Termisk förbehandling av cellulosarika material för biogasproduktion

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Thermal pre-treatment of cellulose rich biomass for biogas production

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Preface
This is a master thesis report comprising 30 credits on D-level and it composes the finishing part of the authors’ master degree in biology. The study was performed within the thematic research project *MicroDrivE* (Microbially Derived Energy) at the faculty of Natural Resources and Agricultural Sciences (NL), Swedish University of Agricultural Sciences in Uppsala.

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*Legere et non intelligere neglegere est – To read without understanding is not to read.*

Ultuna, December 2008
Pauline Demetriades

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Abstract

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Anaerobic digestion is one possible method to produce bioenergy from cellulose rich materials but the process techniques still need refinements to facilitate the production process. In this work the biogas potential from six different plant materials was evaluated and what effect a thermal pre-treatment had on this potential. The biogas production was determined in a batch experiment model with small biogas reactors. The tested substrates were oat straw, meadow grass, aspen, spruce and wet grain residue from two different ethanol production plants in Sweden, all of which were thermally pre-treated with one or two pre-treatment setups and compared in production with untreated materials. Results show that thermal pre-treatment does have an effect on the biogas production but that different materials need different thermal pre-treatment parameters. The experiment also showed that the particle size of the plant material can have an equally large effect on the biogas production as the thermal pre-treatment. Smaller particles give rise to a higher methane production. Of the tested materials the untreated wet grain residue from spirits production showed both the highest degradation rate and total biogas production whereas the thermally pre-treated spruce had the lowest production.

Keywords: biogas production potential, degradation rate, degradation potential, thermal pre-treatment, steam explosion, autohydrolysis, cellulose, lignin, oat straw, meadow grass, aspen, spruce, wet grain residue, batch experiment

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Sammanfattning

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Nyckelord: biogaspotential, rötning, nedbrytningshastighet, nedbrytningspotential, termisk förbehandling, ångexplosion, autohydrolys, cellulosa, lignin, havrehalm, ängsgräs, asp, gran, drank, drankvatten, batch experiment

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Biogas består till största del av metangas och koldioxid och är en restprodukt som bildas då flera olika grupper av mikroorganismer bryter ned organiskt material i syrefria miljöer. Denna nedbrytningsprocess förekommer naturligt i bland annat tarmkanalen hos gräsätare och termiter, i sjöbottensediment samt i mossor och kärr. Biogas kan även produceras under industriella former och processen kallas då ofta för rötning. Det är metangasen som är det energibärande ämnet i biogas och det som används för att producera el, värme och som fordonsbränsle.

Utvinning av biogas genom rötning är ett av de mest effektiva sätten att ta tillvara på energin i cellulosa men för att kunna göra detta på ett tillräckligt effektivt sätt behöver växtmaterialet ofta förbehandlas. Växterna kan till exempel finfördelas, behandlas med het ånga i tryckkammare eller blötläggas i syra eller lut. Det är vanligt att man kombinerar flera av dessa förbehandlingar. Syftet är dock alltid att öka hastigheten på biogasproduktionen genom att bryta upp den fysiska
strukturen. Detta gör näringen mer lättåtkomlig för mikroorganismerna i biogasprocessen vilket ökar både nedbrytningshastigheten och den totala nedbrytningsgraden.

I denna studie genomfördes experiment för att utröna effekten av en termisk förbehandling med het ånga och högt tryck på ett antal växtmaterial med avseende på biogasproduktion. Undersökningen genomfördes på behandlade och obehandlade växtmaterial i småskaliga biogasreaktorer.

Resultaten från undersökningen visade att termisk förbehandling kan ha en positiv effekt på biogasproduktionen från cellulosarika växtmaterial men att olika material reagerar olika på samma behandling. Specifika förbehandlingar behöver därför utvecklas för enskilda växtmaterial. Undersökningen visade också att storleken på partiklarna av det enskilda växt materialet spelar roll för den totala biogasproduktionen och att mindre partiklar ger ett högre utbyte. Partikelstorleken kan ha lika stor effekt som en termisk förbehandling.

Vidare undersökningar behövs för att klargöra om termisk förbehandling är lönsam för specifika substrat och för att optimera termisk förbehandling på olika växtmaterial.
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1. Introduction

The most alarming of all man’s assaults upon the environment is the contamination of air, rivers, and sea with dangerous and even lethal materials.

The clear-sighted words above were written by Rachel Carson in her famous book ‘Silent spring’ (1962) that is said to have been the starting point of the global environmental awareness. The nations of the world started to realize that the unbridled consumption of the natural resources must change to a more sustainable one. Environmental questions quickly became a prioritised issue and the United Nations (UN) started their work by gathering over 100 countries to the so called Stockholm conference in 1972 (SFN, 2008). Twenty years later, in 1992, the next global event was held in Rio de Janeiro which resulted in three main documents – the Rio declaration on Environment and Development, the Convention on Climate Change and the Convention on Biological Diversity. The Rio-declaration contains 27 fundamental principles about human rights and responsibilities and the 8th one about sustainable development reads:

To achieve sustainable development and a higher quality of life for all people, States should reduce and eliminate unsustainable patterns of production and consumption and promote appropriate demographic policies (UN, 1992).

One of the main unsustainable patterns of consumption is the use of petroleum for vehicle fuel, chemicals and plastics. The heavy dependence on petroleum for these different uses has been pointed out as the main source of greenhouse gas emissions thus causing global warming (IPCC, 2007). To break this dependency should be prioritised and there are several possible alternatives. The development of the technologies for biofuel is one important strategy. In the near future biofuels such as ethanol, biodiesel and biogas and techniques for minimising fuel consumption with hybrid engines and batteries will be of great importance (Börjesson & Mattiasson, 2007). The transportation sector has historically favoured liquid fuels (Yang & Wyman, 2007). However the production of bioethanol, biodiesel and DME alone lacks somewhat in resource efficiency in comparison to a more developed system where more of the raw material is used in several steps (Börjesson & Mattiasson, 2007). Development of biogasification techniques to use the ethanol by-products further seems to be a part of the solution where the wet grain residues can be used as biogas production substrate. The government has made large investments in the biogas infrastructure the past years and both production and use is increasing in Sweden.
Biogas is formed when organic material is decomposed under anaerobic conditions (Hobson, 1982). The gas has many applications and can be used to produce heat, electricity or vehicle fuel. Different materials are decomposed to different extent and also produce different amounts of biogas. Biogas production in Sweden was from the beginning a way to reduce the volume of wastewater treatment plant sludge that went to landfills (Börjesson & Mattiasson, 2007). For the time being the current production from household and industrial wastes, energy crops and wastewater treatment plants is enough to satisfy the biogas market in Sweden. To make use of the full capacity of biogas production methods to utilize biogas from cellulose-rich materials must however be developed. The most inexpensive and abundant renewable substrate for biofuels is lignocellulosic biomass (Yang & Wyman, 2007). All plants on Earth produce approximately 100 billion tonnes of biomass annually (Campbell & Reece, 2005). Many plant materials can easily be produced in large quantities and are possible to integrate with several crop systems (Yang & Wyman, 2007).

In the biogas process, the decomposition of cellulose rich materials is often the limiting step in the biogas production. By breaking up the complex structure of the cellulose in a pre-treatment step the biogas process can be speeded up (Yang & Wyman, 2007). There are several possible pre-treatments that have been investigated in other studies (Bougrier et al., 2008; Hongzhang et al, 2005; Mshandete et al, 2006, Yang & Wyman, 2007). In general there are four different categories: biological, chemical, physical and thermal pre-treatments (Yang & Wyman, 2007).

Thermal pre-treatment can be economically unviable because the vast amounts of energy needed to heat the water used in the process. However, this depends on the system the steam-explosion is applied to. If the production unit has access to excess heat that can be re-circulated, this heat can be used for thermal pre-treatment of the substrates without compromising the economical viability. Pre-treatments in general do not always give more in exchange from the substrate compared to the invested energy and the total energy balance must be taken into consideration before applying a method. Compared to other pre-treatment methods steam explosion has a low energy requirement and is considered to be very cost effective (Sun & Cheng, 2001).

Thermal pre-treatment is often a combination of steam explosion after the material has been soaked in acid. This is a common practice for example in ethanol production. According to the substitution principle in the EU decree for Registration, Evaluation, Authorization of Chemicals no. 1907/2006 (REACH) and the precautionary principle in Swedish environmental legislation (SFS 1998:808 Miljöbalk 2:3 §) everyone who manages operations involving actions that might infict harm or damage on people’s health or the environment should use the best available technology to minimise the risks of the operation. Cambi AS is a
Norwegian company with subsidiaries in a number of countries worldwide. They have developed a method for re-circulating steam in the steam-explosion process. While this technical solution makes the biogas production more cost-efficient it still needs to be optimised for cheap and abundant substrates. Furthermore, developers at Cambi AS have constructed a method for thermal pre-treatment which is solely a steam-explosion process. Because the chemicals have been excluded in the process this method can be considered more environmentally friendly than the more commonly used method with acid.

Cambi AS's thermal pre-treatment has previously been evaluated on different industrial and municipal wastewater treatment plant sludge with good results. In this study the effects of thermal pre-treatment will be tested on six different cellulose-rich substrates to evaluate the effect of thermal pre-treatment. The materials in question are spruce, oat straw, meadow grass, aspen wood chips and distillers waste from two different ethanol production plants.

1.1 Hypotheses
Thermal pre-treatment with steam explosion is a treatment that breaks up the crystalline structure of cellulose and thus makes it more available for biological degradation. This should potentially generate more biogas.

1.2 Purpose
One of the overall purposes for the MicroDrivE-project is to optimize biogas production from cellulose rich materials. The purpose of this study was to evaluate if and which of the tested thermal pre-treatment is the better one and to determine the potential for the tested substrates.
2. Background

2.1 Biogas – situation in Sweden
The biogas industry in Sweden developed in the 1950-1970’s when the technology was used to sanitize and reduce volume of sewage sludge (Nordberg, 2006). During the following decades many experiences were made, household recycling was introduced and large scale facilities for decomposition of a variety of different organic wastes were set up. Biogas production has now become the primary objective of several companies and production of agricultural crops solely for this production has also begun to spread. There are around 220 biogas facilities in Sweden and the production has been on a stable level of 1.5 TWh/year the last ten years (Nordberg, 2006). The main part of the production is used as vehicle fuel and local heating. The substrates that contribute for the main body of the production of biogas in Sweden are sewage sludge, municipal organic wastes, biowaste and process wastage from food industries, agricultural crops and animal manure (Nordberg, 2006). If the full potential of these substrates was used the energy production would total up to 15 TWh/year (Linné et al, 2008).

This production and the production from ‘first generation’ biogas substrates, such as different energy crops, will be enough to fill the need of biogas for the next ten years (Börjesson & Mattiasson, 2007). However, in the long run new sources for biofuel production must be found. One potential source is the ‘second generation’ substrates, such as lignocellulosic plant materials. However, these substrates and the technologies to ferment them are still novel techniques and need further research to become fully commercially viable. Lignocellulosic materials are the major group that counts as the ‘second generation’ substrates. For the full potential to be calculated and made use of the industry must find ways to process substrates such as straw and wood. Including the potential from lignocellulosic materials from forestry this figure changes to about 74 TWh/year where the lignocellulosic materials constitute for 80 % (Linné et al, 2008).

2.2 Biogas – formation and microbiology

2.2.1 Biogas
There are several kinds of gasses that are used for energy purposes over the world (Gasföreningen, 2008). These energy gasses have renewable or ending origins. Biogas is a renewable gas formed under anaerobic conditions when organic material is degraded by microorganisms. It consists mainly of methane (CH₄) and carbon dioxide (CO₂) and to some small extent of other gasses, for example hydrogen sulphide (H₂S) and nitrogen gas (N₂) (Svenska biogasföreningen, 2006).

Biogas formation occurs naturally for example in marshes, river beds and in the guts of herbivores (Hobson, 1982; Wall [1], 2008). It can also be produced under controlled conditions in biogas plants with the same kind of microbial cultures.
The basics of this technology have been known for over 80 years and the primary use in Sweden has been to reduce sludge volume and pollution from sewage sludge and municipal wastes (Nordberg, 2006). In other countries, for example China and India, the gas has been used to heat stoves and burnt in lanterns (Levén, 2006). Wastewater treatment plants are the largest producers of biogas in Sweden and biogas from these operations are mostly used for heat and light within the facility (Wall [1], 2008). Major investments are now made in the infrastructure for production and distribution of biogas as a vehicle fuel and estimations show that approximately 1000 GWh will be used as vehicle fuel by 2010 (Nordberg, 2006). Examples of recourses for commercial biogas production are industrial wastewaters from food processing, breweries, distilleries, beverages, pulp and paper production (Wall [1], 2008). Great potential is also found in the organic fraction of municipal solid wastes.

2.2.2 Microbiological background

Four main groups of microorganisms are involved in the biogas process – hydrolytic and fermenting bacteria, acetate-forming bacteria and methanogens, the later belonging to the domain of Archea (Gerardi, 2003; Wall [2], 2008). These groups of microorganisms perform the four main steps of the methane formation process (figure 1). In the first step the degradation of organic material starts when bacteria that is able to perform hydrolysis of complex organic materials (for example polymers of cellulose) with extracellular enzymes break up the material into smaller pieces (monomers like for example glucose). The fermenting bacteria, of which not all are able to perform hydrolysis, degrade the monomers into mainly short fatty acids, alcohols, hydrogen gas and carbon dioxide in the second part of the biogas process. The products from the fermenting bacteria then become substrate for the acetate-forming bacteria which perform the third part of the biogas process. The products previously produced during the fermentation are degraded further by these bacteria and acetate, carbon dioxide and hydrogen gas is formed as their decay products. Together the fermenting and acetate-forming steps of the biogas process are sometimes called acidogenesis.

These components from the acidogenesis make up the substrate for the methanogens in the fourth and last step of the biogas process (Gerardi, 2003; Wall [2], 2008). The methanogens use acetic acid, carbon dioxide as a carbon source and hydrogen to obtain energy while methane, carbon dioxide and water are the final products. Acetate-forming bacteria and methanogens live in a mutualistic symbiotic relationship where both parties benefit from the other. The methanogens compete with sulphate-reducing bacteria which also can be found in anaerobic environments (Gerardi, 2003; Rivard & Grohmann, 1991). Which of the two that wins the competition for living space depends on the ratio between accessible substrate and sulphate in the ingoing substrate.
The methane formed in the process is a waste product and methanogens are the only known organisms that form methane (Gerardi, 2003). Methanogens are found in both aquatic and terrestrial environments and are highly sensitive to oxygen. On a cellular level they differ from other microorganisms in their cell membrane composition and soft cell wall.

![Diagram of biogas formation process](image)

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**2.3 Control parameters of the biogas process**

To successfully control the biogas process proper monitoring of some crucial parameters have to take place. Some substrates can for example contain or form inhibitory compounds which will disturb the microorganisms. To ensure good process efficiency and protect the process from collapsing some of the following measurements can be performed (Nordberg, 2006).
2.3.1 pH
The different microorganisms that interoperate have different pH-optima which mean that they thrive under different conditions (Nordberg, 2006). The acid forming step of the methanogenesis can cause an accumulation of volatile fatty acids (VFA’s) if the methanogens cannot keep up, which can lower the pH-level. The optimal pH in a biogas process is 7-8.5 and if the pH gets below or above this interval the process can be inhibited.

2.3.2 Temperature
In nature anaerobic digestion takes place within three temperature intervals (Nordberg, 2006). These temperature ranges are called the psycrophilic (0-20°C), mesophilic (15-45°C) and the thermophilic (45-75°C). The names refer to the Greek words for ‘cold’-, ‘medium’- and ‘hot’- liking respectively. In conventional biogas digesters there are commonly two temperature intervals in use, the thermophilic at 50-60°C and the mesophilic at 30-40°C. A fluctuation of more than 1-3°C can be crucial to the microorganisms and what temperature that should be used depends on what options there are to insulate the digester and how long duration of stay that is acceptable. Which temperature that is suitable for each plant depends of many factors. A higher temperature results in a better sanitation and killing off pathogens within the process but also costs more energy to keep warm. The process runs faster and more gas can be produced in a shorter time span but the thermophilic process is also more sensitive to temperature fluctuations and inhibitory compounds. The mesophilic temperature span is slower in production rate than the thermophilic but on the other hand commonly more stabile. Also, a reactor run at mesophilic levels needs less heating and thus has lower operational costs.

2.3.3 Nutrient content in the substrate
The basic nutritional needs for the microorganisms are carbon, nitrogen and phosphorous and micronutrients and vitamins for their growth (Nordberg, 2006). The microorganisms cannot just have any amount of nutrients in the substrate. The overall composition does have an impact on the growth of the microorganisms. A common measurement is the relation between carbon and nitrogen, commonly more know as the C/N-ratio. The microorganisms need 10-30 times more carbon than nitrogen. However it is not only the carbon- or nitrogen content of the ingoing substrate that matters for production rate (Osman et al., 2006). A single substrate can be limited regarding its content of micronutrients and studies have shown that co-digestion of for example lignocellulosic materials and animal manure can enhance production with more than 50 % than digestion of a single substrate.

2.3.4 Water content
Water is the solvent of the nutrients in the substrate and also work as contact medium between the microorganisms and the substrate (Nordberg, 2006). The
water content should optimally be between 60-95 %. Degradation of organic material can be done at different water content levels at the ingoing material. Water is normally added to reach adequate levels as well as inoculums of already degraded material to shorten the start-up time for the biogas process. When the water content is above 90 % the process is called a wet process while a process with 60-75 % water content is considered a dry one.

**2.3.5 Hydraulic retention time**

The time the substrate or an element spends in the process is expressed with the measure of Hydraulic Retention Time, also called HRT (Nordberg, 2006). Under mesophilic conditions the process of degradation demands at least 10-30 days while thermophilic conditions need a somewhat shorter time span.

**2.3.6 Organic loading rate**

When a biodegradation process functions and is stable it needs new organic material in a steady slow (Nordberg, 2006). This new addition is defined as Organic Loading Rate (OLR) and is in a biogas process commonly expressed as kg volatile solids (VS) per m³ and day. Sometimes the term COD (Carbon Oxygen Demand) is used instead. Both VS and COD have limitations and advantages. In this study VS is used as a measure of organic content.

**2.4 Operating techniques**

**2.4.1 One- or two-step process**

The four steps of the biogas process are performed by three different organism groups which require somewhat different conditions to work optimally (Nordberg, 2006). In a one-step process all the reactions take place in the same digester. A two-step process is divided with the hydrolysis and acidification in the first digester and the methane-forming step in the second digester with the two steps optimized for the different microorganisms for the two steps.

**2.4.2 Batch wise or continuous process**

In a batch wise process the reactor tank is filled and emptied completely before and after each treatment of a particular substrate (Nordberg, 2006). This method is easy for the handling of the substrate but result in a great variation in biogas production both in quality and quantity of the biogas. On the other hand the batch wise process can allow as much as 100 % degradation of the ingoing organic material. The variations for a biogas plant using a batch wise process can be lowered some by starting reactors at different times and running them in parallel (Nordberg, 2006). In a continuous process addition of substrate is done at the same time as biogas reactor residue is taken out of the reactor. The reactor can be fed continuously, often between 1-8 times per day, which results in a more even gas production. With the continuous process the substrate is never fully degraded because of the parallel continuous outtake. A normal degradation degree can vary
between 50-70 %. The continuous process requires a higher initial investment compared with the simpler batch process.

2.5 Cellulose and cellulose rich materials

Plant materials, such as woods of different kinds, have three main component groups: cellulose (40-50 %), hemicellulose (20-25 %) and lignin and other extractives (5 %) of the total mass (Duff & Murray, 1995).

Cellulose is an organic compound found in the cell walls of plants and in its smallest parts consists of β-1,4-glucose linked together in long chains (figure 2) (Campbell & Reece, 2005). The cellulose-molecules attach to each other with hydrogen bonds and coil together in a tight structure called a microfibril. It is these microfibrils that build up the plant cell walls. This compact structure makes the cellulose resistant to chemical and biological attacks (Taherzadeh & Karimi, 2008).

While cellulose has a rigid and crystalline form hemicelluloses have a more amorphous and randomly branched structure (Taherzadeh & Karimi, 2008). Hemicellulose surrounds the cellulose microfibrils and glues them together (Duff & Murray, 1995). The basic sugars in hemicellulose differ between different woods, especially between soft- and hardwoods.
The third compound in plants is lignin (Campbell & Reece, 2005). Lignin is made out of aromatic units called phenylpropanoids and the most difficult component plant material for microorganisms to degrade.

2.6 Pre-treatment methods
There are several possible pre-treatment techniques for lignocellulosic materials used for biogas production. The purpose of all pre-treatments on cellulose rich materials is to make them more digestible for the microorganisms in the biogas process. A pre-treatment thus results in an increased total accessible surface area and improved levels of available sugars (figure 3) (Taherzadeh & Karimi, 2008). A good pre-treatment should also avoid formation of inhibitory by-products and makes the production more cost-efficient by increasing the biogas yield (Sun & Cheng, 2002).

**Figure 3. Pre-treatment of lignocellulosic materials takes place prior to biogas production to increase the biogas yield (modified after Taherzadeh & Karimi, 2008).**

2.6.1 Biological-, chemical- and physical pre-treatments
In general pre-treatments are divided into four categories: biological, chemical, physical and thermal pre-treatments (Yang & Wyman, 2007). Biological treatment can include the use of brown-, white- and soft-rot fungi to degrade cellulose, hemicellulose and lignin. Another way is to use chemicals to pre-treat the substrates (Taherzadeh & Karimi, 2008). Some bases can be used, such as ammonia, ammonium sulphate and sodium hydroxide, but acids like sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and hydrochloric acid (HCl) are more common. Physical pre-treatments are the most used on cellulose-rich materials. The treatment can be to grind, mill or in any other way comminute the substrate.

2.6.2 Thermal pre-treatment
Thermal pre-treatment is a method where water-containing substrates are subjected to heating under pressure (Liu et al. 2002; Taherzadeh & Karimi, 2008). During the initial state of the treatment organic acids are formed from the acetyl groups in the substrate (Duff & Murray, 1995). These acids catalyze the hydrolysis of the hemicellulose in the material. The material is then rapidly discharged into normal atmospheric pressure which causes an explosion of the macromolecules (Liu et al. 2002; Taherzadeh & Karimi, 2008). This breaks up the structure of the cellulose, removes most of the hemicellulose and increases the total surface area
and therefore making it more accessible for the microorganisms in the biogas process. Another name for thermal pre-treatment is steam pressure disruption or steam explosion which perhaps gives a more precise description of what is done to the substrate.

Four main factors decide the effect of the thermal pre-treatment: residence time, temperature, particle size and moisture content (Sun & Cheng, 2002; Taherzadeh & Karimi, 2008). For an efficient treatment it is important that the optimal conditions are chosen. Furthermore, a too harsh treatment of lignocellulosic materials may result in lower methane yield and longer retention time. The reason behind this is that when lignin is broken up, in for example a pre-treatment, it forms so called furfurals (Rivard & Grohmann, 1991). These aromatic structures are known to inhibit many fermenting microorganisms, including the ones in the biogas process (Negash et al, 1997; Rivard & Grohmann, 1991). In general, softwoods contain higher amounts of lignin than hardwoods and other plant residues (Taherzadeh & Karimi, 2008). In Sweden most of the available biomass for biofuel production is softwoods (Hahn-Hägerdal et al., 2006). This is why the appropriate pre-treatment of cellulose rich biofuel substrates needs to be optimized. Pre-treatments can also be combined in several steps (Taherzadeh & Karimi, 2008). Thermal pre-treatment is for example often combined with addition of sulphuric acid to further improve the recovery of cellulose and hemicellulose and sulphuric acid have been shown to be an effective catalyst for the hydrolyzation of carbohydrates (Sassner et al, 2005; Tahersadeh & Karimi, 2008).
3. Material and methods

3.1 Substrates
The substrates tested in this study were spruce, oat straw, meadow grass, aspen wood chips and wet grain residue (WGR) from two ethanol production plants in Sweden. Both ethanol production plants use cereals as substrate whereas one is producing spirits and the other one is producing ethanol for vehicle biofuel purposes from cereals. Some of the characteristics of the tested materials are shown in table 1 below. WGR was in the form of a thick liquid where the untreated WGR had a light beige colour and the steam exploded WGR was a liquid which had a very dark brown colour. The aspen was grinded to powder, the average size of the untreated meadow grass was approximately 1 mm and the thermally pre-treated meadow grass was approximately 2 cm while the average size of the spruce wood chips was 1 to 5 cm in length. The untreated oat straw was investigated in two size fractions, finely grinded powder, approximately 1 mm, and coarsely chopped, approximately 2 cm, because previous studies have shown that the substrate particle size does have an impact on the biogas production rate (Mshandete, 2006). Coarsely chopped oat straw was used in both thermal pre-treatments of oat straw.

3.2 Thermal pre-treatment
The first pre-treatment consisted of a steam explosion where the substrate was put through a steam gun at 190°C for 10 minutes. Then the pressure was drastically lowered by opening the valve and the material was collected. This treatment will be referred to as T1.

The second pre-treatment consisted of a steam explosion where the substrate was put through a steam gun at 200°C for 5 minutes after which the pressure was drastically lowered by opening the valve and the material was collected. This treatment will be referred to as T2.

Both the T1 and T2 pre-treatments were tested on the oat straw, meadow grass and aspen wood chips. For both the WGR only the T1-treatment was tested and for the spruce only the T2-treatment was evaluated.

3.3 Experiment layout

3.3.1 Batch experiment
The substrates and inoculums dry substance (DS) and volatile solids (VS) content were determined before the start of the experiment using a standard method (APHA/AWWA/WEF, 1995). DS% is the amount of a sample that is left after drying

Figure 4. The bottles in the batch experiment during incubation in a constant temperature room on a shake table.
the sample in 105°C for at least twelve hours compared to the total mass of the sample before drying. VS% is a measure of the organic content of a sample and is measured by weighing in a dry sample before and after heating to 550°C for at least six hours. The remaining ash corresponds to the mineral content of the substrate. Each substrate was investigated in triplicate samples. Table 1 below shows the DS- and VS-measurements of the tested substrates.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>DS% ± Se</th>
<th>VS% ± Se</th>
<th>Ash%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce, untreated</td>
<td>54 ± 2.5a</td>
<td>54 ± 2.5a</td>
<td>0.3</td>
</tr>
<tr>
<td>Spruce, T2</td>
<td>29 ± 0.4b</td>
<td>29 ± 0.4b</td>
<td>0.1</td>
</tr>
<tr>
<td>Oat straw, untreated</td>
<td>95 ± 0.3a</td>
<td>88 ± 0.4a</td>
<td>6.7</td>
</tr>
<tr>
<td>Oat straw, T2</td>
<td>33 ± 2.1b</td>
<td>31 ± 2.1b</td>
<td>2.3</td>
</tr>
<tr>
<td>Oat straw, T1</td>
<td>15 ± 0.2b</td>
<td>14 ± 0.3b</td>
<td>1.3</td>
</tr>
<tr>
<td>Meadow grass, untreated</td>
<td>94 ± 0.2a</td>
<td>89 ± 0.0a</td>
<td>4.7</td>
</tr>
<tr>
<td>Meadow grass, T2</td>
<td>19 ± 0.0b</td>
<td>18 ± 0.1b</td>
<td>0.7</td>
</tr>
<tr>
<td>Meadow grass, T1</td>
<td>16 ± 1.3b</td>
<td>15 ± 1.3b</td>
<td>1.0</td>
</tr>
<tr>
<td>Aspen, untreated</td>
<td>96 ± 0.3a</td>
<td>96 ± 0.2a</td>
<td>0.8</td>
</tr>
<tr>
<td>Aspen, T2</td>
<td>28 ± 1.0b</td>
<td>28 ± 0.8b</td>
<td>-0.1</td>
</tr>
<tr>
<td>Aspen, T1</td>
<td>11 ± 0.4b</td>
<td>11 ± 0.4b</td>
<td>0.2</td>
</tr>
<tr>
<td>WGR spirits, untreated</td>
<td>8.4 ± 0.3b</td>
<td>7.9 ± 0.3b</td>
<td>0.5</td>
</tr>
<tr>
<td>WGR spirits, T1</td>
<td>4.8 ± 0.0b</td>
<td>4.4 ± 0.0b</td>
<td>0.4</td>
</tr>
<tr>
<td>WGR biofuel, untreated</td>
<td>23 ± 0.0b</td>
<td>20 ± 0.6b</td>
<td>2.8</td>
</tr>
<tr>
<td>WGR biofuel, T1</td>
<td>9.9 ± 0.0b</td>
<td>8.8 ± 0.6b</td>
<td>1.1</td>
</tr>
<tr>
<td>Inoculum, old, 2008-08-25</td>
<td>3.8 ± 1.1b</td>
<td>2.4 ± 1.1b</td>
<td>1.4</td>
</tr>
<tr>
<td>Inoculum, old, 2008-10-10</td>
<td>3.8 ± 0.0b</td>
<td>2.2 ± 0.1b</td>
<td>1.5</td>
</tr>
<tr>
<td>Inoculum, new, 2008-10-17</td>
<td>4.4 ± 0.0b</td>
<td>3.0 ± 0.0b</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*a. % from dry sample weight  
b. % of wet sample weight  
c. Ash% corresponds to the estimated DS%-VS%*

Determination of the biogas potential and methane production rate was done using a batch method (Hansen et al, 2004). Each 1120 ml-bottle was loaded with 3 g VS from each respective substrate and triplicate bottles were started for each substrate. The bottles where then flushed with N2-gas while filled with the adjusted amount inoculum. The added amount of inoculum totaled up to ⅔ of the loaded total VS-amount in each bottle. Subsequently each bottle was filled with tap-water up to a volume of 700 ml. The inoculums used in this study came from the VAFAB biogas plant in Västerås, Sweden, and were collected at two separate occasions. DS- and VS-measurements for the inoculum at different age stages can be found in table 1 above. The first collected inoculum, ‘inoculum, old’, was used in the batch experiment used for evaluation of the pre-treatment as well as in a later control experiment (see below). The second collection, ‘inoculum, new’, was only used in a control experiment. Controls with only inoculums and no added substrate were started in each set. The data with accumulated methane production
was adjusted so that the background production of the inoculum was taken into consideration in the analysis.

In the batch method the inoculum is not put into the experiments at once but has to be degassed during incubation at 37°C for at least 4-5 days. The old inoculum was approximately 2 months old at the beginning of the experiments and still active as gas still was produced from endogenous organic material. Sets with groups of test bottles were started with a 2-3 week interval to even out the methane sampling workload. In total three experiment sets and one control experiment were started. The bottles were incubated at 37°C on shake tables running at 130 rpm.

The gas pressure in the batch bottles was measured with a digital pressure meter (Testo 512, Testo AG, Lenzkirch, Germany) and gas samples for measurement of methane concentration (CH₄) were taken at the same time. Gas samples of 2 ml were withdrawn from the test bottle with a syringe and inserted in a glass vial (23 ml) that was pre-sealed with an aluminium cap and a rubber stopper. Methane concentration was determined later with gas chromatography. The batch bottles were then depressurized to atmospheric pressure and the excess gas was collected with a gas bag. Sampling was conducted depending on the expected gas production over time, which means that samples were taken more often in the beginning of the set and more seldom after 1-2 weeks of incubation.

### 3.3.2 Control experiment

Hashimoto (1989) have shown that there is a correlation between the inoculum- and VS-load. To further investigate the affecting parameters of the batch method (Hansen et al, 2004) a control experiment was designed to evaluate the effect from inoculum age and VS-load, with the same batch experiment layout as for the thermally pre-treated materials. In addition the possible effect of using tap water as dilution medium was investigated by using reduced media instead of tap water in one test series (Schnürer et al, 1996).

The new inoculum was approximately 1 week old, inoculum, new, 2008-10-17 in table 1, while the older inoculum was approximately 4 months old at the start of the control experiment, listed as Inoculum, old, 2008-08-25 in table 1. All the bottles were fed with 3 g VS of coarsely chopped oat straw except the controls with only inoculum. The experimental layout can be seen in table 2 below.
Table 2. The control experiment layout showing which inoculum, proportions between inoculum and substrate load, dilution media and substrates that were used. The inoculums load was made on VS-basis in proportion to the VS-content of the oat straw (coarsely chopped).

<table>
<thead>
<tr>
<th>Experimental name</th>
<th>Inoculum</th>
<th>Proportion of inoculums and substrate load</th>
<th>Dilution medium</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>New</td>
<td>1:1</td>
<td>Tap water</td>
<td>Oat straw</td>
</tr>
<tr>
<td>N2</td>
<td>New</td>
<td>2:1</td>
<td>Tap water</td>
<td>Oat straw</td>
</tr>
<tr>
<td>N4</td>
<td>New</td>
<td>4:1</td>
<td>Tap water</td>
<td>Oat straw</td>
</tr>
<tr>
<td>NR</td>
<td>New</td>
<td>2:1</td>
<td>Reduced media</td>
<td>Oat straw</td>
</tr>
<tr>
<td>O2</td>
<td>Old</td>
<td>2:1</td>
<td>Tap water</td>
<td>Oat straw</td>
</tr>
</tbody>
</table>

3.4 Data analysis

3.4.1 Methane analysis

Analysis of methane content was preformed with a gas chromatograph (PerkinElmer ARNEL Clarus 500) with helium as the carries gas at a flow rate of 31 ml per minute. The column used was a 7’ HayeSep N 60/80, 1/8” SF, and the injection temperature was set to 60°C using a Headsampler Turbo Matrix 110. Methane was detected using a flame-ionization detector which operated at a temperature of 250°C. The injected gas sample volume in each glass vial was 2 ml.

3.4.2 Degradation rate

The gas production in each test bottle was analyzed and processed in Excel 2007 so that the mean accumulated methane yield in ml per gram VS over time could be read as seen in the example in figure 5 below. The daily methane production, in ml CH_4/g VS · day, was also calculated and the maximum production per day was used as a comparative value between the different substrates and treatments. Both the figures of daily production rates and accumulated methane production can be used to estimate the period of high production which in turn can be used to predict when the production peak from a certain substrate will occur. In two cases bottles behaved strange and were excluded from the data analysis. One bottle in the T2-treated oat straw showed distinct leakage from the rubber stopper after one month’s incubation time and was excluded for this reason. Another bottle that was excluded was from the untreated aspen where the production of biogas after one month’s time doubled compared to the other two bottles for no obvious reason. One explanation could be that a knob of endogenous material came into the bottle and broke after four weeks time, resulting in the increased biogas production.
Figure 5. Comparison between ml accumulated methane (with standard errors) for untreated and coarsely chopped oat straw and T1-treated aspen. The accumulated methane production differs both after 30 and after 60 days even though the highest mean methane production is approximately the same.

### 3.4.3 Statistical analysis

The statistical analysis was performed in Excel 2007 and Minitab 15. Measurements from the experiments were used to estimate the methane production rate. The gas production results from the different treatments ($X_1$, $X_2$,...,$X_n$) were assumed to be a random samples from a normal distribution with a $N-1$ degrees of freedom ($df$), different $df$ depending on the number of treatments for each substrate. $\sigma$ was estimated from the standard deviation and a 95 % confidence interval was used.

One-way-ANOVA was used to test if there were any significant differences in production for the different substrates. Since time is not an independent factor in the batch experiment layout ANOVAs were executed for the specific time of interest, after 30 days and 60 days accumulated biogas production as well as for the finishing day of incubation of each bottle. In addition a post hoc test (Tukey test) was performed on all ANOVAs to compare the different treatments against each other for each substrate.
4. Results

A summary of the degradation rates and accumulated methane production after 30 days, 60 days and at the last day of degradation can be seen in table 3 below. The degradation rates are illustrated with the maximum mean methane production in ml per g VS and day. The strength of the p-values from the ANOVA is general low due to the low number of replicates in this study. There is no fundamental hindrance to use an ANOVA with a low number of replicates. However, the low strength of the analysis limits the usefulness of the p-values.

4.1 Degradation potentials

The mean maximum production and production potential from a substrate are not always connected and both are of interest to evaluate the possible use in commercial biogas production. To compare the degradation potentials for the different substrates the accumulated methane production in ml methane per gram volatile solids (ml CH₄/g VS) after 30 and 60 days incubation as well as at the total potential is used. The total potential was assumed to have been reached when the methane production had levelled out and the background production from the control was equal with the experiment bottles over at least two measuring points.
Table 3. The highest measured degradation rate for the different substrates over the period with exponential growth rate, measured as the mean methane production in ml per g VS and day (A). Also the mean accumulated methane yield per gram VS at 30 and 60 days as well as the total potential of each substrate at the end of the experiment is shown in this table, all with standard deviation (C). The total potential was assumed to have been reached when the accumulation of methane had levelled out and the highest measured value of accumulated methane was considered to be the total potential. After levelling out in methane production the bottles were terminated from the experiment. The oat straw and meadow grass were terminated after 141 days; aspen, WGR from spirits production and the WGR from biofuel production were terminated after 130 days and the spruce were terminated after 95 days.

<table>
<thead>
<tr>
<th>Substrate treatment</th>
<th>A</th>
<th>B</th>
<th>30 days ± Se</th>
<th>60 days ± Se</th>
<th>Tot. pot. ± Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspen untreated</td>
<td>43</td>
<td>27</td>
<td>76±1</td>
<td>126±3</td>
<td>166±6</td>
</tr>
<tr>
<td>Aspen T2</td>
<td>49</td>
<td>27</td>
<td>121±13</td>
<td>215±2</td>
<td>249±4</td>
</tr>
<tr>
<td>Aspen T1</td>
<td>62</td>
<td>39</td>
<td>140±4</td>
<td>277±6</td>
<td>309±4</td>
</tr>
<tr>
<td>Meadow grass untreated</td>
<td>88</td>
<td>32</td>
<td>180±2</td>
<td>232±2</td>
<td>270±10</td>
</tr>
<tr>
<td>Meadow grass T2</td>
<td>107</td>
<td>32</td>
<td>221±5</td>
<td>254±4</td>
<td>285±4</td>
</tr>
<tr>
<td>Meadow grass T1</td>
<td>100</td>
<td>32</td>
<td>204±4</td>
<td>234±3</td>
<td>262±9</td>
</tr>
<tr>
<td>Oat straw coarse untreated</td>
<td>61</td>
<td>32</td>
<td>129±1</td>
<td>162±1</td>
<td>208±0</td>
</tr>
<tr>
<td>Oat straw fine untreated</td>
<td>75</td>
<td>32</td>
<td>156±7</td>
<td>199±10</td>
<td>248±13</td>
</tr>
<tr>
<td>Oat straw T2</td>
<td>80</td>
<td>32</td>
<td>153±6</td>
<td>204±6</td>
<td>252±7</td>
</tr>
<tr>
<td>Oat straw T1</td>
<td>90</td>
<td>32</td>
<td>185±6</td>
<td>220±5</td>
<td>263±5</td>
</tr>
<tr>
<td>Spruce untreated</td>
<td>9</td>
<td>32</td>
<td>23±11</td>
<td>52±14</td>
<td>102±17b</td>
</tr>
<tr>
<td>Spruce T2</td>
<td>12</td>
<td>25</td>
<td>34±5</td>
<td>53±9</td>
<td>78±30b</td>
</tr>
<tr>
<td>WGR spirits untreated</td>
<td>203</td>
<td>27</td>
<td>412±4</td>
<td>479±3</td>
<td>511±4</td>
</tr>
<tr>
<td>WGR spirits T1</td>
<td>188</td>
<td>27</td>
<td>379±28</td>
<td>429±31</td>
<td>445±32</td>
</tr>
<tr>
<td>WGR biofuel untreated</td>
<td>156</td>
<td>25</td>
<td>332b±50</td>
<td>370±50</td>
<td>375±49</td>
</tr>
<tr>
<td>WGR biofuel T1</td>
<td>147</td>
<td>25</td>
<td>310b±27</td>
<td>388±50</td>
<td>389±4</td>
</tr>
</tbody>
</table>

A. Highest mean CH₄-production (ml CH₄/g VS · day)
B. Day of production peak after the start of incubation
C. Accumulated methane (ml acc. CH₄/g VS) and standard errors (±Se)

a Extrapolated value from mean values between samples taken on day 29 and 32 of incubation.
b Total potential could not be determined within the time frame of the study; value after 95 days incubation.
c Extrapolated value from mean values between samples taken on day 27 and 31 of incubation.

The highest accumulated amount of methane was observed in the WGR from spirits production, 412 ml CH₄/g VS after 30 days, which was higher than the T1-treated WGR from the spirits production that reached a production of 379 ml CH₄/g VS during the same time (figure 6). After 60 days the accumulated methane production had reached 479 ml CH₄/g VS for the untreated WGR from spirits production and 429 ml CH₄/g VS for the T1-treated WGR from spirits production. The statistical analysis showed that there was no significant difference between the untreated and the T1-treated WGR from spirits production after 30 days (p = 0.163) nor after 60 days (p = 0.061). Untreated WGR from biofuel production accumulated 332 ml CH₄/g VS in 30 days and the T1-treated WGR from biofuel...
production gave rise to 310 ml CH\textsubscript{4}/g VS in 30 days (figure 7). After 60 days both WGR from biofuel production had given rise to 388 ml CH\textsubscript{4}/g VS. No significant differences between the production potential of untreated and T1-treated WGR from biofuel production after 30 days was found ($p = 0.672$) nor after 60 days ($p = 0.568$). At the end of incubation of the bottles the untreated WGR from spirits production had given rise to 511 ml CH\textsubscript{4}/g VS in 130 days while the T1-treated WGR from spirits production had given rise to 445 ml CH\textsubscript{4}/g VS in 130 days. The total potential was found to be significantly different between the untreated and T1-treated WGR from spirits production ($p = 0.023$). Untreated WGR from biofuel production was terminated after 95 days and had given rise to 375 ml CH\textsubscript{4}/g VS at that time and T1-treated WGR from biofuel production gave rise to 389 ml CH\textsubscript{4}/g VS in 95 days. The total potential of the untreated and T1-treated WGR from biofuel production was not found to be significantly different ($p = 0.636$).

Figure 6. Accumulated methane production (with standard errors) from WGR from spirits production.
The untreated meadow grass gave rise to 180 ml CH₄/g VS, the T1-treated meadow grass to 204 ml CH₄/g VS while the T2-treated meadow grass gave rise to 221 ml CH₄/g VS in accumulated methane after 30 days of incubation (figure 8). After 60 days the accumulated methane levels had reached 232 ml CH₄/g VS from the untreated meadow grass, 234 ml CH₄/g VS from the T1-treated meadow grass and 254 ml CH₄/g VS from the T2-treated meadow grass. Methane production differed significantly between the untreated, T2-treated and T1-treated meadow grass after 30 days \((p = 0.000)\) as well as after 60 days \((p = 0.000)\). At the end of the study the total potential for the untreated meadow grass was determined to 270 ml CH₄/g VS and the total potential for the T1-treated meadow grass was determined to 262 ml CH₄/g VS whereas the T2-treated meadow grass was determined to 285 ml CH₄/g VS. There was a significant difference in total potential, after 106 days, between the untreated meadow grass and the T2-treated meadow grass \((p = 0.071)\) as well as between T2-treated meadow grass and T1-treated meadow grass \((p = 0.020)\).

The T1-treated oat straw gave rise to 185 ml CH₄/g VS in 30 days which is almost the same amount as given from the untreated meadow grass for the same time (figure 9). After 60 days the T1-treated oat straw had given rise to 220 ml CH₄/g VS. T1-treated oat straw gave rise to a significantly difference in the amount of methane in 30 days compared to the T2-treatment \((p = 0.040)\) and the difference was still significant after 60 days \((p = 0.046)\). There was no significant difference
between the finely grinded untreated oat straw and the T2-treated oat straw after 30 days \((p = 0.234)\) nor after 60 days \((p = 0.604)\). They accumulated 156 ml CH\(_4\)/g VS and 153 ml CH\(_4\)/g VS in 30 days and 199 ml CH\(_4\)/g VS and 204 ml CH\(_4\)/g VS in 60 days respectively. The coarsely chopped oat straw resulted in an accumulated methane yield of 129 ml CH\(_4\)/g VS in 30 days which was significantly less than for the T2-treated oat straw \((p = 0.001)\). After 60 days the coarsely chopped oat straw had given rise to 162 ml CH\(_4\)/g VS, which still was significantly less than the T2-treated oat straw gave rise to \((p = 0.001)\). The total potential for all the oat straw treatments was determined after 106 days of incubation. The total potential for the T1-treated oat straw was determined to 263 ml CH\(_4\)/g VS while the T2-treated oat straw in total had given rise to 252 ml CH\(_4\)/g VS. There was no significant difference in total potential between the T2-treated oat straw and the T1-treated oat straw \((p = 0.110)\). The total potential for finely grinded oat straw was determined to 248 ml CH\(_4\)/g VS and total potential for the coarsely chopped oat straw was determined to 208 ml CH\(_4\)/g VS. There was no significant difference in total potential between T2-treated oat straw and finely grinded oat straw \((p = 0.758)\). However, the total potential of the coarsely chopped untreated oat straw was found to be significantly lower than for the finely grinded untreated oat straw \((p = 0.006)\).

**Figure 8.** Accumulated methane production (with standard errors) from meadow grass.
Figure 9. Accumulated methane production (with standard errors) from oat straw.

Figure 10. Accumulated methane production (with standard errors) from aspen.
The T1-treated aspen resulted in 140 ml accumulated CH$_4$/g VS after 30 days incubation which was significantly more than the T2-treated aspen which accumulated 121 ml CH$_4$/g VS during the same time (figure 10). There was however no significant difference between the T1- and the T2-treated aspen after 30 days ($p = 0.103$). Untreated aspen reached 96 ml CH$_4$/g VS after 30 days which was significantly less than the T2-treated aspen ($p = 0.020$) and the T1-treated aspen ($p = 0.002$). After 60 days the methane production was significantly different between all three treatments ($p = 0.000$). The T1-treated aspen gave rise to 277 ml CH$_4$/g VS in 60 days while the T2-treated aspen gave rise to 215 ml CH$_4$/g VS and the untreated aspen gave rise to 126 ml CH$_4$/g VS in 60 days. After 130 days the methane production from the three aspen-treatments was found to have levelled out and the total potential of the aspen was determined. The total potential for untreated aspen was determined to 166 ml CH$_4$/g VS and for T2-treated aspen to 249 ml CH$_4$/g VS and the difference in total potential was significant ($p = 0.000$). There was also a significant difference in total potential between the T1-treated aspen, which gave rise to 309 ml CH$_4$/g VS, and the T2-treated aspen ($p = 0.000$).

Most modest in production was the T2-treated spruce which accumulated 23 ml CH$_4$/g VS in 30 days and 52 ml CH$_4$/g VS in 60 days, while the untreated spruce gave rise to 34 ml CH$_4$/g VS in 30 days and 53 ml CH$_4$/g VS in 60 days (figure 11). There was no significant difference in methane production between the untreated and T2-treated spruce after 30 days ($p = 0.224$) nor after 60 days ($p = 0.901$). The total potential for spruce could not be determined within the time frame for this study. The untreated spruce had at the end of the study given rise to 102 ml CH$_4$/g VS after 95 days and the T2-treated spruce had given rise to 78 ml CH$_4$/g VS after 95 days which was not found to be significantly different ($p = 0.292$).

**Figure 11.** Accumulated methane production (with standard errors) from spruce.
4.2 Degradation rate

The highest mean methane production rate was observed in the untreated WGR from spirits production, which gave rise to 203 ml CH\textsubscript{4}/g VS \cdot day (figure 12). T1-treated WGR from the spirits distillery gave rise to the second highest methane production, 188 ml CH\textsubscript{4}/g VS \cdot day. Both the WGRs from the spirits production had their production peak after 27 days and the production levels could not be statistically separated (\(p = 0.128\)). The WGR from the biofuel production both peaked after 25 days and reached a maximum mean methane production of 156 ml CH\textsubscript{4}/g VS \cdot day for the untreated and 147 ml CH\textsubscript{4}/g VS \cdot day for the T1-treated (figure 13). However, the difference in mean methane production rate was not statistically significant (\(p = 0.592\)).

![Figure 12. Methane production rates (ml CH\textsubscript{4}/g VS \cdot day) and standard errors from the digestion of untreated and T1-treated WGR from spirits production.](image-url)
Meadow grass treated according to the T2-parameters gave rise to a mean methane production rate of 107 ml CH$_4$/g VS · day at peak production, which occurred after 32 days for all the treatments of meadow grass (figure 14) and significantly more than for the T1-treated meadow grass ($p = 0.012$). The T1-treated meadow grass reached a maximum mean production of 100 ml CH$_4$/g VS · day which was significantly more ($p = 0.001$) than the untreated material which reached up to 88 ml CH$_4$/g VS · day.

The T1-treated oat straw resulted in a maximum mean production rate of 90 ml CH$_4$/g VS · day which is in the same level as the untreated meadow grass (figure 15). Significant difference in maximum mean production rate was found between T1-treated and T2-treated oat straw ($p = 0.031$). T2-treated oat straw and finely grinded untreated oat straw differed little in maximum production rate; they reached 80 ml CH$_4$/g VS · day and 75 ml CH$_4$/g VS · day respectively and could not be statistically separated ($p = 0.229$). Coarsely chopped untreated oat straw gave rise to a maximum production rate of 61 ml CH$_4$/g VS · day which was significantly less than for the finely grinded oat straw ($p = 0.004$). All oat straw treatments reached their production peak after 32 days.
Figure 14. Methane production rates (ml CH$_4$/g VS·day) and standard errors from the digestion of untreated and T2- and T1-treated meadow grass.

Figure 15. Methane production rates (ml CH$_4$/g VS·day) and standard errors from the digestion of untreated coarsely chopped and finely grinded, T2- and T1-treated oat straw.
T1-treated aspen gave rise to a maximum production rate of 62 ml CH₄/g VS · day (figure 16). However, the production peaked after 39 days which is 12 days later than the untreated aspen and T2-treated aspen which peaked in production after 27 days. The untreated aspen and T2-treated aspen reached, after 27 days, a maximum production rate of 43 ml CH₄/g VS · day and 49 ml CH₄/g VS · day respectively which was not a significant difference ($p = 0.077$).

The earliest production peak was observed in the T2-treated spruce, which peaked after 25 days (figure 17). However, the maximum production rate of T2-treated spruce reached only 12 ml CH₄/g VS · day. The untreated spruce reached a maximum production of 9 ml CH₄/g VS · day and peaked in production after 32 days. No significant differences were found either in 25 days ($p = 0.096$) or in 32 days ($p = 0.324$).

![Figure 16. Methane production rates (ml CH₄/ g VS · day) and standard errors from the digestion of untreated, T2- and T1-treated aspen.](image-url)
4.3 Control experiment

A summary of the results from the control experiment are found in table 4 below. The control experiment showed that there was a significant effect on both the degradation rate and the total potential within 30 days of incubation, depending on the age of the inoculum used for digestion and the inoculum load.

4.3.1 Degradation rate and degradation potential

The production rate in the control experiment bottles showed that the highest mean methane production was obtained from the N4-treatment (figure 18), where the inoculum load was doubled compared to the batch bottle experiment layout, previously used in this study. From the N4-treatment the highest mean production was measured to 125 ml CH$_4$/g VS · day. After 30 days the N4-treatment had given rise to 334 ml CH$_4$/g VS (figure 19) which was significantly higher than the NR-treatment ($p = 0.002$) and the N2-treatment ($p = 0.005$). The N4-treatment gave rise to 320 ml CH$_4$/g VS in 60 days which was not found significantly different from the N2-treatment after 60 days ($p = 0.170$) but was found to be significantly different from the NR-treatment after 60 days ($p = 0.022$). At the day of termination of the batch bottles, after 72 days, the N4-treatment had given rise to 321 ml CH$_4$/g VS. This was not significantly higher than the N2-treatment ($p = 0.113$) but significantly higher than the NR-treatment ($p = 0.024$). However, there was no significant difference between the N2- and NR-treatments ($p = 0.221$).
In the NR-treatment, where the inoculums- and substrate load were the same as in the N2-treatment but where the water was exchanged for reduced medium, the production rate reached 108 ml CH\textsubscript{4}/g VS \cdot day and had after 30 days accumulated 279 ml CH\textsubscript{4}/g VS. The N2-treatment had the same substrate load and inoculums load as the batch experiment and reached a maximum production rate of 94 ml CH\textsubscript{4}/g VS \cdot day and gave rise to 274 ml CH\textsubscript{4}/g VS after 30 days. After 60 days the N2-treatment had given rise to 303 ml CH\textsubscript{4}/g VS and the NR-treatment had given rise to 287 ml CH\textsubscript{4}/g VS after 60 days. The NR- and N2-treatments could not be statistically differentiated after 30 days (p = 0.556) nor after 60 days (p = 0.090). At the day of termination of the batch bottles, after 72 days, the N2-treatment had given rise to 300 ml CH\textsubscript{4}/g VS and the NR-treatment had given rise to 289 ml CH\textsubscript{4}/g VS.

In the N1-treatment, which had equal proportions between inoculum and substrate in VS-load, the maximum production reached 59 ml CH\textsubscript{4}/g VS \cdot day and the mean accumulated methane yield had reached 193 ml CH\textsubscript{4}/g VS after 30 days and 226 ml CH\textsubscript{4}/g VS after 60 days. This was significantly lower than for the N2-treatment (p = 0.003) and for the NR-treatment (p = 0.001) but significantly higher than for the O2-treatment (p = 0.007 after 30 days). After 60 days the N1-treatment was found to be significantly lower than the N2-treatment (p = 0.001) and the NR-treatment (p = 0.000) but significantly higher than the O2-treatment (p = 0.002). The total potential of the N1-treatment was determined to 237 ml CH\textsubscript{4}/g VS after 93 days which was significantly higher than from the O2-treatment (p = 0.004) but significantly lower than the NR-treatment (p = 0.000).

The lowest production was observed in the O2-treatment where the treatment gave rise to 137 ml CH\textsubscript{4}/g VS after 30 days and the maximum production reached 22 ml CH\textsubscript{4}/g VS \cdot day at most. After 60 days the O2-treatment had given rise to 185 ml CH\textsubscript{4}/g VS and the total potential was determined to 205 ml CH\textsubscript{4}/g VS after 93 days. The O2-treatment had the same VS-load and inoculums load as the N2-treatment. The production peaked at the same time, after 18 days, for the N1-, N2-, N4- and NR-treatments and after 29 days for the O2-treatment.
Table 4. Results from the control experiment the highest mean methane production per day (A), day of production peak (B), accumulated methane production after 30 and 60 days of incubation as well as the total potential at the end of the experiment (C). The total potential was assumed to have been reached when the accumulation of methane had levelled out and the highest measured value of accumulated methane was considered to be the total potential. After levelling out in methane production the bottles were terminated from the experiment. The bottles N2, N4 and NR were terminated after 72 days incubation. The bottles N1 and O2 were terminated after 93 days.

<table>
<thead>
<tr>
<th>Experimental name</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 days ± Se</td>
<td>60 days ± Se</td>
<td>Tot. pot. ± Se</td>
</tr>
<tr>
<td>N1</td>
<td>59</td>
<td>18</td>
<td>193 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>N2</td>
<td>94</td>
<td>18</td>
<td>274 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>N4</td>
<td>125</td>
<td>18</td>
<td>334 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NR</td>
<td>108</td>
<td>18</td>
<td>279 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>O2</td>
<td>22</td>
<td>29</td>
<td>137 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

A. Highest mean CH₄ production (ml CH₄/g VS · day)
B. Day of production peak after the start of incubation
C. Mean accumulated methane (ml acc. CH₄/g VS)
   a. Extrapolated value from mean values between samples taken on day 29 and 35 of incubation.
   b. Extrapolated value from mean values between samples taken on day 29 and 37 of incubation.

Figure 18. Methane production rates (ml CH₄/g VS · day) and standard error from the digestion in the control experiment where the substrate load and inoculums age differed.
4.3.2 Inoculum

In figure 19 the shape of the accumulated methane curves for the N4-, N2- and NR-treatments in the final phase show a decrease in the accumulation of methane. This decrease is due to that the data from the methane measurements was adjusted for the background methane production of the inoculum. The background production of methane from the inoculum control bottles was at this stage at a higher rate than in the substrate fed bottles.
5. Discussion
The purpose of this study was to evaluate if a thermal pre-treatment process would have an effect on the biogas production from cellulose rich substrates and if so, to evaluate which of the two thermal pre-treatments tested that were the better one compared to untreated cellulosic biomass. Also the total potential of the tested substrates was of interest to reveal.

5.1 Effect of thermal pre-treatment
Does thermal pre-treatment result in a higher biogas yield? This question gets different answers depending on several factors. In this study the main focus has been the substrate and the treatment of the different substrates. For aspen, oat straw and meadow grass the thermal pre-treatments seems to make the substrates more degradable for the microorganisms in the biogas process which therefore can produce more biogas. However, steam explosion does not seem to increase the methane yields for pre-treated WGR from spirits production or WGR from biofuel production.

The experiment results for the oat straw show that a physical treatment with finely grinded oat straw gives the same methane yield as the T2-treated oat straw both after 30 and 60 days of incubation. This could be because the T2-treated oat straw had the same particle size as the coarsely chopped, untreated oat straw (approximately 2 cm). If the finely grinded oat straw had been thermally pre-treated instead of the coarsely chopped oat straw it is likely that the methane yield per gram VS would be higher from the T2-treatment. The thermal pre-treatment clearly had an effect on the oat straw. However, the same increase in methane yield was also achieved by grinding the oat straw. This effect of grinding is in line with the results of a previous study by Mshandete et al (2006). They showed that there is a correlation between increasing methane production with decreasing particle size of sisal fibre in anaerobic digestion. A comparison of the results between the coarsely chopped and finely grinded untreated oat straw show significant differences in both maximum production rates and potential methane yield, which also is in line with the results of Mshandete et al (2006). Thus, pre-treating the oat straw in some way, either by comminuting or by steam explosion, generates a higher biogas yield than putting the oat straw in the biogas process entirely untreated. It is also reasonable to draw the conclusion that thermal pre-treatment of small lignocellulosic particles will give rise to higher amounts of methane than larger thermally pre-treated lignocellulosic particles will do during the same incubation time.

Results for the meadow grass show that the T2-treatment gave rise to a significantly higher amount of methane than both the T1-treatment and the untreated meadow grass. This is not in line with prior studies which have shown that a harsh thermal pre-treatment may result in lower methane yields than a mild one (Taherzadeh & Karimi, 2008). The meadow grass gave rise to higher methane
yields with the harsher treatment in this study. When choosing between the two thermal pre-treatments tested on meadow grass in this study, the T2-treatment should be the better choice from a production point of view.

Aspen showed the largest difference between treatments where the T1-treatment generated more biogas compared to both the T2-treated aspen, which was second best, and the untreated aspen. After 30 days the differences in methane production between the two thermal pre-treatments were not significant which could possibly be explained by the relatively high age of the inoculum used, three months old, for the aspen-batch. The high inoculum age resulted in a longer lag-phase. However, after 60 days the differences in methane production were highly significant between all three treatments. For the spruce the methane production was initially lower from the pre-treated material compared to the untreated. One reason for the lower production of methane in the T2-treated spruce compared to untreated spruce could be that the pre-treatment released inhibitory compounds such as furfurals (Rivard & Grohmann, 1991). Spruce, being a softwood species, contains a higher amount of furfural-forming lignin than aspen (Taherzadeh & Karimi, 2008). This could be one explanation for the differences between the two wood species in this study. Furfurals are known to be inhibitory to the biogas process and according to literature the methanogens can only partly transform this compound (Negash et al, 1997; Rivard & Grohmann, 1991). Some studies have shown that furfurals can be degraded to acetate by the sulphate-reducing bacteria that compete with the methanogens for the living space (Rivard & Grohmann, 1991). These results suggest that the degradation of furfurals in the digester slurry depend on the relation between methanogens and sulphate-reducing bacteria. Commonly the sulphate concentration is not elevated in biogas processes why the sulphate-reducing bacteria in low sulphate processes are outcompeted by the methanogens. Therefore it is not likely that furfurals can be completely degraded, if produced in the process. Another explanation between the differences in methane production between untreated aspen and untreated spruce could be that the particle size differed significantly. The particle size of untreated aspen was approximately 1 mm particles while the untreated spruce pieces were 1-5 cm in length.

Thermal pre-treatment of WGR from both spirits and biofuel production did not show any significant effect on the methane production. WGR contains less cellulose and hemicellulose and a larger proportion of proteins than the other tested materials in this study. A thermal pre-treatment in this case does perhaps not increase the total amount of available sugars for the microorganism to a level where a significant increase in methane production can be measured.

5.2 Degradation rate and biogas potential
There was not always a clear connection between a high degradation rate and a high methane yield per input unit of substrate. One example could be the
comparison between untreated, coarsely chopped oat straw and T1-treated aspen which both had a maximum production rate of approximately 61 ml CH₄/g VS · day. However, T1-treated aspen gave rise to 277 ml CH₄/g VS in 60 days while coarsely chopped, untreated oat straw gave rise to only 162 ml CH₄/g VS after 60 days (figure 5). When the easily digested sugars have been consumed by the microorganisms their production rate levels out as they then have to use the less available sugars, i.e. the cellulose and hemicellulose, in the substrate. Thus, the differences in accumulated production cannot be explained by only comparing the maximum production rates. Instead, the reason for the different accumulated methane yields is that the number of days at peak production rates differs between the substrates (compare for example figure 15 and 16). While the coarsely chopped, untreated oat straw give rise to maximum production rates during approximately 3-4 days the T1-treated aspen gave rise to the same production rates for approximately 6 days which results in a higher methane accumulation over time. Furthermore, the final potential is also dependent on the total amount degradable organic nutrients available in the substrate. However, the degradation rates are still of interest from a production point of view.

When comparing the total potential for the different substrates in this study the untreated WGR from spirits production gave rise to the highest methane production. No positive effect of thermal pre-treatment could be demonstrated for WGR from either ethanol production plants. Pre-treating the WGR is likely to be economically unviable when considering the extra effort and energy cost put into the pre-treatment. A perhaps surprisingly strong effect of thermal pre-treatment was determined for the T1-treated aspen compared both to the T2-treated and untreated aspen. It is of interest to make further investigations on this substrate based on the results from this study since the total potential for aspen was almost twice as high in the T1-treated compared to the untreated material; 309 ml CH₄/g VS and 166 ml CH₄/g VS respectively. A positive effect of thermal pre-treatment on the total potential was also found for both meadow grass and oat straw. The largest difference in total potential was found between the untreated and the T2-treated meadow grass and between the coarsely chopped untreated oat straw and the T1-treated oat straw. T2-treated meadow grass gave rise to approximately 20 ml CH₄/g VS more than the untreated meadow grass whereas the T1-treated oat straw gave rise to approximately 60 ml CH₄/g VS more than the coarsely chopped untreated oat straw. Depending on the availability of oat straw and meadow grass and extra cost for pre-treating these materials steam explosion could be of interest to increase the methane yield. However, further calculations are needed.

Several factors determine if a cellulose rich substrate is interesting for commercial biogas production such as degradation rate, production peak and the total potential. The picture gets more complicated when taking into consideration that most biogas plants are run with co-digestion of several substrates. Co-digestion generally gives a higher biogas yield than if digesting a substrate alone, as have
been done in this study. A common problem with anaerobic digestion of plant materials is that lignocellulosic substrates have low amounts of nitrogen, which results in to high C/N-ratios (Osman et al, 2006). Studies of co-digestion of lignocellulosic materials together with manure have shown higher biogas yields compared to the digestion of both substrates alone. Having in mind that the results in this study applies more on batch wise and single substrate processes the highest production was obtained from the WGR from spirits production. However, the biogas potential from WGR differs depending on its origin and the process it was produced in. The WGR from biofuel production gave a slightly lower production of biogas than the WGR from spirits production which could be due to the use of sulphate in the biofuel production process (Stenströmer Moglia, 2008). WGR is a cellulose rich substrate which contains a higher amount of proteins than the other lignocellulosic materials in this study and this is probably the reason why it was digested at a higher rate and reached its production peak earlier than the other substrates.

5.3 Control experiment and method development
There are many methods for determination of biogas potential which all have different approaches, inoculums of different origin, and different amounts of inoculums load, VS-load, batch- or continuous setups under different conditions (Bougrier et al, 2008; Hansen et al, 2004; Hongzhang et al, 2005; Mshandete et al, 2006; Osman et al, 2006). This makes comparisons of gas production between studies very difficult.

In addition to the thermal pre-treatment study a control experiment was conducted to further evaluate the method used in this study. The control experiment showed that the age of the inoculum used has a significant effect on the degradation rate in the batch digestion process. However, it appears like the methane production potential remains unaffected of the inoculum age and that complete degradation of the substrate just is a matter of time. Methane production rates peaked after 18 days with the new inoculum regardless of dilution medium and inoculum/substrate-ratio, while with the old inoculum the methane production rate peaked after 29 days. A correlation has earlier been found between the inoculum/substrate-ratio (Hashimoto, 1989) and was also seen in this study where the total methane production increased with increased inoculums load.

The control experiment also showed that the initial lag phase was shortened in the batch bottle experiment with an increased inoculums load as the methane production accelerated faster in the beginning of the experiment in the N4-treatment, which had twice the amount of inoculum as for example the N2-treatment. There did not seem to be a lack of micronutrients as no difference was found in the production rates from the NR-treatment and N2-treatments, which differed only in dilution media.
During the final stage of the batch study the N4-, N2- and NR-treatment in the control experiment showed a decrease in the accumulation of methane. It is likely that when the inoculum receives a substrate which contains sugars which are more available for the microorganisms those sugars will be consumed first (Stenströmer Moglia, 2008). This will in turn cause the biogas process to run faster and thus generating more methane. In the control the inoculum was not fed with any substrate and the background production of methane was caused by the endogenous material present in the inoculum. This endogenous material contains sugars that are less available for the microorganisms than the sugars in the added substrate. This causes the degradation process in the inoculum to run slower and producing biogas at a slower rate in the beginning of a batch study than in the batch fed with a substrate. However, the microorganisms in the unfed inoculum control will produce extra cellular enzymes to gain access to the sugars in the endogenous material and the background methane production rate will be higher from the control inoculum than from the substrate fed inoculum at the end of the batch study.

This study shows that the batch method used in these experiments needs further development where the age of the inoculum is taken more into consideration. It is crucial that the origin and age of the inoculum is known and noted as it has a major impact on the results of the degradation rates in the batch experiments. A fresh inoculum will produce biogas at levels closer to the levels in a biogas plant where the reactor material is maintained to be highly active.

5.4 Concluding discussion

The effect of the thermal pre-treatments can still be evaluated; regardless the results from the control experiment. However, the results from the control experiment make it difficult to make any clear statements about the degradation rate in the thermal pre-treatment study.

It is obvious that the degradation rates measured in this study will not be the same as in a large scale operation. Partly because most plants run their processes with co-digestion of several substrates, which in most cases generates a higher methane yield. This has also been shown by Osman et al (2006) where co-digestion gives higher biogas yields than single substrate degradation. Another reason that the degradation rates will differ in large scale operations is because many biogas plants run their production in a continuous process and will therefore not find a lag phase in the biogas production as seen in the beginning of a batch process. It is also likely that inoculums from different operations respond different and different biogas plants will experience different methane production levels from the same substrates. Plant specific microorganism cultures develop in the reactor tanks depending on the substrates fed to the process. A biogas process that is regularly fed with lignocellulosic materials is likely to become more efficient in degrading the cellulose-rich substrate than a biogas process that is not fed with
lignocelluloses as often. However, the substrates tested in this study differ between each other in regards of hydraulic retention time needed for efficient degradation in a biogas reactor. In a large scale operation spruce, aspen and oat straw will most likely need longer time to be degraded than the WGR and meadow grass. To make more certain statements of degradation rate for a particular substrate in a particular biogas plant the substrate in mind must be tested with the inoculum from the biogas plant planning to use the particular material. Also the same temperature and load as in the commercial process should be used in the tests.

Developing and applying the technologies for producing biogas from lignocellulosic materials raises the issue of how to allocate the available natural resources. Even though plant materials are renewable as an energy source there is still a limit for how much is can be used for different purposes. Current and planned uses collide with possible new uses and the authorities must make a levelling of how the available land most efficiently, economically and environmentally safe should be used. Instead of becoming biogas, raw material from forest production might have a greater value as woodworks or for pulp and paper production. Straw, cereals and meadow grass might be of greater value in dairy and meat production. WGR is for example today often used as forage to cattle. The figures that are presented of the total biogas potential or the total bioethanol potential of Sweden are based on the same production figures and could only become a reality if solely one biofuel would be used on the market (Johansson, 2007; Nordberg, 2006). This cannot be considered as a realistic development. There is a need for coordination within the responsible authorities to prioritize and control the production. However, this kind of data is important to make these kinds of considerations.

5.5 Future studies and possibilities
The results in this study show that there still are many issues to address both concerning the thermal pre-treatment of cellulose rich materials and in the refinement of the methodology of the experiments. It would also be of interest to further investigate the effect on biogas production of different particle sizes of different materials in the digestion. Another interesting topic is to investigate the aging of the inoculum and the effects on production peak and the degradation rate for different materials.
6. Conclusions

- Thermal pre-treatment can have a positive effect on the biogas production from cellulose rich materials. However, this effect cannot be expected on all materials.

- Different materials need different thermal pre-treatments to give rise to maximum biogas yields.

- Particle size in the anaerobic digestion also has an effect on the biogas yield, sometimes just as big as the thermal pre-treatment.

- The age of the inoculum used in batch bottle experiments is critical and affects both the methane production rate, the methane production potential and the methane production peak.
7. References

7.1 Text references


Carson, Rachel; 1962; *Silent spring*; Houghton Mifflin Company, USA, p. 6


Gasföreningen, 2008-09-09, *Fakta om gas*,

1. [http://www.gasforeningen.se/FaktaOmGas.aspx]
2. [http://www.gasforeningen.se/FaktaOmGas/Biogas.aspx]


Levén L., 2006, *Anaerobic Digestion at Mesophilic and Thermophilic Temperature – With Emphasis on Degradation of Phenols and Structures of Microbial Communities*, Doctoral Thesis No. 2006:116, Faculty of Natural Resources and Agricultural Sciences, Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden


Stenströmer Moglia E., 2008, *Enzymatic pre-treatment of cellulose rich biomasses for use in the biogas process*, Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden


2. Ferry J.G., Chapter 13 – Acetate-Based Methane Production, pp. 115-170

7.2 Figure references

7.2.1 Figure 1 – Three main steps of the biogas process

7.2.2 Figure 2 – structure of cellulose
Photographer: Pauline Demetriades, 2008-06-27, Austrian Alps

Pictures of the schematic cell, the crystalline cellulose and the chemical structure of cellulose were drawn in BioDraw Ultra 11.01 and ChemDraw Ultra 11.01.

7.2.3 Figure 3 – Pre-treatment of lignocellulosic material

7.2.4 Figure 4 – Batch bottles
Photographer: Pauline Demetriades, 2008-08-30