476ml over 25 days in experiment 3. All the bottles showed the same trend of cumulative biogas production with a rapid increase during the first 10 days and a slight increase till the end of digestion except the bottle with an 85/15 percentage of inoculum and maize without pretreatments stopped producing gas after day 5, and the bottle with the same percentage with chemical pretreatment obtained a sharp increase during day 11 to day 23. (Fig. 3.4.1)

![Cumulative biogas production graph](image)

**Fig. 3.4.1** Cumulative biogas production of different pretreated maize with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)

The dry matter content of maize was 41% of ww with 98% volatile solids. The variations of pH after digestion were in a range of 5.2-7.7. The bottle with an 85/15 percentage of inoculum and maize was inhibited, with the result of a low pH value. The highest methane yield was obtained in the bottle with a 95/5 percentage of inoculum and maize with ultrasonic pretreatment at value 710 ml(gVS)^{-1}. The C/N ratio tested after digestion varies from 10.1 to 12.6. (Tab.3.4.1)
Tab.3.4.1 Summary of parameters after digestion in experiment 3 with maize

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<tr>
<th>Maize percentage</th>
<th>pretreatment</th>
<th>pH</th>
<th>TS%</th>
<th>VS%</th>
<th>C/N</th>
<th>CH₄ vol-%</th>
<th>Biogas yield ml(gVS)¹</th>
<th>Methane yield ml(gVS)¹</th>
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</thead>
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<td>10.9</td>
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The highest methane production is obtained in the bottle with an 85/15 percentage of inoculum and maize with chemical pretreatment. All the pretreatments at different percentages of inoculum and maize contributed to the methane production. Ultrasonic pretreatment had better efficiency than other pretreatments when dealing with low percentage of substrates. The differences of methane productions between different pretreatments were not significant. The best efficiency of pretreatments obtained at 90/10 percentage of inoculum and maize with thermal, ultrasonic and chemical pretreatment which increased the methane production by 48%, 80% and 49%, respectively. (Fig. 3.4.2)

Fig.3.4.2 Methane production of different pretreated maize with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)
3.5. Effect of different pretreatments on ensiled ley during experiment 3

The bottle with an 85/15 percentage of inoculum and ensiled ley with thermal pretreatment obtained the highest cumulative biogas production at 16505 ml over 25 days in experiment 2.

All the bottles showed the same trend of cumulative biogas production with a rapid increase from the beginning to day12 then a steady increase till the end of digestion period. However, all the bottles with chemical pretreatment obtained relatively lower biogas productions. (Fig.3.5.1)

![Graph showing cumulative biogas production](image)

**Fig. 3.5.1** Cumulative biogas production of different pretreated ensiled ley with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)

The dry matter content of the ensiled ley was 31% of ww with 91% volatile solids. The variations of pH after digestion were in a range of 7.6-7.8. The highest methane yield was obtained in the bottle with a 95/5 percentage of inoculum and ensiled ley with ultrasonic pretreatment at 566 ml(gVS)⁻¹. The C/N ratio tested after digestion varies from 9.4 to 11.6. (Tab.3.5.1)
Tab.3.5.1 Summary of parameters after digestion in experiment 3 with ensiled ley

<table>
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<tr>
<th>Ensilged ley percentage</th>
<th>pretreatment</th>
<th>pH</th>
<th>TS%</th>
<th>VS%</th>
<th>C/N</th>
<th>CH4 vol-%</th>
<th>Biogas yield ml(gVS)</th>
<th>Methane yield ml(gVS)</th>
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<td>10.1</td>
<td>42.7</td>
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<td>Ensilged ley</td>
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<td>—</td>
<td>31</td>
<td>91</td>
<td>11.8</td>
<td>—</td>
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<td>—</td>
</tr>
</tbody>
</table>

The highest methane production is obtained in the bottle with an 85/15 percentage of inoculum and ensiled ley with thermal pretreatment. In this case pretreatments did not give any contributions to methane production. Moreover, chemical pretreatment decreased the methane production in all the percentages of inoculum and ensiled ley. (Fig. 3.5.2)
3.6. Effect of different pretreatments on straw during experiment 4

Since the experiment was downscaled 4 times, the biogas production should be multiplied by 4.

The bottle with a 85/15 percentage of inoculum and straw with chemical pretreatments obtained the highest cumulative biogas production at 7253 ml during 25 days in experiment 2. All the bottles showed the same trend of cumulative biogas production with a steady increase from the beginning to the end. The bottles with chemical pretreatments obtained a relatively higher biogas production. (Fig.3.6.1)

![Cumulative biogas production graph](image)

Fig. 3.6.1 Cumulative biogas production of different pretreated straw with different percentage of inoculum and substrates (T=Thermal, U=Ultrasonic, C=Chemical)

The dry matter content of the straw was 81% of ww with 94% volatile solids. All the pH values were under 7 within a range of 6.8-7. The highest methane yield was obtained in the bottle with a 95/5 percentage of inoculum and straw with chemical pretreatment at 249 ml(gVS)\(^{1}\). The C/N ratio tested after digestion which varies from 9.8 to 15.4. (Tab. 3.6.1)
Since the experiment was downscaled 4 times. The methane production should be multiplied by 4.

The highest methane production was obtained in the bottle with an 85/15 percentage of inoculum and straw with chemical pretreatments. Ultrasonic pretreatments did not give contributions to the methane production. Thermal and chemical pretreatments increased methane production at 95/5 percentage of inoculum and straw by 78% and 142%, respectively. Comparing the methane production between 90/10 percentage of inoculum and straw with and without water, the bottle with the water increased methane production by 27%. (Fig.3.6.2)
3.7. ANOVA test of different pretreatments with different substrates

In the group of different substrates, only ensiled lay and straw showed significant differences between the pretreatments with p-values <0.05 (0.026, 0.000 respectively). (Tab.3.7.1.)

Tab.3.7.1 ANOVA test of different substrates with different pretreatments

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Sig. (p-value)</th>
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<tbody>
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<td>Sugar beet</td>
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</tr>
<tr>
<td>Sugar beet and sugar beet leaves</td>
<td>0.724</td>
</tr>
<tr>
<td>Straw/sugar beet leaves</td>
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</tr>
<tr>
<td>maize</td>
<td>0.453</td>
</tr>
<tr>
<td>Ensiled ley</td>
<td>0.026</td>
</tr>
<tr>
<td>Straw</td>
<td>0.000</td>
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</table>

In the case of ensiled ley, the chemical pretreatment was significantly different from the other pretreatments and reference with p-value < 0.05

In the case of straw, chemical and thermal pretreatments were significantly different from the other pretreatments and the reference with p-values < 0.05. (Tab.3.7.2)

Fig.3.7.1 difference of mean methane yield between different pretreatments in ensiled ley with standard deviation
Comparison of different pretreatments

Chemical pretreatments increased the most methane yield with sugar beets/sugar beet leaves and straw by 68% and 102% respectively while it had a negative effect on the methane yield of the sugar beets and the ensiled ley by 23% and 39%, respectively. All the thermal pretreatments had a positive effect on methane yield especially with straw which increased methane yield by 54%. Ultrasonic pretreatments had a positive effect on the methane yield of most substrates with the highest obtained with sugar beets/sugar beet leaves and maize which increased methane yield by 43% and 41% respectively. (Fig. 3.8.1)
The highest methane yield was obtained in sugar beets (618 ml(gVS)⁻¹) with a thermal pretreatment while the lowest methane yield was obtained in straw (109 ml(gVS)⁻¹) with ultrasonic pretreatment.

The methane yield decreased by 52% after adding sugar beet leaves to sugar beets, however, the methane yield increased by 173% after adding sugar beet leaves to straw (Fig. 3.8.2)
4. Discussion

4.1. Chemical pretreatment

Chemical pretreatment gave the lowest methane yield compared to other pretreatments and reference with sugar beets. This may have happened because after the chemical pretreatment, the pH-value remained high during the whole digestion process which slowed down the acid-forming stage, as the optimum pH-value is 5.5-5.6 for acidogens. This also explained the low biogas production rates of chemical pretreatment of sugar beets during the first 10 days. However, anaerobes can be grouped into two separate pH groups: acidogens and methanogens. The high pH-value met the methanogens requirement as being optimum is 7.8-8.2 which explains the methane contents with chemical pretreatment were higher than other pretreatments and reference.

Sugar beets are carbohydrate-rich and contains simple sugars which are broken down very quickly and easily resulted in rapid hydrolysis and an acid-forming stage.(Fig. 3.3.1) This explained the rapid increase of biogas production during first the 5 days. The rapid hydrolysis and acid-forming stage made pretreatments useless. However, as a consequence, the methanogenic stage becomes the bottleneck with high amounts of sugar beet added because methanogens do not have time to consume the quantities of organic acids produced with an accumulation of acids is the result. If the pH of the digester drops, the process may eventually fail or in extreme case like the 85/15 percentage of inoculum and sugar beets, microorganisms were killed and the process collapsed. The same happened for the 80/10/10 and 80/15/5 percentage of inoculum, sugar beets and sugar beet leaves. When a certain quantity of sugar beets were added into the digesters this caused the acidification which eventually collapse the process. However, the ones with chemical pretreatments at the same percentage avoided the pH dropping and maintained the optimal pH value for methanogens. When the lignin content is high, chemical pre-treatments have a clear positive effect on the biodegradability, but when it is low, this effect is lower or even absent. (Dien et al., 2006)

After adding sugar beet leaves for co-digestion with sugar beets, chemical pretreatment increased methane yield by 68%. Sugar beet leaves contains more fiber which extended digestion time, however, chemical pretreatment decreased the degree of polymerization and disrupted the intermolecular ester bonds crosslinking hemicellulose and lignins. This makes the substrate more accessible for enzymes and bacteria which can lead to a rapid increase in biogas production.
The same happened for straw as straw is considered as a high lignin content substrate. The degradation of lignin is very tough for its amorphous heteropolymer character and for being non-water soluble (Fengel and Wegener, 1984). However, chemical pretreatment increased the methane yield of straw by 102%. Because of the hard degradability of straw, it still cannot give high methane yield. The ANOVA test of straw also supported that chemical pretreatment with straw showed significant difference compared to other pretreatments and the reference.

Chemical pretreatment did not give any contribution to the co-digestion of the mixture of straw and sugar beet leaves with ratio 86:14 which was unexpected. Both straw and sugar beet leaves contain high quantities of lignin and cellulose. We might say that cellulose contents of the mixture of straw and sugar beet leaves were higher than straw. Therefore we could not get high biogas amount and when the experiments finished, the bottles were still producing biogas. If the experiment could last longer, the biogas production would be higher. In the meantime, ultrasonic pretreatment of this substrate increased the methane yield by 22% as will be explained later.

Ensiled ley which has a similar content as straw with extra fiber, gave totally contrary results compared to straw. During the ANOVA test, ensiled ley had significant differences between different pretreatments which turned out to be the negative effect by chemical pretreatment of decreasing methane yield by 39% which was unexpected. The reason could be after being ensiled, organic acids and alcohols increased during ensiling (Christiane Herrmann, 2011) which made ley easily digested without pretreatments. With chemical pretreatment can only cause the relevantly high pH-value during first step of digestion which decreased the biogas production at first step. Finally decrease the methane yield. Another explanation could be a decrease of lignin solubilization because the treatment with lime caused interaction between negatively charged lignin molecules and positively charged calcium ions which reported by Xu et al. (2010).

Consider of full-scale applications, Chemical treatment with CaO has a high potential to decrease the concentration of ammonia in the substrate. Because of the risk of inhibition of the microorganisms involved in the biogas process, removal of ammonia may be required at full-scale biogas plants digesting substrates, such as manure that have a high content of organic nitrogen or ammonia. Although the chemical treatment with CaO resulted in the highest methane yield gain, the advantage of this treatment has to be evaluated carefully. The analysis will have to take into account the costs of chemicals and the need for extra investments such as mixers (thorough mixing is required to ensure homogenous distribution of CaO on the biofibers) and storage (the storage volume is proportional to the reaction time of the treatment) (Emiliano Bruni, 2010)
4.2. Thermal pretreatment

Although the effect of thermal pretreatment was not significant, it had positive effect on the methane yield with all the substrates especially with straw which increased methane yield by 54%. Straw contains rich lignin and hemicelluloses, this is hard to degrade. During thermal pretreatment, a part of hemicellulose is hydrolyzed and forms acids during thermal processes. These acids catalyzed the further hydrolysis of the hemicellulose (Gregg, 1996). Thermal pretreatments can also enhance hydrolysis rates and reduce HRT days which became clear in most results that after thermal pretreatment, the increasing rate of biogas production is higher than the untreated ones. According to Rafique (2010) that thermal pretreatment showed enhancement in the temperature from 50 ºC to 100 ºC. Also Deublein and Steinhauser (2008) found 30% increase of biogas yield if substrate is thermally treated before it entered the reactor.

Although, chemical pretreatment had positive effect on methane yield of all kinds of substrates, harmless to the residual and easily operation, the energy input of thermal pretreatment should also be taken into consideration if applied in large-scale.

4.3. Ultrasonic pretreatment

Ultrasonic pretreatment was obtained the highest methane yield in sugar beets and sugar beet leaves, straw/sugar beet leaves and maize with 43%, 22% and 41% increased respectively. Ultrasonic pretreatment can increase the specific surface area, reducing the degree of polymerization. All the factors increase the total hydrolysis yield of the lignocellulose. Furthermore, ultrasonic pretreatments had a positive effect on the methane yield of most substrates with the highest obtained with sugar beets/sugar beet leaves and maize which increased methane yield by 43% and 41% respectively.

Ultrasonic pretreatment did not give contributions to the methane yield of ensiled ley and straw due to their character of high content of lignin which is hard to be break down in such a short time of the ultrasonic treatment.

Nowadays, ultrasonic pretreatments are usually applied in a bypass where 30% of the sweage is treated. (Deublein and Steinhauser, 2008) Also according to Kim (2003) methane production was found to increase 34% by ultrasonic-treated sludge compared to untreated sludge.

However, the high energy requirements make some kinds of mechanical pretreatment not economically feasible. Ultrasonic pretreatment is a straightforward treatment
method, but the energy input may become higher than the energy content of the extra methane produced. (Taherzadeh and Karimi, 2008).

In experiment 4, the low pH-value of all the bottles indicated the methanogenic bacteria were inhibited in the process. The reason is not so clear. According to Fig.3.6.1, at the end of digestion, biogas production still increased. It can be suggested that extending digestion time of straw can result in higher biogas production. This could be the reason of low pH-value as digestion was not finished yet.

5. Conclusion

Different pretreatments have different effect on different substrates with different mix ratios of inoculum and substrates.

From this study, it can be concluded that chemical pretreatment is not suitable for carbohydrate-rich and easily degradable substrates such as sugar beets. After ensilation, organic acids and alcohols are formed. These products increased the degradability of ley, therefor chemical pretreatment will decrease the methane yield. However, chemical pretreatments increased the most methane yield with sugar beets/sugar beet leaves and straw by 68% and 102% respectively which is an impressive increase. Chemical pretreatment is a good option with highest increased methane yield and a low-cost operation, however, it can also be expensive if storage and chemical costs are considered.

Although all the thermal pretreatments had a positive effect on methane yield especially with straw which increased methane yield by 54%, the difference of thermal pretreatment and reference was not significant. Considering the high energy input and high requirements of technology for full-scale applications, according to Steinhauser D (2008), the process only runs economically with regenerative heat exchange between the supply and the downpipe of the thermo reactor.

Although ultrasonic pretreatments had a positive effect on the methane yield of most substrates with the highest obtained with sugar beets/sugar beet leaves and maize which increased methane yield by 43% and 41% respectively, the difference of thermal pretreatment and reference was not significant. Considering the high energy input and high requirement of technology of full-scale applications, according to Taherzadeh and Karimi (2008) that ultrasonic pretreatment is a straightforward treatment method, the energy input may become higher than the energy content of the extra methane produced.

At full-scale application, energy input should be considered. Three pretreatments have different energy demands and different effects on biogas production. The optimal pretreatment depends on the substrates and technology used in the biogas plant.
However, not every pretreatment brings benefits. Sometimes without pretreatment can reach higher profits if consider the cost of pretreatments.

6. Acknowledgement

I am sincerely thankful to my supervisor Marie Mattsson for the precious opportunity to work in my interested field under her supervision and her patient and generous guidance.
I am deeply indebted to my co-supervisors Johan Rundstedt and Niklas Karlsson for their sincere help and inspiring advice during this study.
To the group, I would like to thank all of them for spending time and involving me in one of their projects
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Europe Commission, 2011 COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL {COM(2011) 31 final}


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