Anaerobic digestion of pre-treated biological sludge from pulp and paper industry using heat, alkali and electroporation

Lina Cardell
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The biological sludge formed in the pulp and paper wastewater treatment constitutes a costly problem to dispose off due to poor dewaterability. It is often incinerated or used as soil conditioner improvement. By using anaerobic digestion of the biological sludge, the dewaterability can be increased. Thanks to the formation of biogas, the sludge volume is decreased and energy can be recovered as methane. By pretreating the sludge, the biogas production can be increased, making the anaerobic digestion more economically feasible.

Eleven samples of biological sludge from six Swedish pulp and paper mills, chosen to represent different types and sizes of mills available in Sweden, were pre-treated with alkali (NaOH, pH12), heat (80°C, 1 hr) and electroporation (2000 pulses, 10 kV/cm). Initial methane production rate and methane potential of all sludges and pre-treatments were determined using batch experiment. A combination of two sludges (from the same mill) pre-treated with alkali and heat was further investigated in a semi-continuous digester experiment.

The batch experiments showed that alkali pre-treatment had the greatest positive effect on methane production. Heat treatment performed second best, whereas electroporation had no or little effect. Overall, pre-treatments increased the initial methane production rate, but with insignificant effects on the methane potential. Heat pre-treatment showed no difference in biogas production compared to the control in the semi-continuous digester experiment. Alkali treatment was shown to inhibit biogas production and cause high accumulation of acetate. It could not be concluded whether it was an effect from hydroxide or sodium ion addition. Further analysis of the NaOH impact on floc structure, toxicity and bioavailability is suggested to determine the suitability of alkali-treated sludge for anaerobic digestion.

Keywords
Alkali, heat, electroporation, anaerobic digestion, biological sludge, pre-treatment, pulp and paper industry
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ABSTRACT

The biological sludge formed in the pulp and paper wastewater treatment constitutes a costly problem to dispose off due to poor dewaterability. It is often incinerated or used as soil conditioner improvement. By using anaerobic digestion of the biological sludge, the dewaterability can be increased. Thanks to the formation of biogas, the sludge volume is decreased and energy can be recovered as methane. By pre-treating the sludge, the biogas production can be increased, making the anaerobic digestion more economically feasible.

Eleven samples of biological sludges from six Swedish pulp and paper mills, chosen to represent different types and sizes of mills available in Sweden, were pre-treated with alkali (NaOH, pH12), heat (80°C, 1 hr) and electroporation (2000 pulses, 10 kV/cm). Initial methane production rate and methane potential of all sludges and pre-treatments were determined using batch experiment. A combination of two sludges (from the same mill) pre-treated with alkali and heat was further investigated in a semi-continuous digester experiment.

The batch experiments showed that alkali pre-treatment had the greatest positive effect on methane production. Heat treatment performed second best, whereas electroporation had no or little effect. Overall, pre-treatments increased the initial methane production rate, but with insignificant effects on the methane potential. Heat pre-treatment showed no difference in biogas production compared to the control in the semi-continuous digester experiment. Alkali treatment was shown to inhibit biogas production and cause high accumulation of acetate. It could not be concluded whether it was an effect from hydroxide or sodium ion addition. Further analysis of the NaOH impact on floc structure, toxicity and bioavailability is suggested to determine the suitability of alkali-treated sludge for anaerobic digestion.

Keywords: Alkali, heat, electroporation, anaerobic digestion, biological sludge, pre-treatment, pulp and paper industry
**SAMMANFATTNING**


Denna studie har undersökt effekten av förbehandling för elva bioslam från sex svenska pappers- och massabruksverk, valda att representera olika typer och storlekar på svenska bruk. Behandlingen gjordes med alkali (NaOH, pH12), värme (80˚C, 1 h) och elektroporering (2000 pulser, 10 kV/cm). Effekten av förbehandling på initial metanproduktionshastighet och metanpotential undersöktes med hjälp av satsvis utrötning (batch) av alla slam. En kombination av två slam från samma bruk utvärderades i ett semi-continuerligt rötningsförsök efter förbehandling med värme och alkali.


**Nyckelord:** Alkali, elektroporering, anaerobisk rötning, bioslam, förbehandling, pappers- och massaindustri
PREFACE
This thesis concludes my degree in Master of Science in Engineering Biology, with a profile in Industrial Biotechnology and Production, at Linköping University. The work has been carried out at Scandinavian Biogas Fuels AB during the spring term of 2010.
Lina Cardell, June 2010
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1 INTRODUCTION

The environmental impact arisen from the utilization of fossil fuels has probably escaped no one and has led to an increased interest in developing new ways of obtaining renewable energy. The process of converting biomass to energy through anaerobic digestion has received particular attention and is gaining new grounds by the day. Different substrates are constantly being investigated in order to find new suitable recourses, from which biogas (methane) and fertilizer can be formed. Commonly used substrates are agricultural wastes, manure and industrial wastewater (de Mes et al. 2003).

Biological sludge constitutes one of the by-products in pulp and paper wastewater treatment, and has during recent years become more and more problematic to dispose off. An interesting and promising approach to deal with biological sludge is anaerobic digestion, this way two advantages can be gained; the decomposable organic fraction is transformed into methane, thus, constituting a substrate for energy carrier generation, and the amount of sludge is consequently significantly decreased and has an improved dewaterability (Truong et al. 2010). In order to make anaerobic digestion of biological sludge more economically feasible, it is of great interest to find means of enhancing the production of methane from the substrate. A very well practised way is pre-treatment.

ABBREVIATIONS

C-digester Control digester fed with untreated substrate
GC-FID Gas chromatograph with flame ionizing detector
HRT Hydraulic retention time
OLR Organic loading rate
PS-digester Digester fed with pre-treated substrate
TS Total solids (also called dry matter, DM)
VFA Volatile fatty acid
VS Volatile solids
2 BACKGROUND

2.1 PAPER MILL WASTEWATER TREATMENT

Large amounts of sludge are produced daily in the pulp and paper industry from the wastewater treatment plant (WWTP). The sludge contains organic materials and, hence, is prohibited from landfill disposal by law since 2005. The sludge is therefore either combusted or transformed into some kind of product, e.g. for soil conditioner improvement. Two principally different kinds of sludges are produced in the wastewater treatment process. The primary sludge, i.e. fibre sludge, is produced in a first step of the process through mechanical treatment. The remaining water is then treated biologically (aerobic conditions) in a second step, in which microorganisms convert the organic material to carbon dioxide, water and new cell mass, i.e. biological sludge (biological sludge is often referred to as waste activated sludge – WAS, excess sludge, biosludge etc.). Sometimes a third step is applied, using chemicals, which results in a chemical sludge. Due to low TS content, the sludges must be dewatered or dried before disposal, which often implies high costs. (Höglund et al. 2001; Truong et al. 2010)

The main component of biological sludge is microorganisms, which hold a lot of water (Figure 1), thus making the biological sludge hard to dewater (Höglund et al. 2001). One strategy of dealing with this has been to mix biological sludge with fibre sludge, since the fibre sludge is easier to dewater. However, at many mills, overall decreased production of fibre sludge has again revealed the dilemma of dewatering of the biological sludges (Gillberg et al. 2003). Anaerobic digestion holds new hopes of treating biological sludge and making final disposal less expensive. However, one of the problems using biological sludge for anaerobic digestion is the disposal of the digestate. At present, there are no certification regulations for use of the digestate from pulp and paper industry residues as fertilizer (Truong et al. 2010).

![Figure 1 Sludge particle](image-url)

Figure 1 Sludge particle Microorganisms hold a lot of water, the illustration shows how the water can associate with sludge particles. Modified after Gillberg et al. (2003)

In year 2000 approximately 270 000 tons of biological sludge was produced within the Swedish forest industry. The main part (48%) was combusted, 31% was used as soil conditioner improvement, 15% as covering material (e.g. for sand repository) and 6% was disposed on landfills. Dryness, energy content and presence of unwanted substances decide how the biological sludge is disposed off after it has been dewatered and dried. (Ek and Westling 2003)
2.2 Biogas Production from Anaerobic Digestion

Anaerobic digestion is the biological conversion of organic matter into methane (CH$_4$) and carbon dioxide (CO$_2$). Gas composition is 55-75 vol% CH$_4$ and 25-45 vol% CO$_2$. The biogas can be upgraded to vehicle gas (96-97 vol% methane) or used for production of heat. A part of the generated biogas can be used at the biogas plant itself in order to provide for the internal energy requirement. Except for gas, a digestate is formed, which can be used as fertilizer. (Appels et al. 2008; de Mes et al. 2003)

Anaerobic digestion might be part of the answer to the problem of disposing biological sludge from pulp and paper industry. Anaerobic digestion is able to reduce the amount of sludge by transforming the organic material into biogas. In the process, the dewaterability is improved and a stabilisation, i.e. odour reduction, of the sludge is obtained. Today, dewatering of biological sludge is often performed using sedimentation, filtration, polymer addition and centrifugation (Gillberg et al. 2003; Truong et al. 2010). Anaerobic digestion posses several advantages compared to aerobic digestion (presence of oxygen), such as lower nutrients requirement, lower operation costs and lower sludge production. Aerobic digestion converts about 50% of the carbon and energy from organic compounds into new bacterial cells or sludge, corresponding percentage in anaerobic digestion is about 5%. Instead, much of the organic material is converted into methane. However, in terms of biological wastewater treatment, anaerobic digestion would not be able to replace the aerobic degradation of several types of organic materials. Aerobic digestion also possesses lower concentrations of suspended solids and nutrients in the supernatant compared to the anaerobic digestion (Gerardi 2003).

2.3 Biology of Anaerobic Digestion

The anaerobic digestion is fairly complex, and can only take place under strictly anaerobic conditions. Four main steps, each one involving different groups of microorganisms, are generally distinguished to make up the degradation pathway, where the final product form one step constitutes the substrate for the next one as described in Appels et al. (2008):

- **Hydrolysis** – conversion of insoluble organic materials into soluble organic substances
- **Acidogenesis** – formation of volatile fatty acids and CO$_2$
- **Acetogenesis** – formation of acetate, CO$_2$ and H$_2$
- **Methanogenesis** – formation of CH$_4$ and CO$_2$

The hydrolysis is considered to be the overall rate-limiting step in anaerobic digestion on sludge (Climent et al. 2007; Gavala et al. 2003).

2.3.1 PROCESS PARAMETERS

A number of parameters and settings must be monitored to evaluate and ensure that the process is stable (i.e. steady pH, gas production etc), but also to detect changes as early as possible. The ones in focus in this thesis are described below.

**pH and temperature**

Anaerobic digestion can take place under psychrophilic (10-20°C), mesophilic (20-40°C), or thermophilic (50-60°C) conditions, but methane production has an optimum between 35 and 37°C (de Mes et al. 2003). The pH optimum varies among the different groups of
microorganisms. The methanogenic bacteria constitute the group that is the most sensitive, and has an optimum pH in the range of 6.5-7.2 (Appels et al. 2009).

**Alkalinity**

In order to keep pH neutral and stable it is necessary to retain a steady alkalinity, which is a measure of the buffer capacity of the process. Bicarbonate in the liquid phase constitutes the major source of alkalinity, and is in equilibrium with carbon dioxide in the gas phase. (Appels et al. 2009)

**Total solids (TS), volatile solids (VS) and organic loading rate (OLR)**

The amount of organic material that enters the digester and becomes accessible to the microorganisms is determined by the organic loading rate (OLR). Too low OLR results in a stagnated process and too high OLR will cause overload, since the microorganisms cannot keep up with the incoming substrate. The accessible organic material is usually stated as the amount of volatile solids (VS). To determine VS, the amount of total solids (TS) (also called dry matter –DM) must be known (Schnürer and Jarvis 2009). TS is defined as the mass percentage of the substrate that is left after drying in 105°C. VS is then calculated as the mass percentage of TS that is combusted in 550°C (Swedish standard (SIS), SS 02 81 13). The OLR is often given as g VS/L/day.

**Hydraulic retention time (HRT)**

The average time for the liquid sludge to stay in the digester is called the hydraulic retention time (HRT; Appels et al. 2008), and is determined as the ratio between the working volume and the daily input.

**Volatile fatty acids (VFA)**

Volatile fatty acids (short fatty acids) are important to monitor on a regular basis, since a rise in VFA concentration is an important indication on process disturbance. Propionic acid is especially important to keep track of. VFA accumulation may depend on substrate overload, or inhibition of the methanogenic bacteria. (Schnürer and Jarvis 2009)

**Rheology and viscosity**

Rheology is a useful tool in sludge handling, and “describes the deformation of a body under the influence of stress”. A “body” in this case refers to a fluid, but could also be a solid or a gas. Limit viscosity (η), shear rate (γ) and shear stress (τ) are a few of the many rheological parameters that can be measured with a rheometer and used to characterize reactor liquids and residual sludge (cf. Appendix C). (Schramm 1994; Tixier et al. 2003a)

**Methane potential**

The methane potential of a substrate can be decided by digesting it completely, i.e. until the gas production has ceased. This is performed using batch digestion, since complete digestion seldom occurs in continuous digestion. The methane potential is therefore not reached in continuous digestion, and is instead used to determine the yield of the continuous digestion by comparing its gas production to the complete digestion.

### 2.4 Pre-treatment of substrate

The major part of the organic fraction of biological sludge is enclosed within the microbial cell membranes. The rigid cell envelope make up an efficient defence against biodegradation, and hydrolysis is therefore recognized as the rate-limiting stage in anaerobic digestion of biological sludge (Kim et al. 2003; Lin et al. 2009; Weemaes and Verstraete 1998). By pre-treating the sludge, floc structure is ruptured and both intracellular and extracellular materials
can be more easily released through destruction of the microorganisms. This results in a faster and more efficient conversion of the organic materials leading to an increased biogas production (Kopplow et al. 2004; Lin et al. 2009). Other advantages achieved by pre-treatment are reduced foam formation in the digester, increased dewaterability and improved pathogen reduction (Davidsson et al. 2008).

Several different approaches have been investigated to pre-treat sludge, including thermal (Gavala et al. 2003; Wang et al. 1997), chemical (Kim et al. 2003; Lin et al. 1997), thermochemical (Tanaka et al. 1997; Vlyssides and Karlis 2004), ultrasonic (Kim et al. 2003; Truong et al. 2010) and mechanical (Barjenbruch and Kopplow 2003).

Full scale pre-treatment of sludge, mainly thermal (the Cambi-process; a combination of high temperature and pressure) and ultrasonic treatments are utilised within the Nordic region (Davidsson et al. 2008). According to Lin et al. (2009) there are no full scale biogas plants for digestion of solid residues within the pulp and paper industry.

2.4.1 ELECTROPORATION PRE-TREATMENT

Electroporation (pulsed electric fields) is widely used in the field of genetic engineering as a means of transferring genetic material into cells by temporally creating pores in the cell membrane using electric pulses. By increasing the pulse intensity the pores can be made permanent, which is commonly practiced on vegetables and fruits within the food industry (Carlsson and Lagerkvist 2008). The experience of using electroporation in the context of pre-treatment for anaerobic digestion, especially when conducted on sludge substrate is low. Thus, Kopplow et al. (2004) report that the method is not yet competitive due to high energy input levels. Carlson and Lagerkvist (2008) concluded that electroporation pre-treatment of sewage sludge did not increase production of methane. Higher field intensity (>24 kV/cm) was suggested for future experiments. A so called “discharge treatment” was advised by the manufacturer due to the small size of sludge cells, making them hard to penetrate. Choi et al. (2006) demonstrated that use of a pulse power technique for pre-treatment of waste activated sludge could increase the biogas production 2.5 times.

2.4.2 HEAT PRE-TREATMENT

Literature mostly report studies where high temperature (150 - 200°C) has been used (Appels et al. 2008; Bougrier et al. 2008; Elliott and Mahmood 2007). Temperatures exceeding 200°C have been found to induce formation of refractory compounds (Climent et al. 2007). In addition, high temperature pre-treatments are more difficult to operate and more energy consuming, which makes low thermal, (<100°C) more attractive (Gavala et al. 2003). Several studies report successful outcomes when pre-treating sludge at 70°C (Climent et al. 2007; Ferrer et al. 2008; Skiadas et al. 2005) and a few also underlie the use of short pre-treatment times (~1 hr; Davidsson and Jansen, 2006; Wang et al. 1997), thus, reducing energy consumption even further.

Lüdtke (2010) found that 80°C was the most optimal temperature (also evaluating 55°C and 120°C). He also found that duration time of heat pre-treatment, including the time to reach the desired temperature, did not have as great effect on the methane production as was expected: 6 hrs (230 ml methane/g VS), >12 hrs (220 ml methane/g VS), >2 hrs (210 ml methane/g VS). The common asset of 70-80°C waste heat on paper mills constitutes another reason to investigate the effect of pre-treatment at this temperature.
2.4.3 **ALKALI PRE-TREATMENT**

The almost exclusively used chemical for alkali pre-treatment is sodium hydroxide (NaOH). Several studies confirm greater effect of NaOH upon COD solubilisation and gas production compared to other bases such as KOH, Ca(OH)_2 and Mg(OH)_2 (Kim et al. 2003; Penaud et al. 1999; Ray et al. 1990).

Some studies show a clear tendency of improved performance in terms of methane production, VFA-concentration and protein solubilisation when raising the pH (Chen et al. 2007; Hu et al. 2009). In his master thesis, Lüdtke (2010) concluded from batch experiments that the methane potential and the methane production rate were higher for biological sludges treated with NaOH at pH 12 than at pH 10 and pH 11. This result is of special importance, since similar biological sludges with the same origin, i.e. pulp and paper industry, have been the subject of this study.

However, it is not evident that a high pH gives a significantly better outcome on anaerobic digestion. It is also desirable to minimize the use of chemicals in terms of cost. Studies investigating the effect of increasing NaOH dosage often find a limit when it is no longer meaningful to increase concentration (Heo et al. 2003; Kim et al. 2003; Lin et al. 1997).
3 PROSPECTS AND OBJECTIVES

The aim of this master thesis is to evaluate the methane potential from anaerobic digestion of eleven different biological sludges from pulp and paper industry using three different pre-treatment methods: low thermal (80°C), chemical (alkali) and electroporation.

3.1 HYPOTHESIS

Hypothesis 1
Pre-treatment will enable an increase of methane potential for biological sludges when performing batch experiments.

Hypothesis 2
Larger effect from pre-treatments will occur for sludges with low methane potential (~100 mL/g VS) than for sludges with high inherent methane potential (~200 mL/g VS).

Hypothesis 3
A pre-treatment that increases the initial methane production rate will give an increase of the methane production in semi-continuous digester experiments, even though the pre-treatment does not increase the methane potential.

Research questions
To answer the hypotheses above, the following research questions will be used as a guideline:

a) How does the effect of pre-treatment on methane potential vary among the different biological sludges and pre-treatment methods?
b) Can pre-treatment increase initial methane production rate?
c) Which paper mill biological sludge is most suitable for anaerobic digestion?
d) Does polymer addition in dewatered sludges contribute as a substrate to methane production in a significant manner?
e) Will gas production be inhibited by NaOH pre-treatment in the semi-continuous experiment?
f) Will carbon dioxide production be sufficient to buffer high pH substrate in the digester?
g) How is viscosity of digester fluid affected from pre-treated substrate feeding?

3.2 DELIMITATIONS

This thesis is primarily based on a screening approach, to determine the effects of the pre-treatments in question. Evaluation of results in relation to raw materials or wastewater treatments processes of the six mills is therefore not covered.
4 METHODOLOGY

4.1 SLUDGE COLLECTION

The biological sludges used in this study were collected from six Swedish pulp and paper mills (1-6), which were chosen to represent different types and sizes of mills available in Sweden. From mill 2, 3, 5 and 6, samples were taken at two (or three) different locations in the wastewater treatment process (Table 1), overall resulting in eleven sludge samples henceforth referred to as 1, 2A, 2B, 3A, 3B, 4, 5A, 5B, 5C, 6A and 6B. B and C-samples are withdrawn after dewatering of the biological sludge, thus, holding higher values of TS content than corresponding A-sample. TS varied considerably among the different sludges most of them ≤ 3%, but up to 16% was recorded (Table 2).

Table 2 Sludge TS.

<table>
<thead>
<tr>
<th>Sludge</th>
<th>TS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2A</td>
<td>1</td>
</tr>
<tr>
<td>2B</td>
<td>3</td>
</tr>
<tr>
<td>3A</td>
<td>3</td>
</tr>
<tr>
<td>3B</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>5A</td>
<td>1</td>
</tr>
<tr>
<td>5B</td>
<td>0.6</td>
</tr>
<tr>
<td>5C</td>
<td>5</td>
</tr>
<tr>
<td>6A</td>
<td>16</td>
</tr>
<tr>
<td>6B</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

4.2 EXPERIMENTAL SET-UP

Four laboratory-scale batch and one semi-continuous digester experiment were performed in order to determine the effect of low thermal (80°C), chemical (NaOH) and an electroporation pre-treatment. Batch experiments are conducted to investigate the methane potential of a substrate, by measuring the methane production in sealed bottles at 5-7 occasions during a period of time (normally around 40 days). Batches are generally performed in order to screen for suitable substrates, and precedes digester trials. Semi-continuous digester experiments are then set to further investigate the substrate in a scale that is more similar to the full scale, which includes daily feeding and outtake, analysis etc.

4.2.1 BATCH EXPERIMENT

Batch 1 and 2 included all sludges exposed to thermal, chemical or no pre-treatment and Batch 3 and 4 all sludges pre-treated with electroporation and untreated samples. Batch experiments were executed according to Scandinavian Biogas Fuels AB standard procedure as presented below.

Inoculum

Inoculum for the batch experiments consisted of digester outtake (40-60%) from different Scandinavian Biogas Fuels AB lab-scale continuously stirred reactors (CSTR) and sludge from Nykvarn sewage treatment plant (Tekniska Verken, Linköping) (40-60%) to provide a seeding material. Thus, digester material used was a combination of oat, rape, household garbage, cow manure or on fibre and biological sludge from sewage plants or paper mill wastewater treatment.

Batch start-up

All treatments were carried out in triplicates, using 320 mL glass flasks. Each sample bottle contained 20 g inoculum, 2 mL nutrients solution (NH₄Cl, NaCl, CaCl₂ · 2H₂O and MgCl₂ · 6H₂O) and 0.3 mL of reducing Na₂S-solution (added after change of gas phase). Due to different TS content of the biological sludges, the amount of sludge added varied depending on the desired OLR. This resulted in different additions of oxygen free MilliQ-water needed to achieve a working volume of 100 mL. Inoculum, MilliQ-water and nutrients solution were added, while flushing with N₂ to ensure an anaerobic environment.
# Paper mill overview

Source: Truong et al. (2010)

<table>
<thead>
<tr>
<th>Mill</th>
<th>Pulping process</th>
<th>Raw material</th>
<th>Production</th>
<th>Treatment of sludge before sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mechanical</td>
<td>Spruce, recycled paper</td>
<td>Newspaper and catalogue paper</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>Chemical (sulphite)</td>
<td>Wood from coniferous tree and deciduous tree</td>
<td>Fine paper</td>
<td>450</td>
</tr>
<tr>
<td>3</td>
<td>Chemical (sulphate)</td>
<td>Wood from coniferous and deciduous tree</td>
<td>Market pulp</td>
<td>430</td>
</tr>
<tr>
<td>4</td>
<td>Chemical (sulphate) and CTMP – Chemico-Thermomechanical Pulping</td>
<td>Wood from coniferous tree</td>
<td>Cardboard</td>
<td>700</td>
</tr>
<tr>
<td>5</td>
<td>Chemical (sulphate)</td>
<td>Wood from coniferous and deciduous tree</td>
<td>Cardboard</td>
<td>400</td>
</tr>
<tr>
<td>6</td>
<td>Mechanical</td>
<td>Coniferous tree</td>
<td>Printing paper</td>
<td>700</td>
</tr>
</tbody>
</table>

* Only performed upon request for digester trials.
After sealing the bottles, using EPDM rubber stoppers and aluminium caps, a change of gas phase to 20% CO$_2$ and 80% N$_2$ was performed.

Control flasks, also in triplicate, were prepared as follows:

- **Seed blank** – determines the contribution to methane production from inoculum only (Inoculum 20 g, MilliQ-water 78 mL and nutrients solution 2 mL)
- **Positive control** – tests the state of the inoculum (Whatman Filtration paper No.3 (Whatman Limited, England) 0.5 g, inoculum 20 g, MilliQ-water 78 mL and nutrients solution 2 mL)
- **Incubated methane control** – tests the reliability of the instruments by knowing the amount and concentration of methane in the bottle (MilliQ-water 100 mL and methane 50 mL)

Bottles were placed in 37°C and gas samples were withdrawn days 1, 3(4), 7, 14, 20, 40 and 60.

### 4.2.2 SEMI-CONTINUOUS DIGESTER EXPERIMENT

Two laboratory-scale semi-continuous digesters, one control (C) and one fed with pre-treated substrate (PS), were carried out with a working volume of 4 L and a 20 day retention time under mesophilic (37°C) conditions. Each digester had a start inoculum of 4 L consisting of 1.8 L from a process running on biological sludge and 2.2 L digested sludge from Nykvarn sewage treatment plant (Tekniska Verken, Linköping). The digesters were fed once a day (200 mL substrate) and stirred 15 minutes every hour (400-500 rpm). Before feeding, 200 mL of digester liquid was withdrawn and blended into the food portion to facilitate feeding. During an 11 day start-up period both digesters were fed with untreated substrate with an OLR at 2 g VS/L/day the two first days and at 3 g VS/L from day three. At day 11, heat-treated substrate feeding of the PS-digester began and continued for 27 days. The heat-treated substrate was replaced by alkali pre-treated substrate at day 38, which continued for 39 days (two retention times). Food portions consisted of a mixture of sludge 5B and 5C in order to achieve the OLR desired.

### 4.3 BIOLOGICAL SLUDGE PRE-TREATMENT

#### 4.3.1 ELECTROPORATION

Electroporation was performed using equipment from KEA-TEC GmbH (Germany). Substrate was poured into a 250 mL cylinder cuvette (length 20 cm, diameter 4 cm). Due to the cuvette design, some biological sludges had to be diluted to enable handling and prevent the formation of air bubbles in the cuvette. Parameter setting was U=36 kV and I=40 mA giving rise to a frequency of ~10.5 Hz. Field strength was ~10kV/cm. Number of pulses used was 2000 (2 x 1000, due to equipment limitation), each pulse corresponding to an energy consumption of 54.4 J, i.e. each treatment consumed 0.11 MJ. Temperature and conductivity were measured before and after electroporation.

#### 4.3.2 THERMAL PRE-TREATMENT

**Batch experiment**

250-300 mL of sludge was incubated in a 105°C oven using plastic tubes of 400 mL covered with a lid. Every 20 min, samples were stirred to reduce temperature differences within the
samples and to avoid drying of the surface layers, which was a problem especially for samples with a high TS content. During the last hour of heating, temperature was monitored after stirring. After approximately 2 hrs of heating, sample temperature had reached 80˚C, at which point they were transferred to an 80˚C oven and incubated for one hour. Samples were left to cool (without lid) overnight in a fume cupboard.

**Digester experiment**

Heat-treated substrate sludge used during the ten first days of feeding was heated up using a water bath (i.e. a big kettle) and a stainless bowl covered with a kettle lid. To reach 80˚C took 80 min under constant supervision, why the method was abandoned. Instead, food portions were prepared before treatment and heated at 105˚C. The temperature increase was monitored electronically. Both heating procedures suffered from water losses, which were compensated (17 mL for water bath and 5 mL for 105˚C oven).

4.3.3 **CHEMICAL PRE-TREATMENT**

**Batch experiment**

Alkali treatment was performed using NaOH (1M or 2.5M) to adjust pH >12 and then incubated in a fume cupboard (~17 hrs) before being neutralized to pH<8 using HCl (1M). pH adjusting was carried out in a glass cup with a pH-meter monitoring pH, while adding acid or alkali. Stirring was performed manually for sludges with high TS content using a spoon or by a magnetic flea whenever possible (TS ≤ 2%). Three high TS content sludges had to be diluted (3B 25%, 5C 40% and 6B 10%) before alkali treatment to enable an even distribution of the chemicals. Alkali consumption and measurement data are shown in Figure 2 and Appendix A.

**Digester experiment**

Alkali pre-treatment for digester trials was conducted similarly to the batch experiments. pH was adjusted to ~12 after food portion preparation, without being neutralised. Food portions for seven to ten days were prepared at a time. pH of food portions was measured days 1, 4 and 7 before feeding the first week of alkali pre-treatment.

4.4 **TRIAL ON POLYMER IMPACT ON METHANE POTENTIAL**

Since polymer addition is a common way of increasing the dewaterability of sludge, it is of value to investigate its impact on the methane potential. Therefore, a polymer solution used at mill 5, was used as a substrate in batch tests as described above. Due to low TS- and VS content (0.3% resp. 50%) a loading rate of only 0.5 g VS/L/day could be achieved.

4.5 **ANALYSIS**

4.5.1 **BATCH**

Production of biogas was determined by measuring gas pressure using Testo digital pressure meter (Testo AG, Germany). A sample of 1 mL of biogas was taken and injected to a 31.7 mL glass vial from which 0.3 mL was analysed by gas chromatography to determine the methane content of the biogas. A GC-FID HP 5880A (Hewlett Packard, USA) equipped with a Poraplot T column and with nitrogen as the carrier gas (130 mL/min) and a mixture of air (250 mL/min) and hydrogen (30 ml/min) in the flame ionizing detector (FID) was used with oven at 80˚C, injector at 150˚C and at detector 250˚C.

Methane content was determined via a standard curve at start of every measurement occasion, using methane gas standards of 0.07, 0.63 and 1.71%. Gas standards were renewed at least
once every 14 days. Mean area of gas standards was determined from five injections and from three injections of sample, both times after rejecting the most deviating value.

Conductivity analysis at electroporation pre-treatment was carried out with an EC 300 conductivity instrument (WTW, Germany).

4.5.2 SEMI-CONTINUOUS DIGESTER

**Analysis routine**

VFA analysis was performed using a GC-FID HP 6890 (Hewlett Packard, USA), using settings as follows; injector temperature 150°C, oven temperature starting at 80°C increasing to 200°C according to temperature programme, and detector temperature 250°C. GC carrier gas was helium (2 ml/min). FID-detector carrier gas was a mixture of air (250 ml/min) and hydrogen (25 ml/min). Gas composition was determined with a gas Check Biogas instrument (Geotech, United Kingdom). pH analysis was carried out using a Inolab 730 pH-meter (WTW, Germany) equipped with a Polilyte Bridge Lab pH electrode (Hamilton, Switzerland). Amount of produced gas was measured with Scandinavian Biogas Fuels AB’s special constructed gas meters based on the principal of water displacement. TS- and VS concentrations were determined using an ATL-423 milligram scale (Acculab, Germany). Analysis frequency is described in Table 3.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Method</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas volume</td>
<td>Water displacement</td>
<td>Once/day</td>
</tr>
<tr>
<td>pH</td>
<td>pH-electrode</td>
<td>Twice/week</td>
</tr>
<tr>
<td>VFA</td>
<td>GC-FID</td>
<td>Once/week</td>
</tr>
<tr>
<td>Gas composition</td>
<td>Gas analyser, GC-FID</td>
<td>Once/week</td>
</tr>
<tr>
<td>TS- and VS percentages</td>
<td>Milligram scale</td>
<td>Once/week</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Rheometer</td>
<td>Once/month</td>
</tr>
</tbody>
</table>

**VS-reduction**

VS-reduction measures the conversion of the organic material into biogas, and was calculated using formula 1. A high VS-reduction indicates an efficient degradation in the digester.

\[ 1 = \frac{\text{Volume}_{out} \cdot \text{TS}_{out} \cdot \text{VS}_{out}}{\text{Volume}_{in} \cdot \text{TS}_{in} \cdot \text{VS}_{in}} \]  

(Formula 1)

**Viscosity analysis**

Viscosity measurements for both digesters were made before changing to a new substrate pre-treatment of the PS-digester: at day 10 (untreated substrate), day 38 (heat-treated substrate) and day 65 (alkali-treated substrate). Analysis was performed using a Dinamic/Rotation Rheometer (DIN/ISO) (Anton Paar, Austria) and Rheoplus Software.
4.6 **Statistical Analysis**

Statistical analysis, using Minitab® 15 (Minitab Inc., USA 2007) was performed to compare the pre-treatment methods in terms of methane production and methane potential.

*Graphical presentation of batch results*

Electroporation pre-treatment was performed in batches separate from alkali and heat pre-treatments, thus, rendering its own set of controls i.e. untreated samples. An example of the accumulated methane production for sludge 5B and 5C during batch experiments, when pre-treated with alkali and heat, can be seen in Figure 3. In order to facilitate interpretation of all pre-treatments of a specific biological sludge using the same graph, the result of pre-treatment are presented after calculation using formula 2 (Figure 4).

Average CH4 production of treated sludge — average CH4 production of untreated sludge

(Formula 2)

*F-tests and construction of Tukey confidence intervals*

When comparing more than two methods (i.e. treatments), it is advisable and time saving to start making a test in order to determine, if there is a significant difference among the methods at all. If there is a significant difference, confidence intervals are constructed to find and determine the difference/es. The normal distribution of the data allowed use of F-tests and construction of Tukey confidence intervals (cf. Appendix B).
5 RESULTS AND DISCUSSION

This section is divided into three parts. The first (5.1) discusses the effect from the pre-treatments themselves, i.e. before anaerobic digestion. The second (5.2) and third (5.3) parts discuss the results from batch and semi-continuous digester, respectively.

5.1 GENERAL EFFECTS AND OBSERVATIONS FROM PRE-TREATMENT

5.1.1 ELECTROPORATION PRE-TREATMENT

All biological sludges gained an increase in temperature from the electroporation pre-treatment to ~50°C. This temporary heating had likely no hydrolytic effect, since treatment only lasted for ~4 min. In five of eleven sludges the conductivity had increased 30-100 μS/cm after treatment (data not shown). The remaining six sludges were too thick or sedimented too fast to enable conductivity measurements.

5.1.2 ALKALI PRE-TREATMENT

**Batch**

All sludges experienced a drop in pH during the ca 17 hrs of pre-treatment. This could be a result of protein hydrolysis, forming amino acids which lower pH (Harris 1999). A trend of further decrease of pH and lower NaOH consumption was observed with increasing TS content (Figure 2; cf. Appendix A). A higher alkali consumption for the low TS sludges could be explained by a larger area per sludge particle that can bind the hydroxide ions. The pH drop was greater for high TS-sludges, likely due to larger amounts of proteins present.

![Graph](image)

**Figure 2** The response in NaOH consumption and pH upon alkali treatment at different sludge TS contents

When performing alkali treatment, a minimum use of chemicals (base and acid) is desirable. Therefore, large decrease in pH during alkali treatment hydrolysis is advantageous by reducing the potential need of a neutralising acid. The long pre-treatment time during this experiment may limit the benefits of the observed decrease in pH. However, the pH drop over time was not investigated, but most of the change likely occurred during the first hours of treatment, as supported by observations in similar studies by Heo et al. (2003).

High TS content sludges possess the benefits of both low alkali consumption and a large pH drop. However, the high TS of the sludge mostly means that the sludge already has been...
dewatered. When choosing between low consumption of alkali or a large drop in pH, the former would probably be of advantage, since a high pH is more compatible with subsequent anaerobic treatment (Ray et al. 1990; Weemaes and Verstraete 1998).

Adding alkali decreased the VS percentage for all sludges (cf. Appendix A). The reason is that NaOH constitutes inorganic solids, which add up to the non combustible part of total solids, hence, lowering the fraction of volatile solids. TS remained relatively unchanged except for diluted sludges.

_Digester_
During the first week of feeding with alkali-treated sludge, the pH of the sludge prior to feeding had decreased to 10.7 (day 1), 10.4 (day 4) and 10.3 (day 7). This indicates that most hydrolysis took place during the first 24 hrs of treatment. The amount of NaOH required to increase pH to ~12 varied between 5-9 g NaOH/100 g TS.

5.1.3 _HEAT PRE-TREATMENT_
After heat treatment, sludges showed signs of better phase separation, i.e. an increase of the water phase, which was somewhat more pronounced for sludge at high TS contents.

5.2 _BATCH RESULTS_
As described in 4.6, treatments were performed on separate occasions, and results are therefore presented using _formula_ 2 (cf. Figure 3 and Figure 4).

5.2.1 _ELECTROPORATION PRE-TREATMENT_
Sludge 3B was the only one responding well to the electroporation pre-treatment. Instead, electroporation treatment seemed to have a negative effect on methane production for several sludges, which is difficult to explain (cf. Figure 4, Table 4 and Appendix A). To improve electroporation performance, higher field strengths would be of interest (cf. Carlson and Lagerkvist 2008).

5.2.2 _HEAT AND ALKALI PRE-TREATMENT_
In general, both alkali and heat pre-treatment generated an increase in initial methane production rate (Figure 4). Mostly, the alkali treatment exceeded heat treatment. The effect is most visible for days 7-14, but fading around day 20. However, the methane potentials at day 60 were mainly at the same levels, thereby rejecting _Hypothesis 1_. Numeric values of methane potential are given in Table 4. Twelve of all combinations of biological sludges and pre-treatments ended up in a significant difference in methane potential compared to the control. Remarkably, nine of these, involving sludges 1, 2A, 2B, 5C and 6A, generated methane potentials under that of corresponding controls. Refractory compound formation might explain the results derived from NaOH pre-treatment (Penaud et al. 1999), but not in the case of heat pre-treatment, since substantial refractory compound formation in sludge is
Figure 3 Accumulated methane in batch trials with a) sludge 5B b) sludge 5C pre-treated with alkali and heat.
observed to occur first at temperatures around 200°C (Climent et al. 2007). The results with sludge 6B are consistent with the findings by Lüdtke (2010), who found that the very same sludge to be difficult to pre-treat. It was suggested that efficient removal of cellulose fibres resulted in a high degree of digestion in the biological cleaning steps, thereby producing a sludge rich in biomass hard to degrade.

**Sludge candidates for semi-continuous digestion experiment**

Sludge 1, 2A, 2B, 3A, 3B and 5A showed the highest increase in initial methane production, after pre-treatment with alkali and/or heat according to the statistical test (cf. Appendix A), whilst sludge 4, 5B, 5C, 6A and 6B were less affected. Because of the low TS content, high enough OLR could not be reached using sludge 1, 2A 2B or sludge 5A, and these sludges were therefore not suitable for the semi-continuous experiment. However, the increase for sludge 5A, when treated with heat should still be noted. Judging from the increase of initial methane production rate and methane potential in batch trials (Figure 4 and Table 4), sludge 3A and 3B treated with alkali were the best candidates. However, because sludge from mill 3 already had been the subject to a similar digester study (Lüdtke 2010), sludges 5B and 5C from mill 5 were chosen for the continued digester trial as it was the second most suitable (cf. section 5.3).

**Correlation between methane potential and pre-treatment effect**

There were no indications that sludges with low methane potential respond better to pre-treatment than sludges with high methane potential in terms of initial methane production rate or methane potential. Together with the fact that two of the three sludges with increased methane potential from pre-treatment can be considered to be high methane potential sludges, Hypothesis 2 is rejected.

**Further observations**

Based on the averages in Table 4, the methane potentials were generally higher for high TS content sludges than for low TS content sludges, when comparing untreated sludges from the same paper mill. One reason might be that polymer addition during dewatering give rise to an increase in methane production. It is also possible that the dewatering steps have a positive influence on the hydrolysis, making high TS content sludges more easily biodegradable.

Four low TS-sludges (1, 2A, 4 and 5A) gave less methane production than the control at day 40 (data not shown) after treatment with alkali. The sludges in question belong to those showing the highest alkali consumption (Figure 2). Penaud et al. (1999) found that biodegradability decreased at high NaOH levels, and that it was an effect of the hydroxide addition (not the sodium). Hydroxide ions were proposed to cause formation of refractory compounds, which might explain the decrease in methane production.
Figure 4 Batch result overview  Methane production of pre-treated substrate subtracted by the corresponding control according to $\text{PS} - \text{C}$ (mL CH$_4$/g added VS at 273K) over time (days).
5.2.3 Methane Potential of Polymer

No conclusions can be drawn from polymer batch experiments. Due to the low OLR, caused by low TS- and VS content, methane production in the polymer amended treatments was about the same as for the inoculum (data not shown). Thus, the possible effect of the polymer in assays of the methane potential of sludge amended with polymer can be neglected.
Table 4 Methane potentials of biological sludges from batch trials (day 60) Average methane production values (mL/g added VS) are given for triplicates ± standard deviation a)-b). Electroporation pre-treatment is presented separately from heat and NaOH treatments, since it was performed on different occasions, i.e. having separate controls (b). Methane potential increase over control (mL/g added VS) (c) has been determined with 95% confidence interval. The same statistical procedure was applied to accumulated methane production at days 7, 20 and 40 (Appendix A). Statistical tests resulting in not significant differences are denoted “n.s.”

<table>
<thead>
<tr>
<th>Sludge</th>
<th>1</th>
<th>2A</th>
<th>2B</th>
<th>3A</th>
<th>3B*</th>
<th>4</th>
<th>5A</th>
<th>5B</th>
<th>5C**</th>
<th>6A</th>
<th>6B***</th>
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<tbody>
<tr>
<td>TS (%)</td>
<td></td>
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<tr>
<td>Pre-treatment</td>
<td>Methane production (mL/g added VS)</td>
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<td></td>
<td>-</td>
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<td>130(±2)</td>
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<td>160(±4)</td>
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<td>130(±3)</td>
<td>130(±5)</td>
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<td></td>
<td></td>
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<td>-</td>
<td>-</td>
<td>150(±2)</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>70(±10)</td>
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<tr>
<td>Untreated</td>
<td>150(±10)</td>
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<td>170(±4)</td>
<td>170(±4)</td>
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<tr>
<td>NaOH</td>
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<td>[-32 -6]</td>
<td>n.s.</td>
<td>[4 28]</td>
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<td>n.s.</td>
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<td>[-24 -1]</td>
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<td>n.s.</td>
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<td>n.s.</td>
<td>n.s.</td>
<td>[-24 -0.3]</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

(*) represent diluted sludges with water content of *25%, **40% and ***10% when treated with alkali.
5.3 SEMI-CONTINUOUS DIGESTER RESULTS

Semi-continuous digesters were fed with biological sludge from mill 5. This mill was chosen because a high TS content sludge was needed to achieve a sufficient OLR. The batch result of 5B gave rise to an interesting hypothesis (Hypothesis 3). As shown in Figure 3a), the final methane potential is approximately the same for sludge 5B when treated with alkali, heat and no treatment. However, the initial rate of methane production is higher for treated sludges, and therefore might be able to generate a higher biogas production in a digester. The reason is that each “sludge unit” only stays a certain period of time in the digester, and a treated sludge that produce methane more quickly has a bigger chance to reach its methane potential before leaving the digester. Unfortunately, this could not be observed in the PS-digester, for either pre-treatment (alkali or heat), and Hypothesis 3 was therefore rejected. The differences in methane production rate were probably not big enough to be shown in methane production in this experiment. The following headings will present an evaluation of the digester performance.

5.3.1 BIOGAS PRODUCTION

No substantial difference in biogas production could be observed between the digesters (Figure 5). Figure 6 describes the relative difference between the PS-digester and the C-digester as (PS-C)/C. Due to a decrease in the TS content of sludge 5C the feeding with this sludge was increased from 50 g to 110 g. Since NaOH and heat pre-treatment had no effect on the 5C-sludge, Figure 3b), this increase is likely the major cause why no difference in biogas production occurred between the C and PS-digesters. The higher initial methane production rate of 5B pre-treated sludge compared to that of 5C was not enough to overcome this obstacle in the mixture. From the present data set it is not possible to judge whether the alkali treatment of 5B sludge would have come through if only this sludge had been used in the digester experiment.

Gas production reached a maximum of ca 3.3 L during days 15-25 in both digesters (Figure 5). The decrease is probably an effect from the high viscosity sludge substrate, causing a more inefficient stirring and therefore a lower biogas production. The biogas production of the digesters stabilised at around 2.3 L/day. However, after day 58 the biogas production of PS-digester started to decrease with a concomitant increase in VFA levels (Figure 7). This is probably an effect of the NaOH addition used in the alkali pre-treatment, which had been ongoing for one retention time when ca 60% of the reactor material has been replaced by alkali pre-treated substrate.

The biogas potential of untreated sludge 5B varied significantly between different batch trials, making it difficult to compare it with specific biogas production in digester. The combination of both 5B and 5C sludge in digester substrate complicates evaluation even further. However, it is safe to say that sludge substrate generated less biogas in the semi-continuous digester trial, partly due to the continuous withdrawal (section 2.3.1), but probably more likely due to inefficient stirring. Table 5 summarizes the biogas potential in batch trial and digester.

Table 5 Biogas production comparison between batch and digester trials  Values represent biogas production (mL biogas /g added VS at 273K). Note that biogas production from heat pre-treated substrate in digester is overestimated, since it has not yet reached a stable production.
Figure 5 Produced biogas in digesters

Dashed vertical lines mark time passed in hydraulic retention times.

Figure 5 Produced biogas in digesters Dashed vertical lines mark time passed in hydraulic retention times.
5.3.2 VFA

Acetate and propionate concentrations in the C-digester remained low (around 2 mM or less) throughout the whole period and in the PS-digester until day 41, when the acetate concentration started to rise (Figure 7). However, propionate levels remained low, around 2 mM. The increase of the acetate levels took place directly after starting feed of NaOH pre-treated sludge (day 38), which indicated an inhibition caused by hydroxide or sodium ions. One reason could be that hydroxide ions bind trace elements, making them inaccessible for the methanogens. A trace element- and nutrients solution was therefore added on days 66 and 71, but no change in biogas production (Figure 5) or acetate accumulation occurred before ending the experiment. Another reason can be that NaOH addition generated a sodium toxicity, which is known to occur at sodium concentrations reaching 3500-5500 mg/L (Appels et al. 2008). The levels in the NaOH-treated substrate amounted up to 4000 mg/L. However, sodium toxicity can be reduced by simultaneous addition of calcium and potassium according to Appels et al. (2008). In further studies this should be kept in mind.
Initial pH in both digesters decreased due to the lower pH of the substrate than of the inoculum (Figure 8). However, pH in the C-digester remained relatively constant (6.9-7.2) during the whole period. pH of PS-digester was not affected by heat-treated substrate (day 11-37), but increased when fed with alkali-treated substrate (day 38-75), as expected. When considering buffer capacity of produced CO₂, increased acetate accumulation must be taken into account since acetate repress the high pH derived from the alkali pre-treated substrate. The pH stabilised around day 60 (Figure 8) at about the time when VFA concentrations started to increase (Figure 7). This makes it difficult to determine whether the produced CO₂ was enough to buffer the high pH substrate or not.

Gas composition, measured by gas collection in balloons for 24 or 48 hrs, had a high fraction of unidentified gas (~20%). Knowing the oxygen content of the gas, it is reasonable to assume that most part of the unidentified gas was nitrogen, which is then due to air leakage. Thus, the methane fraction is probably underestimated. In spite of this, all measurements experienced the same problem, why comparisons are still possible.

Methane content of biogas from PS-digester started to rise around day 38. However, this increase is at least partly an effect from the alkali treatment, since carbon dioxide trapped by the bicarbonate/carbonate buffer system at high pH. During the last week of the digester experiment, the methane content was determined by GC-FID. The results from the two methane measuring methods are compared in Table 6. The gas analyser results are lower, since they represent an average of gas produced during 48 hrs. GC-FID samples reflect the methane composition just before feeding, which is slightly higher. Still, GC-FID results confirm that methane content was higher for PS-digester, but also show that the methane content of the C-digester might be underestimated in Figure 8.
Table 6 Comparison of methane analysis methods

<table>
<thead>
<tr>
<th>Day</th>
<th>C-digester</th>
<th>PS-digester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gas analyser</td>
<td>GC-FID</td>
</tr>
<tr>
<td>70</td>
<td>43%</td>
<td>49%</td>
</tr>
<tr>
<td>73</td>
<td>42%</td>
<td>48%</td>
</tr>
</tbody>
</table>

5.3.4 VS-REDUCTION
The TS contents of the digester fluid were similar for the two digesters; starting at 4.5% and increasing to ~7% as a consequence of the mixing in of substrate, having a TS percentage of ~7.5%. The VS content was also similar, until alkali pre-treated feeding started of the PS-reactor. As observed in batch trials (section 5.1.2 and Appendix A), VS fraction decreased as a result of NaOH addition. The increasing TS content of the digestate affected the VS-reduction calculations, why the most reliable values are those obtained when most of the starting material has been replaced, i.e. after approx 60 days (three retention times). Before steady state, VS-reduction was 30-40% in both digesters, decreasing to ~20% in the end of the experiment. This is half of what was observed by Truong et al. (2010) for digestion of sludge of the same origin, and is yet another effect from inefficient stirring resulting in poor distribution of newly added substrate.
5.3.5  VISCOSITY ANALYSIS

Effect from sludge substrate

The limit viscosity and yield stresses (cf. Appendix C) increased (Figure 9) as a consequence of introduction of the sludge substrate, having higher viscosity than the starting material.

Limit viscosity

The first measurement showed a similar viscosity in the C- and PS-digesters (day 10) Table 7; Figure 9). After 27 days of feeding with heat-treated sludge, the viscosity was slightly higher in the PS-digester (day 38), which is an indication of a more stable flock structure (Paula Segura de la Monja, pers. comm.). A pronounced difference in viscosity between the digesters was observed after 27 days of feeding the PS-digester with alkali pre-treated sludge (day 65). The lower viscosity in the PS-digester is likely a result of the NaOH addition, which probably disturbed the floc structure. A similar result was observed by Tixier et al. (2003b), who found that cation addition, using sodium chloride and calcium chloride, generated viscosity decrease caused by decreased interparticle interactions. NaOH addition also resulted in a sticky and slime-like consistency, but was still easier to handle at feeding compared to untreated sludge. This is an important factor to consider in full scale applications.

<table>
<thead>
<tr>
<th>Day</th>
<th>Yield stress (Pa)</th>
<th>Limit viscosity (Pa·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-digester</td>
<td>PS-digester</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>38</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>65</td>
<td>59</td>
<td>40</td>
</tr>
</tbody>
</table>

Figure 9 Limit viscosity comparison diagram
5.4 SUGGESTIONS FOR IMPROVEMENTS AND CONTINUATION

Gas composition was the only parameter suffering from uncertain measurements. This was probably an effect of the small amounts of gas that were produced during 24 hrs (and 48 hrs). A better way to determine the methane content is to take gas samples directly from the digester and analyse them using GC-FID, which was done during the last week. This method, combined with Gas analyser, would give better knowledge about gas composition.

To be able to get more VS-reduction data, it would be optimal to start from digesters which have reached steady state, i.e. after three retention times of sludge feeding. Further improvements would be to use a more efficient stirrer, higher field strength at electroporation pre-treatment and to also examine the dewaterability of the digestate e.g. by through centrifugation.

An interesting continuation is to combine heat and alkali pre-treatment, i.e. pH increment followed by heating. This is supported by the studies by Kim et al. 2003; Penaud et al. 1999; Tanaka et al. 1997 revealing that thermo-chemical pre-treatment performs better than either pre-treatment alone.

To be able to investigate the suitability of NaOH pre-treated sludge for anaerobic digestion, further analysis of the impact of sodium and hydroxide ions on floc structure, toxicity and bioavailability is needed. Effects of sodium addition on digestate disposal opportunities should also be considered. Better performance of NaOH treatment compared to heat treatment in batch experiments might therefore not be applicable in practice, as indicated by the semi-continuous experiment, unless measures are taken to compensate for the negative impact derived from the NaOH addition. Further, the cost of the potential measures must be considered in relation to the profit of increased methane production. Considering this, heat treatment might be a more suitable pre-treatment, especially when excess heat is available at the paper mill, and since no inhibition occur from the treatment itself.

As reviewed by Appels et al. (2008) and Davidsson et al. (2008), and observed in this study, pre-treatment results vary considerable depending on the quality and origin of the sludge. Individual optimisation in combination with deeper knowledge of the raw material and wastewater treatment processes of each sludge would therefore be of importance in order to find the best conditions for anaerobic digestion of biological sludge.
CONCLUSIONS

Batch

- Pre-treatment of biological sludges showed an effect on the initial methane production, but insignificantly on the methane potential for most of the sludges.
- Alkali treatment (pH 12 using NaOH, ~17 hrs) was the best method among the ones tested and had the highest impact on the initial methane production rate, but not on the methane potential.
- Heat treatment (80°C, 1 hr) had significant impact on the initial methane production from some of the biological sludges.
- No or little effect on initial methane production rate or methane potential was achieved from electroporation pre-treatment (2000 pulses at 10 kV/cm).
- No correlation between methane potential of the sludge and effect from pre-treatment could be found.
- Sludge from mill 3 responded best to all pre-treatments. Due to project time limitations the reason for this was not further investigated.
- The effect on methane potential from polymer addition in dewatered sludges could not be determined in batch experiments at the operational concentrations used in the tests.

Digester

- Heat and NaOH pre-treatments did not increase the methane potential in the digester experiment. Largely, this was due the choice of substrate mix, where the main sludge component did not respond to pre-treatment in batch trials was used in order to obtain the desired OLR level.
- Higher limit viscosity in the reactor fed with pre-treated substrate compared to control indicated a more stable floc structure when substrate had been heat pre-treated prior to feeding. NaOH pre-treatment generated a significantly lower limit viscosity compared to the control, which is suggested to be an effect from decreased interparticle interaction caused by cation addition.
- It could not be decided whether carbon dioxide in the produced biogas was able to provide enough buffer capacity to handle the high pH of substrate, due to high levels of acetate. Still, methane content of the gas in the reactor fed with pre-treated substrate was higher compared to the control reactor indicating that carbon dioxide was consumed as buffer.
- NaOH pre-treated substrate generated high accumulation of acetate and decreased biogas production. It could not be concluded whether it was an effect from hydroxide or sodium ion addition.
ACKNOWLEDGEMENTS
This master thesis would not have been possible, or as much fun, if it was not for a number of great people. I especially want to thank my supervisor, Xu-Bin Truong, for always having time for my questions. Paula Segura de la Monja performed the viscosity analysis, which added a valuable and interesting dimension to my results. Martin Ohlson, at Department of Mathematics at Linköping University, helped me refresh my knowledge in statistics. Many thanks to my opponent, Susanne Tumlin, and my examiner, professor Bo Svensson, for commenting and improving my report. Last but not least, I want to thank the staff and master thesis workers at Scandinavian Biogas Fuels AB and Department of Thematic Studies – Water and Environmental Studies at Linköping University for all our fun discussions, and Peter, for support and cooking dinner all the times I was stuck in the lab.
REFERENCES


SIS (1981) SS 02 81 13 Metod för analys av torrsubstans (TS) och glödförlust (VS)


**PERSONAL COMMUNICATIONS:**
Paula Segura de la Monja, master student at Energy and Environmental Engineering

**UNPUBLISHED MASTER THeses:**
APPENDIX A

BATCH RAW DATA

Table 8 TS, VS and OLR at batch trials

TS and VS analysis were made in duplicates. TS values varied ±0.2 or less and VS values ±2.0 or less. Italic and none italic values derive from two separate pre-treatment occasions, and are therefore not comparable. TS and VS for electroporated biological sludge were assumed to be the same as the untreated control sludge, since pre-treatment took place in a closed container (cuvette). Organic loading rate (OLR) is stated as means of three bottles. For low TS-sludges, OLR becomes limited. Variation of OLR was ±0.1 or less.

<table>
<thead>
<tr>
<th>Sludge</th>
<th>1</th>
<th>2A</th>
<th>2B</th>
<th>3A</th>
<th>3B</th>
<th>4</th>
<th>5A</th>
<th>5B</th>
<th>5C</th>
<th>6A</th>
<th>6B</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
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<td>1</td>
<td>3</td>
<td>4</td>
<td>13</td>
<td>1</td>
<td>0.6</td>
<td>5</td>
<td>8</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Thermal</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>14</td>
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<td>5</td>
<td>9</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Chemical</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>9</td>
<td>1</td>
<td>0.8</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>8</td>
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<td>Electroporation</td>
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<td>0.9</td>
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<td>3</td>
<td>8</td>
<td>3</td>
<td>1</td>
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<td>8</td>
<td>1</td>
<td>4</td>
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<table>
<thead>
<tr>
<th>VS (% of TS)</th>
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<th></th>
<th></th>
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<td>80</td>
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<td>80</td>
<td>90</td>
<td>90</td>
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<table>
<thead>
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<th>OLR (g VS/L)</th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
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<td>Untreated</td>
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<td>6</td>
<td>3</td>
<td>11</td>
<td>12</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Thermal</td>
<td>5</td>
<td>8</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>6</td>
<td>4</td>
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<td>13</td>
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<td>3</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>10</td>
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<tr>
<td>Electroporation</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>13</td>
<td>19</td>
<td>5</td>
<td>10</td>
<td>12</td>
<td>7</td>
<td>10</td>
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</tbody>
</table>
Table 9 Alkali pre-treatment data  Initial substrate pH varied 6.6-7.6. Pre-treatment took place in room temperature over night, in average 17 hrs. Low TS-sludges tended to cause smaller pH drop, and to some extent consume more alkali.

<table>
<thead>
<tr>
<th>Sludge</th>
<th>1</th>
<th>2A</th>
<th>2B</th>
<th>3A</th>
<th>3B*</th>
<th>4</th>
<th>5A</th>
<th>5B</th>
<th>5C**</th>
<th>6A</th>
<th>6B***</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>13</td>
<td>1</td>
<td>0.6</td>
<td>5</td>
<td>16</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>At pre-treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Initial pH</td>
<td>7.5</td>
<td>7.6</td>
<td>6.9</td>
<td>6.8</td>
<td>7.2</td>
<td>6.6</td>
<td>7.4</td>
<td>6.8</td>
<td>6.8</td>
<td>7.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Final pH</td>
<td>12.2</td>
<td>12.1</td>
<td>12.1</td>
<td>12.1</td>
<td>12.2</td>
<td>12.2</td>
<td>12.5</td>
<td>12.2</td>
<td>12.3</td>
<td>12.1</td>
<td>12.1</td>
</tr>
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<td>At neutralisation</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Initial pH</td>
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<td>11.3</td>
<td>10.5</td>
<td>10.1</td>
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<td>11.8</td>
<td>12.3</td>
<td>11.2</td>
<td>11.9</td>
<td>10.6</td>
<td>10.1</td>
</tr>
<tr>
<td>Final pH</td>
<td>7.5</td>
<td>7.8</td>
<td>7.7</td>
<td>7.7</td>
<td>7.7</td>
<td>7.6</td>
<td>7.5</td>
<td>7.6</td>
<td>7.7</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Duration time of treatment (h)</td>
<td>16</td>
<td>17</td>
<td>17</td>
<td>17.5</td>
<td>17.5</td>
<td>16.5</td>
<td>17.5</td>
<td>16.5</td>
<td>17.5</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>pH drop during treatment period</td>
<td>0.8</td>
<td>0.8</td>
<td>1.6</td>
<td>2.0</td>
<td>2.2</td>
<td>0.4</td>
<td>0.2</td>
<td>1.0</td>
<td>0.4</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Alkali consumption (g NaOH/100g TS)</td>
<td>12</td>
<td>12</td>
<td>15</td>
<td>8</td>
<td>6</td>
<td>11</td>
<td>31</td>
<td>7</td>
<td>5</td>
<td>9</td>
<td>6</td>
</tr>
</tbody>
</table>

(*) represent diluted sludges with water content of *25%, **40% and ***10%
Table 10 Statistical analysis of methane production using F-tests and Tukey confidence intervals

Numbers within brackets describe a 95% confidence interval of methane production (ml/g added VS) of pre-treatment (A-alkali; H-heat; E-electroporation) over control. Negative values represent higher methane production of control. Non significant differences are denoted “n.s.”

<table>
<thead>
<tr>
<th>Mill 1</th>
<th>Sludge A</th>
<th>Mill 2</th>
<th>Sludge A</th>
<th>Mill 2</th>
<th>Sludge B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>H</td>
<td>E</td>
<td>A</td>
<td>H</td>
</tr>
<tr>
<td>7</td>
<td>[25 47]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>[21 36]</td>
<td>[10 25]</td>
</tr>
<tr>
<td>40</td>
<td>[1  33]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mill 3</th>
<th>Sludge A</th>
<th>Mill 4</th>
<th>Sludge A</th>
<th>Mill 4</th>
<th>Sludge C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>H</td>
<td>E</td>
<td>A</td>
<td>H</td>
</tr>
<tr>
<td>7</td>
<td>[64 76]</td>
<td>[44 57]</td>
<td>n.s.</td>
<td>[65 75]</td>
<td>[47 57]</td>
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<td>20</td>
<td>[30 48]</td>
<td>[19 36]</td>
<td>n.s.</td>
<td>[37 52]</td>
<td>[19 33]</td>
</tr>
<tr>
<td>40</td>
<td>[8  30]</td>
<td>[6  27]</td>
<td>n.s.</td>
<td>[15 38]</td>
<td>[3  25]</td>
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<tr>
<td>60</td>
<td>[4  28]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>[9  26]</td>
<td>n.s.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Mill 5</th>
<th>Sludge A</th>
<th>Mill 6</th>
<th>Sludge A</th>
<th>Mill 6</th>
<th>Sludge B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>H</td>
<td>E</td>
<td>A</td>
<td>H</td>
</tr>
<tr>
<td>7</td>
<td>[9  25]</td>
<td>[15 31]</td>
<td>n.s.</td>
<td>[8  30]</td>
<td>n.s.</td>
</tr>
<tr>
<td>20</td>
<td>n.s.</td>
<td>[4  41]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>40</td>
<td>n.s.</td>
<td>[3  43]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>60</td>
<td>n.s.</td>
<td>[9  46]</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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</table>

<table>
<thead>
<tr>
<th>Mill 6</th>
<th>Sludge A</th>
<th>Mill 6</th>
<th>Sludge B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>H</td>
<td>E</td>
</tr>
<tr>
<td>7</td>
<td>[5  14]</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>60</td>
<td>[-21 -11]</td>
<td>[-24 -14]</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
APPENDIX B

STATISTICAL ANALYSIS

METHANE PRODUCTION RATE AND PROGRESS
Analysis was performed using Minitab® 15 (Minitab Inc., USA 2007).

Normal distribution test
‘ANOVA Test for equal variances’ (Levene’s test)
$H_0$: Data is normal distributed against $H_1$: Data is not normal distributed
If $P > 0.05$, $H_0$ cannot be rejected and normal distribution is assumed.

F-test and construction of Tukey intervals
Assuming data is normal distributed, ‘ANOVA One way analysis of variances’ can be performed to test
$H_0$: Treatments are equal against $H_1$: Treatments are not equal

Example (Minitab)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14</td>
<td>2</td>
<td>2422.3</td>
<td>1211.1</td>
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<tr>
<td>Error</td>
<td>6</td>
<td>113.3</td>
<td>18.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>2535.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If $F > F_{0.05}(2, 6) = 5.14$, $H_0$ can be rejected at the level of significance of 5%. Confidence intervals according to Tukey are then constructed to locate the differences.

Tukey 90% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of C14
Individual confidence level = 95.45%

C14 = Control subtracted from:

C14 = A subtracted from:

From this it can be concluded that both alkali (A) and heat (H) treatment is better than control (i.e. intervals do not hold 0), but there is no significant difference between the alkali and heat treatment.
APPENDIX C

VISCOITY ANALYSIS

BASIC RHEOLOGICAL PARAMETERS
Shear rate ($\gamma$) is the velocity gradient of the cylinder that spins in a cup, holding the sludge sample (Figure 10) and has the unit s$^{-1}$. The shear stress ($\tau$) is the force needed to be applied to the cylinder area to make the fluid flow, and is measured in Pa (Schramm 1994; Paula Segura de la Monja, pers. comm.). For a Newtonian fluid, such as water, the relationship between shear rate and shear stress is linear:

$$\tau = \eta \cdot \dot{\gamma} \quad (Formula 3)$$

where $\eta$ is the viscosity (Pa·s). The viscosity makes up the internal resistance of the fluid, and affects the velocity of the flow for a given force. Flocculation state, bound water and extracellular polymeric substances are factors that affect the viscosity of sludge. In a rheogram, the viscosity of a Newtonian fluid can be interpreted as the slope ($\omega$) (Figure 11). However, sludge is not a Newtonian fluid, and the shear stress and shear rate are therefore not proportional. Instead, an apparent viscosity, $\eta_{app}$, is calculated for each specific shear rate setting. Depending on how the sludge is behaving, different mathematical models are used to determine $\eta_{app}$. Regardless the model, the limit viscosity, $\eta_\infty$, is then determined as described in Figure 12. The yield stress, $\tau_0$, is defined as the stress that must be exceeded to make the fluid flow (Figure 11). (Pevere et al. 2006; Schramm 1994; Tixier et al. 2003a)

---

**Figure 10 Princip of a cylindric rheometer** A motor runs a cylinder submerged in the sludge sample. The internal resistance of the fluid, i.e. the viscosity, affects the amount of stress that must be applied to make the cylinder spin.
**Figure 11** Flow curve rheogram describing shear stress ($\tau$) as a function of shear rate ($\gamma$) for different types of liquids Modified after Schramm (1994).

**Figure 12** Viscosity curve rheogram Used to determine the limit viscosity, $\eta_{\infty}$, for non-Newtonian liquids. The limit viscosity is defined as the value of the viscosity when it no longer changes when increasing the shear rate.
**RHEOGRAMS**

Figure 13 Rheograms combining flow and viscosity curves from a) C-digester and b) PS-digester. Left axis: shear stress (τ) as a function of the shear rate (γ). Right axis: apparent viscosity (η_{app}) as a function of τ and γ. The flow curves describing the shear stress are constructed by first increasing the shear rate, and then decrease it back to zero, which give rise to the twin appearance of each graph. The space between the twin graphs is a sign of “poor memory”, i.e. instability of the sludge.
Figure 14 Flow and viscosity curves from day 65 The measurements from day 65 are here presented separately for clarity, but can also be found in Figure 13. Higher yield stress, shear stress and limit viscosity can be seen for the C-digester. This is a consequence of the NaOH addition in the PS-digester, which lower the viscosity.