



UNIVERSITY OF BORÅS
SCHOOL OF ENGINEERING

Optimization of Pre-hydrolysis conditions for the production of Biogas

Ignatius Ekwe, Awasume (S091427@student.hb.se)

Abel Prince Jabakumar, Sathiyaraj (X080215@student.hb.se)

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University of Borås
School of Engineering
SE-501 90 BORÅS
Telephone +46 033 435 4640

Examiner: Ilona Sárvári Horváth

Supervisor's name: Ilona Sárvári Horváth

Supervisor's address: University of Borås
School of Engineering
University of Borås
School of Engineering

Client: University of Borås

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Abstract

Hydrolysis and solubilisation of organic material is known to be the rate limiting step during anaerobic digestion. In this study, the effect of pre-hydrolysis was investigated. The substrate had a composition of the following wastes streams: fish and slaughterhouse waste, cleaning waste, doggy meat, doggy sludge, doggy dry food, mink (fur), Norway slurry, SOR2-Industrial waste, and municipal solid waste (MSW) from households in Borås community.

Analyses were made on two batches of experiments performed at two different mesophilic temperature conditions; 34 °C and 42 °C and with or without the addition of mesophilic sludge obtained from a waste water treatment plant. One of the substrate maintained the original carbon/nitrogen (C/N) ratio, and for the other substrate the carbon nitrogen ratio was then slightly increased by adding 7.98g of special shredded paper which contains 82% volatile solids (VS). The experiments were run for 7days with samples collected regularly after 0 day, 4 days, 5 days, 6 days and 7 days retention time. Chemical oxygen demand (COD), pH, total nitrogen, and volatile fatty acids (VFAs) as process indicators were analyzed.

The results showed that at 42 °C the rate of hydrolysis was faster with a higher COD release and removal. The optimum retention time for hydrolysis was 4 days and any longer time caused some of the carbon to be lost to carbon dioxide. The effect of adding mesophilic sludge had no significant effect. An increased C/N ratio resulted in a balanced system with lower VFAs produced compared with that from the original substrate mixture.

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1.0 Introduction

The consumption of natural resources by human and animals through a series of activities generate wastes after the intended use of the resource. Most often this waste is heterogeneous and in a solid form; referred to as solid waste. By definition it is the unwanted and useless parts of animal and human activities which are basically solid. Waste is a consequence of life and varies from one society to another. However solid waste generally arises from agricultural, industrial and social activities. The part of this waste generated from the municipality is termed municipal solid waste (MSW) and usually contains discarded material like papers, plastic bags, glass, metal fine earth particles, ash, sewage sludge, yard trimmings, food residues/leftovers and dead animals (Kumar et al., 2009). Pollution problems like foul odor-attracting vermin (wild mammals and birds which are harmful to crops, farm animals, or game, and which carry diseases), toxic gases, groundwater contamination and wasting landfill capacity are a consequence of unattended or poorly managed waste (Komemoto et al., 2009). Therefore an environmentally friendly way of waste disposal is a call for concern; giving rise to anaerobic digestion process (for the organic fraction) and combustion (for the inorganic fraction) of waste to produce sustainable energy.

Municipal solid waste (MSW) in the city of Borås is managed by the Energy Company called “Borås Energi och Miljö AB”. The waste from the households which has been sorted into white bags and black bags are collected and separated at Sobacken. Here, the white bags containing inorganic waste and can be burned are crushed and sent to the Rya plant where they are used as fuel in the combustion facility. The biodegradable waste in black bags, left behind at Sobacken is then fermented and turned into biogas,-a renewable fuel at the biogas facility (www.borasenergimiljo.se).

In addition to the MSW from the households, waste from slaughterhouses and other industrial waste streams are often used in the production of biogas for the Borås community. These waste streams are first mixed and diluted in a buffer tank prior to biogas production. However, because the supply of waste might be limited or not regular and based on the fact that each component of waste (starch, cellulose and protein) is degraded under different environmental conditions; with starch degradation increasing at low pH, cellulose at high retention time and protein at both high retention time and neutral pH (7-9), the Sobacken biogas plant seeks to optimize some biological and physicochemical parameters (adjusting the environmental conditions) during hydrolysis and acidification process in the buffer tank.

It is believed that if the degradation process is enhanced during this stage, the quantity and quality of biogas produced would be improved. This hypothesis is supported in the GTI final technical report on economically and environmentally friendly production of fuel and power from hog manure using HIMETSM (for high methane) two stage anaerobic digestion technology (Saber, 2009).

Also, on grounds that there are differences in nutritional requirements, kinetic growth rates, physiologies, sensitivity levels and environmental tolerances between the fermentative bacteria operating during hydrolysis and acidification and the more sensitive microorganism responsible for Methanogenesis, a two phase anaerobic digestion is important (Saber, 2009). In the same line, (Komemoto et al., 2009), underscores a two phase digestion configuration as a more rational one because it provides suitable environments for acidification and methanisation in two separate steps. In addition, (Pavan et al., 2000) concluded that good methanisation yields (specific gas production around 0.6 m³/kg Total volatile solids (TVS) can be achieved in a two phase system with mesophilic temperature of the hydrolytic reactor and a thermophilic temperature in the methanogenic reactor. The conclusions were made in experiments using organic fraction of municipal solid waste (OFMSW) as the feed and 12days of overall hydraulic retention time (HRT). A two stage approach has previously been successfully applied to fruits and market waste (Vituria et al., 1989), municipal solid waste (Pavan et al., 2000), agro-industrial residues), and solid potato waste .The question left to ponder about is “which of the three possible two-stage systems is best applicable?”

The performance of three temperature-phase system; (I) mesophilic first stage (37°C) + mesophilic second stage (37°C), (II) mesophilic first stage (37°C) + thermophilic second stage (55°C), and (III) thermophilic first stage (55°C) + thermophilic second stage (55°C) were investigated. Results showed that after specific OLR were established in the second stage of all the systems; from 2.2g to 11.0g COD/l/d in system I, from 4.5g to 22.3g COD/l/d in system II, and from 1.3g to 36.0g COD/l/d in system III, system I and III showed signs of overloading of the second or methanogenic reactor due to the accumulation of VFAs. However, in system two, no overloading was observed because the solid potato (feedstock) load was quickly degraded completely before the OLR reached a level high enough to cause failure (Sundin, 2008). Satisfactorily enough, high methane content and biogas was achieved in all the systems.

1.1 Purpose of Study (Objectives and scope)

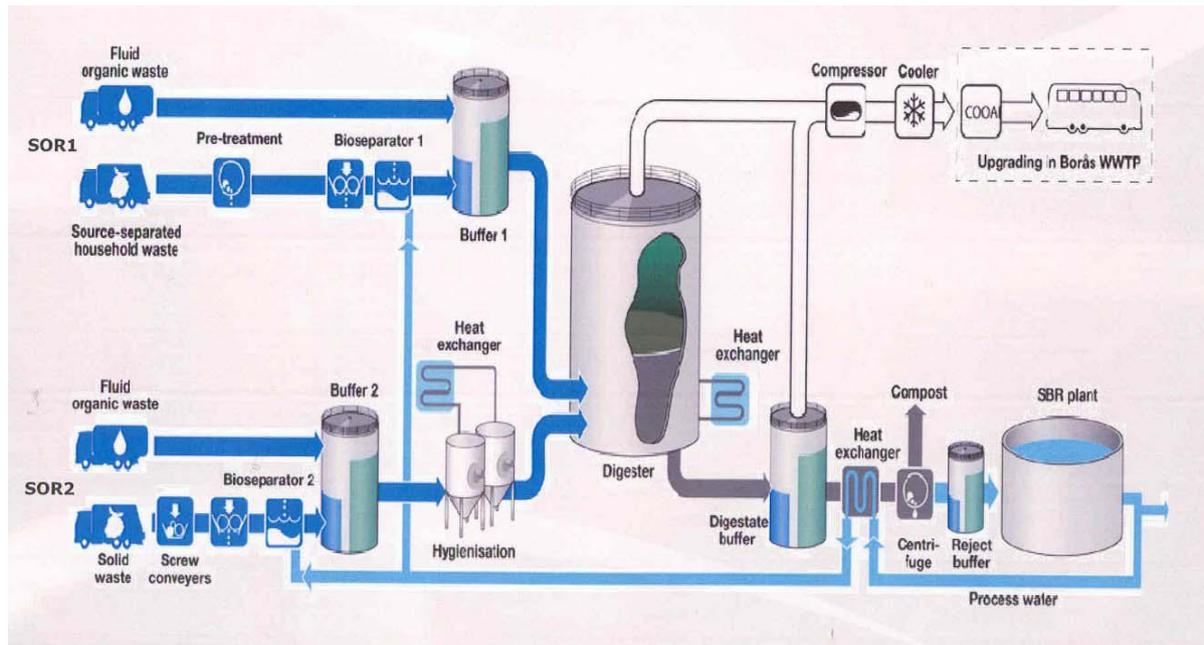
The limitations of conventional combined digestion method of biogas production would be overcome if the process parameters are controlled during hydrolysis and fermentation (Saber, 2009). Generally, hydrolysis is considered to be the rate limiting step of anaerobic digestion if the substrate is in particulate form (Veeken et al., 2000). And hydrolysis is a function of the process parameters. (Komemoto et al., 2009) has indicated that hydrolysis can be enhanced through the use of physico-chemical and biological pretreatment methods, and that the more direct and economical way of improving hydrolysis is to employ microorganisms with high degrading activity.

Focus in this study is placed on the optimization of process parameters like pH, temperature, retention time and C: N ratio during the pre-hydrolysis conditions in the buffer tank. These parameters will be evaluated by regular measurements of process indicators such as; volatile fatty acid (VFA) concentration, concentration of free ammonia and dissolved chemical oxygen demand (COD). The effect of microorganisms during hydrolysis and fermentation by added mesophilic sludge as inoculum is also investigated.

Furthermore, to achieve an optimum hydrolytic and fermentation process in the buffer tank, these parameters must be carefully controlled since biological processes require a constancy of living conditions (Deublein and Steinhauser, 2008).

Although hydrolysis is said to be the rate limiting step, acetogenesis or methanogenesis can be the rate limiting step in anaerobic digestion at high organic loading rates of easily degradable substrates due to the accumulated VFA which results from the rapidly degradable components. Presently this assertion is still lacking substantial backing since a more complex structured model which has not been developed has to be used (Komemoto et al., 2009).

2.0 Anaerobic Digestion (AD) for Biogas production at Sobacken



1. Figure 1: the flow chart of biogas production process at Sobacken.

Figure 1.0 shows substrate flow into the Sobacken biogas plant. The plant receives substrates in two lines. Line 1 substrates (SOR1) are solid and fluid organic waste which has been optically separated as the black plastic bags, and line 2 substrates (SOR2) are industrial waste from grocery stores, slaughterhouses, food production and animal waste which are both in solid and liquid form (Rousta, 2008).

Generally, all types of waste aimed at anaerobic digestion are pre-treated in order to enhance the quality of the substrates and improve biodegradation. At Sobacken, this pre-treatment is done in various stages. Initially, the drum sieve machine separates the black plastic bags from the waste which is transferred to the magnetic separator to remove any available metals from the stream. The shredder machine then reduces the size of the waste to a desired size and the biomass which is made into fluid by the bio separator is later transferred into buffer tank one. Any liquid waste from line one (SOR1) is directly being pumped to the buffer tank 1 where all the waste is treated at 55 °C for 6 hours before entering the methanizer or digestion tank.

The industrial waste from line two (SOR2) usually in solid forms is shredded and sent into the bio separator where it is blended and separated from the packaging material before being

transferred into the buffer tank 2. At this stage, it is sanitized for one hour at 70 °C before transferred into the digestion tank (Rousta, 2008).

In a simpler model, the processes taking place at Sobacken and a clear illustration of the scope of this work can be seen as illustrated in Figure 2. Our project had a focus on optimization of the pre-hydrolysis conditions.

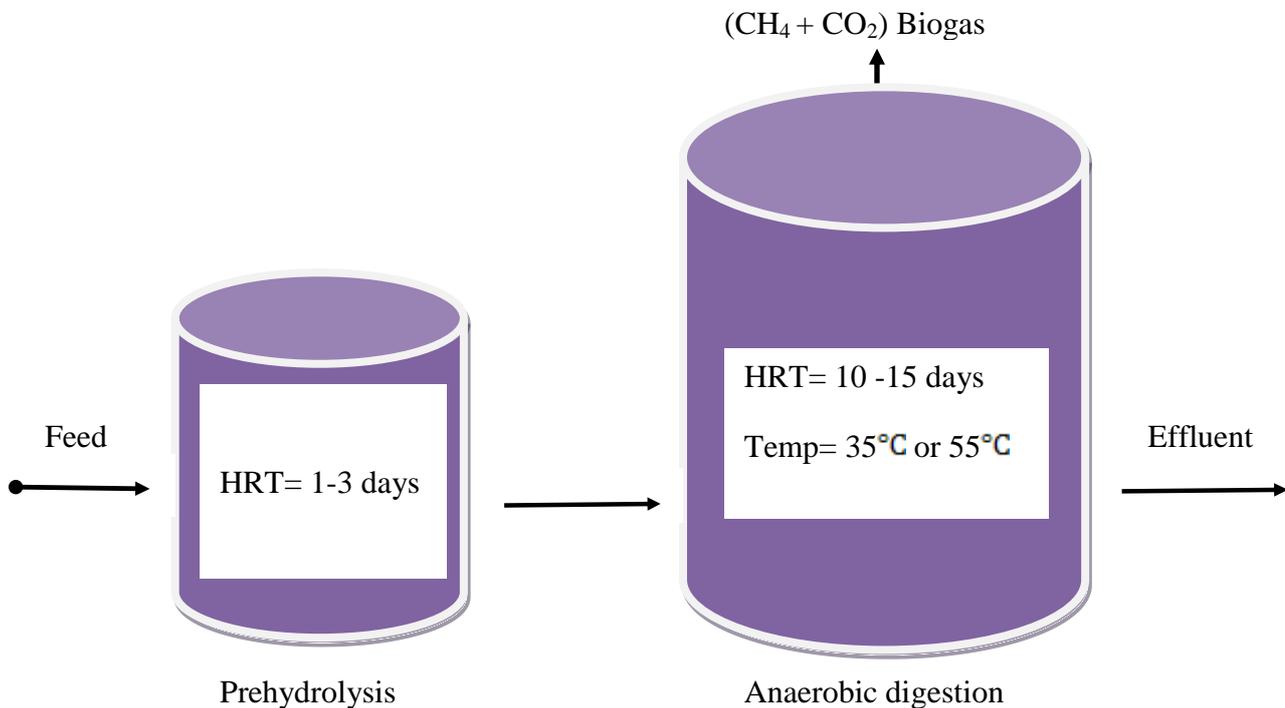


Figure 2. The scope of the work.

2.1 Biochemical Reactions

Anaerobic digestion (AD) is a series of chemical reactions during which organic material is decomposed through the metabolic pathways of naturally occurring microorganisms in an oxygen free environment. The degradation process usually occurs in some form of a tank called the digester. The reaction is said to be complete upon the production of a colourless, odourless gas called methane (CH₄) and carbon dioxide (CO₂); while humus which is normally returned to the soil emerges as a by product (Saber, 2009). The composition of biogas produced in terms of percentage methane depends on the composition of the waste used.

AD occurs in four stages or sub processes which include: hydrolysis, acidification or fermentation, acetogenesis and finally methanogenesis. Each stage is being carried out by specific microorganisms; and the intermediates of each stage are required to be in equilibrium. This means that the rate of products formation in one step should be balanced with the rate of those products consumed in another step. If equilibrium is not achieved, the process will be unstable with low efficiency or the metabolic process will collapse (Saber, 2009).

2.1.1 Hydrolysis

During this stage, complex organic compounds (carbohydrates, proteins and fats) are broken down into their respective constituent monomers (molecules which are more soluble and transportable through the cell membrane) by hydrolytic enzymes secreted by specific groups of bacteria.

Polysaccharides such as cellulose are hydrolysed to glucose by cellulase enzyme; hemicellulose and Pectin are converted to monomers like xylose, glucose, galactose, pentoses, arabinose and mannose by pectinases and hemicellulases, while starch is converted to glucose by amylase enzyme(Saber, 2009, Ostrem et al., 2004).

Proteins are generally hydrolysed to amino acids by protease enzymes secreted by *Bacteriodes*, *Butyrvibrio*, *Selenomonas* and *Streptococcus* (Saber, 2009, Ostrem et al., 2004).

Extracellularly produced lipases secreted by lipolytic bacteria (clostridia and micrococci), convert lipids to long chain volatile fatty acids (Ostrem et al., 2004)

An approximate chemical formula suggested by (Ostrem et al., 2004)can be used to represent a hydrolysis reaction of carbohydrates into simple sugars. In the case of glucose, the reaction can be illustrated by the equation 1 below.



(Sugar polymer) + (water) → (glucose) + (hydrogen)

2.1.2 Acidification/Fermentation (Acidogenesis)

This is the second step in AD process. And at this stage, acid forming bacteria which are facultative anaerobic and being able to grow in acidic environment, break down the products of hydrolysis into acetic acid (CH₃COOH), hydrogen (H₂), carbon dioxide (CO₂) and alcohols. Other lower weight simple organic acids like propionic acid and butyric acids which are in turn converted to acetic acids can also be produced at this stage. (Yadvika et al., 2004)

Below are some typical reactions that occur during acidogenesis. In equation (2), glucose is converted into ethanol and in equation (3), propionic acid is formed from glucose (Yadvika et al., 2004) .



(Glucose) ↔ (ethanol) + (carbon dioxide)



(Glucose) + (Hydrogen) ↔ (propionic acid) + (water)

2.1.3 Acetogenesis

This is the step during which obligate hydrogen producing bacteria convert fatty acids to acetate as the main product and hydrogen gas. However, some acetate (≈20%) and hydrogen (≈4%) are produced during acidogenesis. As well, acetogenesis is the path way through which biological oxygen demand (BOD) and chemical oxygen demand (COD) are reduced in AD process. Although the presence of hydrogen is of critical importance to anaerobic digestion process, its concentration has to be kept low enough (60ppm - 200ppm) to thermodynamically allow the conversion (Sterling, 2001). If the concentration of hydrogen as measured by the hydrogen partial pressure is not low, the further reactions will be inhibited. But the presence of hydrogen consuming bacteria during methanogenesis lowers the concentration of hydrogen and ensures the feasibility of the conversion of all acids (Salminen and Rintala, 2002).

2.1.4 Methanogenesis:

This is the last AD step where the soluble products of acidogenesis and acetogenesis (acetic acids, carbon dioxide and hydrogen) are converted into a mixture of methane and carbon dioxide (Biogas). The methanogens responsible for this conversion are divided into two groups: H_2/CO_2 and acetate consumers. The acetate consumers; mainly *Methanosarcina* and *Methanotrix* produce about 70% of methane from acetic acid while 30% is produced from carbon dioxide by reduction with hydrogen. Methanogens are very sensitive to changes and prefer a neutral or slightly alkaline environment (Saber, 2009), (Sterling, 2001).

Figure.3 shows a conceptual diagram of the biological processes involved in anaerobic digestion (AD) of organic matter. It indicates the percentage of various products through one bio-process to another.

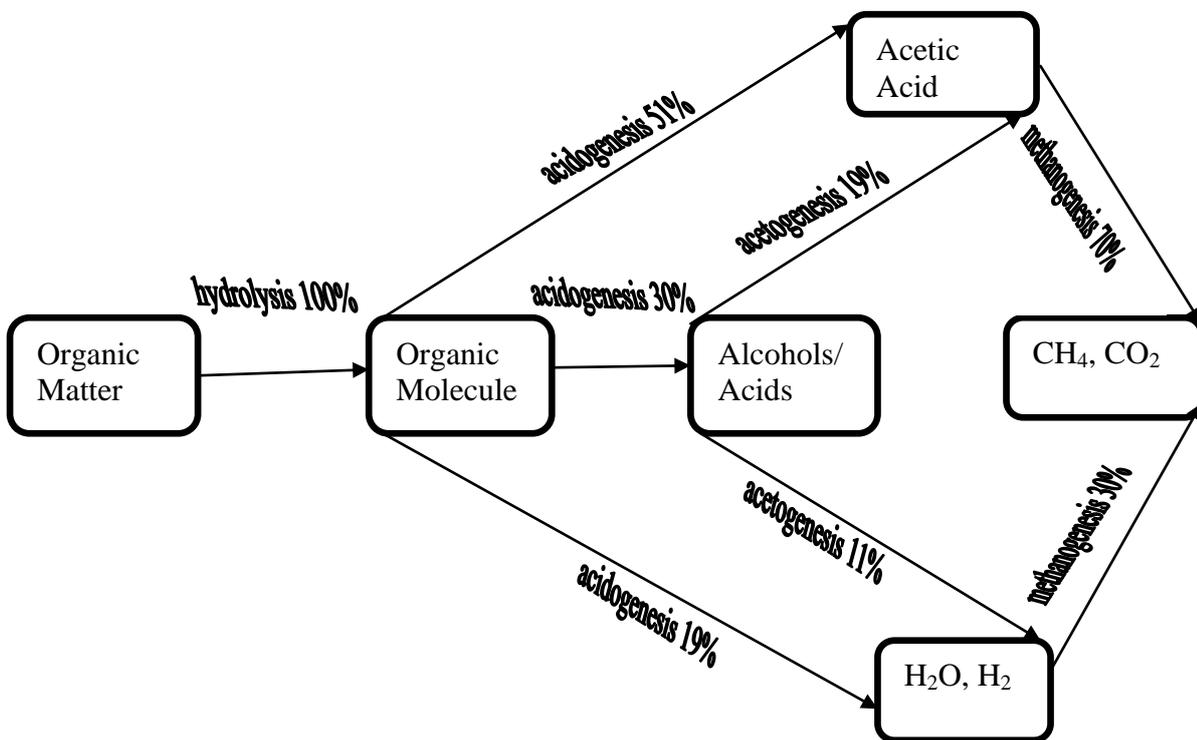


Figure. 3 Schematic of biochemical processes in Anaerobic Digestion.(Saber, 2009)

3.0 Process Parameters

In each step of biogas process, the gas production and the decomposition rates of organic waste is influenced by environmental factors. In a conventional single-tank (i.e. combined phase) digestion, both fermentative bacteria and methanogenic archaea coexist in the same vessel. The operations under these conditions represent a compromise of conditions for growth and feedstock conversion as there are differences in their nutritional, kinetic growth and environmental tolerances. For example, whereas the optimum pH for acid forming phase is approximately pH 6, the single digester is usually operated at pH 7 to maintain stability of the methane forming microorganism (Saber, 2009). In order to avoid this compromise and optimize the gas production, the parameters listed below are closely monitored in effort to improve the anaerobic treatment of waste and methane production.

3.1 pH

pH is usually used to measure the acidic, alkaline or neutral nature of a solution. It is defined as the negative logarithm of hydrogen ion concentration or more precisely, H^+ ion concentration in a solution ($pH = -\log [H^+]$). A pH of 7 represents neutral conditions in an aqueous solution at $25^{\circ}C$, for equilibrium constant for the ionization of water into H^+ and OH^- (K_w)= 1.0×10^{-14} , and $H^+ = 10^{-7}M$ and $OH^- = 10^{-7}M$. At a higher H^+ concentration, the pH will be below 7 (acidic solution) and at a higher OH^- concentration, pH will be higher than 7 (alkaline solution) (Deublein and Steinhauser, 2008). The growth of microbes during anaerobic fermentation is affected by changes in pH. Therefore, an ideal pH range is necessary to ensure that the process is optimized. Methane forming microorganisms will function optimally at a desired pH range of 6.7-7.5 (Deublein and Steinhauser, 2008). Some other literature suggests that fermentation process will be optimal at a pH range of 6.8-7.2 (Yadvika et al., 2004). Fluctuations of pH out of this range would have a negative effect in the formation of methane. The growth of methanogenic microbes will greatly reduce at a pH below 6.6 or if the pH reduces below this value, organic acids are produced which leads to a further decrease of the pH and eventually stops the degradation process. And on the other hand, if the pH is excessively alkaline, microbial granules will disintegrate and the process will subsequently fail (Deublein and Steinhauser, 2008).

The concentration of volatile fatty acid and the amount of carbon dioxide produced during the anaerobic process affects the pH of the digester content. And for anaerobic fermentation to proceed normally, the concentration of volatile fatty acids particularly acetic acid should be below 2000mg/l (Yadvika et al., 2004). (Shanmugam and Horan, 2009) conducted a study to evaluate the combined effect of C: N ratio with pH control; since the C: N ratio is important in determining free ammonia concentration. The Bio-methane potential (BMP) evaluation was carried out at a C: N ratio of 15 with controlled pH values of 4.5, 5.5, 6.5, 7.5, and 8.5 to study the impact on NH₃-N toxicity on biogas yield. Results showed that over this pH range (4.5-8.5), the cumulative biogas production ranged from 1258mL at pH 8.5 to 6518mL at pH 6.5. The minima biogas production at pH 8.5 coincided with the highest (3473mg/L) ammonia production while at pH 6.5 where the volume of biogas yield was 6518mL; the ammonia produced was 817mg/L. Their results were in line with the works of (Shanmugam and Horan, 2009) who observed that ammonia toxicity only occurred at pH values greater than 7.6.

It is also indicated that, methane yield is not only a function of ammonia concentration as the lowest concentration of 515mg/l was observed at a pH of 4.5. And at this pH, the VFA concentration/toxicity is said to be the limiting factor. This is because the VFA concentration through a chemical equilibrium influences the pH in the waste; and for specific waste composition, the VFA concentration and the pH can be related to each other (Veeken et al., 2000). (Sterling, 2001) concluded that when ammonia is added to the digester, the pH was increased until a chemical equilibrium was established.

(Yadvika et al., 2004) found that the efficiency of methane production was more than 75% when the pH was above 5.0. In the same line as stated in (Ghaly, 1996) the two phase anaerobic reactor using cheese whey and dairy manure as substrate operated as a single phase reactor when the pH of whey was not controlled. But when pH was controlled, it operated as a two-phase reactor (Yadvika et al., 2004). When the anaerobic digestion process is separated into two phase as with the HIMET process, the fast growing acidogenic bacteria prefer a low pH range of 4.5 to 5.5. While the slow growing and the more sensitive methanogenic bacteria prefer neutral to slightly alkaline environment of pH > 7 (Saber, 2009).

3.2 Particle size

The sizes of feedstock particles have an influence on the gas production though it is not as important parameter as pH and temperature of the digester content. If the particle sizes are too large, the digester might be clogged and also, the digestion of the particles will be difficult for microorganisms. On the other hand, smaller particles would provide large surface area for absorbing the microorganisms and enzymes. This will lead to increased microbial activity and hence increased gas production (Yadvika et al., 2004).

(Sharma et al., 1988) studied the effect of particle sizes on biogas production using particles of 0.088, 0.40, 1.0, 6.0 and 30.0mm. They found out that maximum quantity of biogas was produced from particles with smaller size (0.088mm and 0.40mm). However, succulent materials such as leaves with larger sizes could be used though materials such as straw and other larger particles would decrease the production of biogas. In a nut shell, the results suggested that a physical treatment such as grinding could significantly reduce the volume of the digester without decreasing the biogas production (Moorhead and Nordstedt, 1993).

3.3 Volatile Fatty acids (VFA)

The concentration of volatile fatty acids and their relationships receive a great deal attention during anaerobic digestion. This is because they are precursors of methane production and secondly, they act as inhibitors to the process when in excess (Hill and Holmberg, 1988). In anaerobic degradation of complex substrates, a diversity of microorganisms (facultative and obligatorily anaerobic bacteria) is involved. In the case of slaughterhouse waste and other waste streams, fermentative bacteria particularly proteolytic *Clostridium* species, hydrolyze proteins to polypeptides and amino acids. Lipids are hydrolyzed via β -oxidation to long chain fatty acids (LCFAs) and glycerol and polycarbohydrates hydrolyze to sugars and alcohols. After this break down, the intermediates are converted to short chain organic acids-volatile fatty acids (VFAs) with C1-C5 molecules, hydrogen (H₂), and carbon dioxide (CO₂). Examples of these VFAs are: acetic acids, butyric acid, propionic acid, valeric acid and lactic acid (Salminen and Rintala, 2002), (Deublein and Steinhauser, 2008), (Komemoto et al., 2009). Butyrate's are reported to be produced mainly from the degradation of starch, acetic acid from the degradation of cellulose and propionic acid, valeric acid and acetic acids from protein degradation (Hill and Holmberg, 1988).

The VFAs, hydrogen and carbon dioxide are then converted to acetic acids by acetogenic hydrogen producing bacteria. During methanogenesis, the hydrogen is consumed by the acetogenic hydrogen consuming bacteria to establish a balance. If this balance is not established, the degradation process of the organic material is inhibited. The degradation of the LCFAs may be rate limiting to AD because of the slow growth of the LCFAs consuming/hydrogen producing bacteria. As a result, there would be slow degradation of the LCFAs or lack of degradation of the degradation products (hydrogen). This can lead to the accumulation of organic acids and alcohols which is an indication of the process imbalance (Hill and Holmberg, 1988).

Volatile fatty acids concentrations have quite frequently been used as performance indicators. (Hill and Holmberg, 1988) states that the short chain propionic and acetic acids and their ratio Propionic/Acetic (P/A) have always been used as stress/failure indices while the long chain butyric, Iso butyric, valeric and Iso valeric have received little attention. He based his proposition on the fact that the long chains are completely no detectable or they are in very low concentrations. He used the concentration of acetic acid level alone to make predictions of the digester health; stating that failure is eminent when that concentration is greater than 800mg/l.

Furthermore, he mentioned that in order to have a stable/non inhibitory operating system, concentrations of total volatile fatty acids of less than 2000mg/l must be maintained. And that it is likely to observe an impending failure of a digester whose propionic acid to acetic acid ratio is more than 1.4 (Hill and Holmberg, 1988).

The inhibition of the AD process by LCFAs is due to their toxicity to the anaerobic microorganisms particularly acetogens and methanogens or due to their tendency to form floating scum which cause fouled gas collection pipes and scum overflow. Another mechanism of inhibition of LCFAs is by adsorption to the microbial cells. And adsorption together with other mechanisms of inhibition depend on the concentration of LCFAs, the type of bacteria present, the type of reactor, type of input material (feedstock) and specific surface area of the sludge (Hill and Holmberg, 1988). Gram-positive microorganisms (with defined cell wall) and suspended sludge are said to be more vulnerable than the Gram-negative microorganisms and granular sludge. The concentration of volatile acids in a reactor is affected by the temperature, hydraulic retention time and the waste type. The higher the temperature and/or the longer retention time the lower the concentration of volatile acids (Ghaly, 1996). It is also noted that VFAs accumulation occurs because of the rapidly

degradable materials like grains; which brings about severe inhibition in the acidogenic phase due to pH drop and product inhibition. A reduction of such material in the feedstock and decrease in dilution rate of the substrate is a more proximate solution to this (Ghaly, 1996).

The most inhibitory LCFAs are the saturated with 12-14 carbon atoms and the unsaturated with 18 carbon atoms. In addition, the toxicity effect of fatty acids increases in the presence of another (Poulsen, 2003), (Salminen and Rintala, 2002). Some examples of these LCFAs are: Oleic acid, Stearic acid, Palmitic acid, and Myristic acid (Salminen and Rintala, 2002).

Even though LCFAs might be this far inhibitory to anaerobic digestion of organic material, there are yet other compounds that reduce their toxicity. Albumin, starch, bile acids and cholesterol may reduce the toxicity of fatty acids by forming complexes or competing with them on cell wall adsorption. Other substances which prevent LCFAs inhibition are bentonite (because of its ability to flocculate-causing small lumps) and its cations such as calcium. These cations and calcium from other sources also reduces the inhibitory effect of LCFAs due to its ability to form precipitates and increase surface tension (Viturta et al., 1989).

In a two phase anaerobic digestion where the hydrolytic reactor was operated firstly at mesophilic conditions and secondly at thermophilic conditions, it was found that the production of total volatile fatty acids (TVFAs) was less in the hydrolytic phase of the thermophilic condition than the hydrolytic phase of mesophilic condition (Viturta et al., 1989).

The efficiency of acidogenic fermentation can be evaluated by degree of conversion of volatile fatty acids from the substrate. This can be illustrated by equation 4 below.

$$\text{Acidification \%} = \frac{\text{VFAP}}{\text{VFAt}} \dots\dots\dots(4)$$

Where VFAP is the concentration of volatile fatty acids produced, and VFAt is the concentration of the total volatile fatty acids (Viturta et al., 1989).

3.4 Temperature

The temperature of the digester is another important and critical parameter considered during AD of organic waste for biogas production. This is mainly due to the strong dependence of the digestion rate. Generally, there are three temperature ranges during which AD can operate. These categories are determined by the species of bacteria which operate in each of the three temperature ranges: the psychrophilic range (0°C to 30°C), the mesophilic range (30°C to 42°C) and the thermophilic range (50°C - 60°C). However, most AD operations are carried out under mesophilic or thermophilic temperatures during which enzyme activity is high with optima at 35°C and 55°C respectively (Yadvika et al., 2004). Mesophilic digesters are the most common and they operate stably. This might be because of the variety of mesophilic bacteria that tolerate greater changes in environmental parameters including temperature; than the thermophilic bacteria. (Poulsen, 2003) reports that of more than 1500 European biogas plants only 5% are psychrophilic, 8% are thermophilic and 87% are mesophilic.” The only European country as at the year 2001; having more thermophilic than mesophilic biogas plants is Denmark. Italy and Switzerland are the only known countries operating biogas reactor plants under psychrophilic conditions (Poulsen, 2003).

The choice to operate a digester in either mesophilic or thermophilic temperature ranges involves some trade-offs. Digestion at thermophilic temperatures allow high loading rates, fastest bacterial growth, fastest degradation of substrates (volatile solids), increase in the destruction of weeds, seeds and pathogens; ensuring efficient hygienisation and thus greater production of biogas. In a three system two phase anaerobic digestion experiment: (I) mesophilic first stage (37°C) + mesophilic second stage (37°C), (II) mesophilic first stage (37°C) + thermophilic second stage (55°C), and (III) thermophilic first stage (55°C) + thermophilic second stage (55°C), it was observed that the digestion period of potato waste which was used as the feedstock was shorter by 24% in system II and by 34% in system III than in system I, indicating an advantage of digestion at thermophilic conditions (Poulsen, 2003). However, they require higher energy demand to increase the temperature above ambient conditions. And during cold seasons, the energy demand is even higher and therefore not economical to the process. More so, thermophilic methanogens are more temperature-sensitive than mesophilic microbes. A small change in temperature causes substantial decrease in activity. In addition, (Pavan et al., 2000) states that when the hydrolytic reactor temperature (23°C) was raised to a thermophilic range (55°C); with other experimental variables kept constant, the production of total volatile fatty acids (TVFAs) was 10 times

lower than when a mesophilic temperature was used. The reason is associated with the kinetics of the bio-reactions at the thermophilic range wherefore some TVFAs are consumed at the start up of the first phase. However, the 10 times difference in the TVFA, induced only a small difference in the biogas yield at the thermophilic-methanogenic reactor (Ostrem et al., 2004). Thermophilic conditions are also criticized on the higher concentration of VFAs found in the effluent than there is with the mesophilic conditions (Poulsen, 2003). In a study, it was observed that the COD removal in laboratory experiments using a 2.8 l up flow Anaerobic Sludge Blanket (UASB) of proteinaceous wastewater under mesophilic (37⁰C) conditions was about 84% while only 69-83% was removed under thermophilic (55⁰C) conditions. And COD is a good indicator of the completeness of the degradation process, since any undigested material will require oxygen (in an aerobic environment) to complete degradation. Since the optimum temperature for methanogenesis may not necessarily be the optimum for hydrolysis, acidification nor acetogenesis, multi-stage digesters that separate processes taking place in respective tanks like the two-phase digester could be used (Yadvika et al., 2004).

In order to keep the temperatures at desired range, it is good to coat the digesters with insulating materials during installations. The construction of biogas plant facing the sun has advantages as this protects them from cold winds. In this way biogas yield would be increased when temperature drops in digesters. In (Yadvika et al., 2004) it has been mentioned that a charcoal coating of ground around the digester results in improved biogas production in KVIC plant by 7-15%. The use of hot water in the preparation of slurry also helps to improve gas production and in the same light, solar assisted plants are found to produce higher gas yields during winter months (Yadvika et al., 2004).

3.5 C/N ratio

The substrate used for AD process is the source of material for biodegradation. It contains total solids (TS) of which 82% to 85% is volatile solids (VS) which is available for microbial degradation in the case of manure (Poulsen, 2003). The microorganisms responsible for this degradation process require specific nutrients as do all living organisms, with carbon (C) and nitrogen (N₂) being the most important. The gas production and smooth functioning of the process in terms of ammonia/ammonium (NH₃/NH₄⁺) toxicity is dependent on the ratio of carbon to nitrogen. This problem is mostly pronounced when animal manure which contains high concentration of urea is used as the substrate (Poulsen, 2003).

For efficient plant operation, the C/N ratio must be properly maintained in the composition of feedstock. During anaerobic digestion microorganisms utilize carbon 25-30 times faster than nitrogen. This means that microbes need a 25-30: 1 ratio of C to N with and most literature citing 25: 1 as the ideal level. If the C/N ratio is very high, nitrogen will be consumed rapidly by the methanogens for meeting their protein requirement. In this case, there will be no more nitrogen left to react with the left over carbon in the feedstock. As a result, gas production will be low. On the other hand, if the C/N ratio is very low; nitrogen will be liberated and will accumulate in the form of ammonia (NH₃) which is the inhibiting component (Poulsen, 2003). The equilibrium reaction involved during the production of NH₃ is represented by equation 5.



At high temperatures, the equilibrium reaction will shift to the left (the dissociation of ammonium into hydrogen ion and ammonia is faster than its formation) there by producing more NH₃ and eventually increasing the inhibition. NH₃ will increase the pH value of the digester and when it is beyond 8.5, the environment becomes toxic to methanogenic bacteria population. In addition, the compost derived from the digestate will be of less quality because of the high ammonium content (Poulsen, 2003).

3.6 Retention time

This is the amount of time the feedstock stays in the digester before it flows out as effluent. If the feedstock is liquid; it is referred to as hydraulic retention time (HRT). And it is defined as the ratio of the digester volume to the amount of digested material per day; as measured by the COD and BOD of exiting effluent. E.g. 1m³ /day to 10m³ tank has a HRT of 10 days (Poulsen, 2003), (IMTE, 2007),(Ostrem et al., 2004). But if the feedstock is solid; it is known as solid retention time (SRT). It is defined as the ratio of the mass of solids held in the digester to the mass of solids removed per unit time. Since the amount of solids removed from the digester is always in liquid form (effluent), there exists a relationship (ratio) of the concentration of substrate still in the solid form in the digester (*C_d*) to the concentration of solid that has been converted into waste as effluent (*C_w*). If the ratio (*C_d*)/ (*C_w*) is 1, SRT= HRT. However in some digester designs, SRT>HRT if (*C_d*) > (*C_w*). Another measure of the time scale on which the digester operates is the microbial retention time (MRT). It is defined as the ratio of the microbial mass held in the digester to the microbial mass removed per unit

time. Because bacteria have a high tendency to attach to surfaces, it would be reasonable to assume that a large fraction of microbes are attached to the surfaces of solids in the digester. This way, MRT tends to follow the changes in the SRT. Therefore if the SRT is increased relative to HRT, it applies the same to MRT (Saber, 2009).

The hydraulic retention time is the determining factor in the conversion efficiency of anaerobic digestion process where the substrate in volatile solid (VS) content is converted into biogas by bacteria. The amount of methane produced is therefore influenced by the hydraulic retention time of the substrate.

The longer the retention time, the more bacterial growth that can occur; with corresponding increase in the bacterial activity on the substrate and the larger the substrate is converted to biogas within a given temperature range and/or category of bacteria (psychrophilic, mesophilic, or thermophilic). This means that the longer a substrate is kept under proper reaction conditions, the more there will be complete degradation. However at some point, the rate of reaction will decrease with increasing retention time. This indicates that there is an optimum time that will achieve the benefits of digestion in an optimum way. The appropriate time depends on the feedstock, environmental conditions that influence: - (a) the growth of the bacterial populations, (b) the types of the by-products of all classes of bacteria and their competition. It also depends on availability of the food sources and the intended use of the digestate (Saber, 2009). Typical periods of growth can span from a fraction of an hour to a day or more.

The retention time for most dry processes ranges between 14 and 30 days and for wet processes can be as low as 3 days; though the optimal value varies according to the specific technology, the process temperature and the waste composition (Ostrem et al., 2004).

For mesophilic bacteria the retention times are within 15-30days and 12-14days for the thermophilic bacteria (Saber, 2009).

Changes in feedstock and changes in temperatures might eventually cause a variation in retention times of the substrate in the digester. For changes in feedstock, retention time may vary from day to day while with changing temperatures, retention time changes from season to season. The digestion of substrate (volatile solids) after 10 hours during recent research has been observed to be between 64% and 85%. But 10 days were typically chosen for complete digestion (Ostrem et al., 2004).

The size of a digester or reactor affects retention time. And it is cost saving to have a digester with low retention time and thus high gas production rate per digester volume unit. However, there is always an overall lower degradation of the feedstock which compromises with the total volume of the gas produced and the quality of other digestion products. It is therefore important that the two factors be balanced in the design of a full scale reactor (Ostrem et al., 2004).

There are some generally accepted practices which aid in reducing retention times. A number of them are employed without having the retention time as the fundamental reason. Two of the famous ones are continuous mixing and using low solids (Ostrem et al., 2004).

The recirculation of water and biogas in the digester to keep material moving and minimize settling within the tank is the most common technique of mixing. This ensures stable environmental conditions within the digester as well as increases the accessibility of the digestible surfaces of the feedstock to the bacteria. A well mixed digester uses the entire volume of the tank or a greater working volume and is void of stratified layers or scum.

When the feedstock has a low solid content, bacteria can easily access the digestible portion of liquid substrate and in addition, the fact that the reactions require water, retention time will be reduced. Another important aspect is the complete mixing that is achieved with liquid substrates than with solid substrate. In addition, the separation of the stages of digestion into individual chambers optimizes the activity of specific bacteria population and hence reducing retention time. Another option is to introduce surfaces or a combination of surfaces on which bacteria would permanently live in order to reduce the population that is washed out (Ostrem et al., 2004). A fifth option is to improve circulation within the chamber by alternating the flow pattern. Furthermore, the pretreatment of waste in order to increase digestibility can be considered as the sixth option to reduce retention time (Ostrem et al., 2004). It is worth noting that each of these options are designed and proved to be effective with specific feedstock's though all could be combined for MSW. In a two stage fermentation process to produce hydrogen and methane under thermophilic hydrolysis/fermentation (60°C) and methanogenesis (55°C) the hydraulic retention time was optimized at 8 days (hydro genesis, 1.2d and methanogenesis; 6.8d). At this retention time, 5.4m³/m³/d of hydrogen and 6.1m³/m³/d of methane was produced (Ostrem et al., 2004).

3.7 Chemical oxygen demand (COD) concentration

This parameter measures the quantity of oxygen used in chemical oxidation of materials. . However, the organic component predominates and it is of greater interest (IMTE, 2007). Furthermore, COD test together with VS are the most important method to determine the quantity of organic matter in the slurry, sludge or any feedstock for anaerobic digestion. This is why the feeding of the digester with substrate in terms of organic loading rate (OLR) is usually expressed per mass of COD or VS (for example 7.5g VS/l/d or 6.5g COD/l/d). In most cases, COD test are done for water samples with low TS content and on the other hand, VS is used to measure the organic content of sludge with higher TS (Sundin, 2008). According to Poulsen; 2003, changes in the amount of volatile solids of the input to the output of the digester, does not give information about the stage of the biological process of anaerobic digestion. However, the analysis of this change can indicate process imbalance. A failing process will be indicated by a drop in the VS reduction from inlet to outlet but can only be seen if the process is strongly out of balance (Poulsen, 2003). Moreover, measuring the soluble COD amount can give information about the degree of hydrolysis and solubilisation achieved by acidogenic bacteria (Sundin, 2008).

COD measurements during hydrolysis and acidification will decrease with increasing hydraulic retention time. This means that the COD concentrations will be higher at a shorter retention time than a longer retention time or the COD removal efficiency will be low during hydrolysis/fermentation. This is because, organic substances (VFAs and alcohols) remain in the process; and methane production which results from the decomposition of these organic substances had not occurred (Sundin, 2008). COD concentration measured in an experiment during hydrolysis and acidification of solid potato waste initially increased and then stabilized before declining during the process (Sundin, 2008). In the same literature, it was observed that the COD removal by a thermophilic hydrolysis/acidification reactor was greater than in a mesophilic hydrolysis/acidification reactor. The better performance of the thermophilic hydrolysis/acidification reactor was attributable to the fact that thermophilic microorganisms have higher growth rates than the mesophilic ones (Sundin, 2008).

4.0 Materials and Methods

A representative sample of feedstock utilized at Sobacken facility is used during this study and it is a combination and mixture of the following ten different waste components.

Table.1. Composition of substrate by waste type and proportion

	Type or source of waste	Volume (l)	Concentration (%)
1	Fish	0.88	4.9
2	Slaughterhouse waste	1.09	6.1
3	Cleaning waste	0.00	0.0
4	Doggy meat	0.37	2.1
5	Doggy sludge	0.28	1.6
6	Doggy dry food	0.42	2.3
7	Mink (fur)	0.07	0.4
8	Norway slurry	0.70	3.9
9	SOR2 (Industrial waste)	4.38	24.3
10	Municipal solid waste (MSW-Borås)	9.82	54.5
	Total	18.0	100

4.1 Batch Experiments.

A homogeneous mixture of the above portions of these waste types was prepared and used, in two different batch experiment series. In the first batch, two samples, each 200ml of the substrate, were prepared in a 500ml volumetric flask. One of the samples contained even mesophilic sludge with additional 5% volume (10ml) of the substrate. The sludge was taken from Gässlösa municipal waste water treatment plant in Borås city.

In the second batch, 147.2 g of the substrate with 8.9% VS was mixed with 7.98g of special shredded paper containing 82% VS in a 500ml volumetric flask to increase the C/N ratio of the substrate. The volume of each sample was then adjusted to 200ml level with Milli-Q water. Each batch of experiment was duplicated for statistical reasons.

The flasks were corked with cotton plugs and then placed in two separate water bath shakers (OLS 200 Grant), operating at mesophilic temperature conditions, one at 34⁰C and the other at higher temperature of 42⁰C. In both shakers the rotation was kept at 120 rpm to provide better

contact between the substrate and the microorganisms. The aerated condition during this pre-hydrolysis provides that a mix culture of microorganisms presented in the samples would work effectively.

Both experimental series were followed up by taking samples of 5ml from each flask regularly (at the start point and then after 4days, 5days, 6days, and 7days), from which 1ml experimental unit was used for the analysis of the process parameters.

4.2 Analytical methods

Total solid content (TS) was measured by weighing the samples before and after oven-drying at 105⁰C for 24 hours to remove the moisture in the substrate. After oven-drying, the samples were allowed to cool at room temperature in a desiccator prior to weighing. The calculation was done by dividing the dry weight of the sample by the wet or total weight as shown in equation 6.

$$TS (\%) = \frac{\text{Weight after drying } 105 \text{ } ^\circ\text{C}}{\text{Wet weight } 25^\circ\text{C}} \times 100 \dots\dots\dots(6)$$

The volatile solids content (VS) expressed in either g/l, g/m³ or in % of TS, is also known as loss on ignition (LOI). It is the content of organic matter in the waste and is measured by keeping the oven-dried samples at the temperature of 550⁰C± 25 until a stable weight can be obtained. It was measured by weighing the oven-dried sample before and after ignition at 550⁰C ± 25 for 2½ hours in an electric furnace. After this period, all of the organic matter in the waste sample is ignited and the weight of the crucible with the mineralized residue (GR) remains constant. In the same way as with TS measurements, a desiccator was used to cool the samples to reach room temperature prior to weighing. The value was derived by using the mathematical expression described by equation 7.

$$VS = \frac{\text{TS weight} - \text{weight after heating at } 550 \text{ } ^\circ\text{C}}{\text{Wetweight}} \times 100 \dots\dots\dots(7)$$

In continuation of the operation, VFAs concentrations, pH, soluble COD and ammonium nitrogen (NH₄-N) were measured regularly to monitor the progress of the hydrolysis. NH₄-N was measured by HACH LANGE DR-2800 instrument after diluting the samples with milli-Q water at a dilution ratio of 1: 10. VFAs were monitored with a High Performance Liquid Chromatography (Varian 9000 HPLC, Walnut Creek, CA, USA), using a Bio-Rad column 125-0115 (Biorad, Hercules, CA, USA). All samples except those for the analysis of pH were centrifuged at 10,000 rpm for 5 minutes and stored at 5⁰C until further analysis. Those for VFAs were further filtered through a 0.2µm filter before the analysis by HPLC. COD was measured using commercial reagents, COD reactor and COD analyzer (DR-2000; HACH Co.). The samples were diluted with milli-Q water at a dilution ratio of 1:5 in order to be within a measurable range of the reagent (0mg/l-15,000mg/l). The pH was measured using a pH meter (Thermo Russell).

5.0 Results.

Table .2. Summary of batch experiments and experimental conditions.

Conditions	Substrate							
	Normal C/N ratio				C/N ratio increased			
Temperature	34°C		42°C		34°C		42°C	
Addition of mesophilic sludge (+)								
No Mesophilic sludge (-)	+	-	+	-	+	-	+	-

The two batches of experiments performed under two different mesophilic temperature conditions of 34⁰C and 42⁰C and with two different substrates, one with an original C/N ratio , as it is found in the mixture and the other with an increased C/N ratio. Both series were performed with and without the addition of mesophilic sludge to the samples as it is summarized in Table 2. The results are presented in the figures and tables that follow. The experiments were performed to be able to investigate the differences and similarities caused by the effects of temperature conditions, mesophilic sludge addition, treatment time and change in C/N ratio, on the process of hydrolysis. As it was mentioned earlier, the concentration of dissolved COD, NH₄-N and VFAs were measured and used as process

indicators to evaluate the suitability of the operational conditions. Detailed data from the performed batch experiments are shown in appendix III to X at the end of the report.

5.1. Effect of Temperature

Figure 4 and Figure 5 present the concentration of various process indicators, measured during the hydrolysis of original substrate mixture at two different temperatures of 34 °C and 42 °C, respectively.

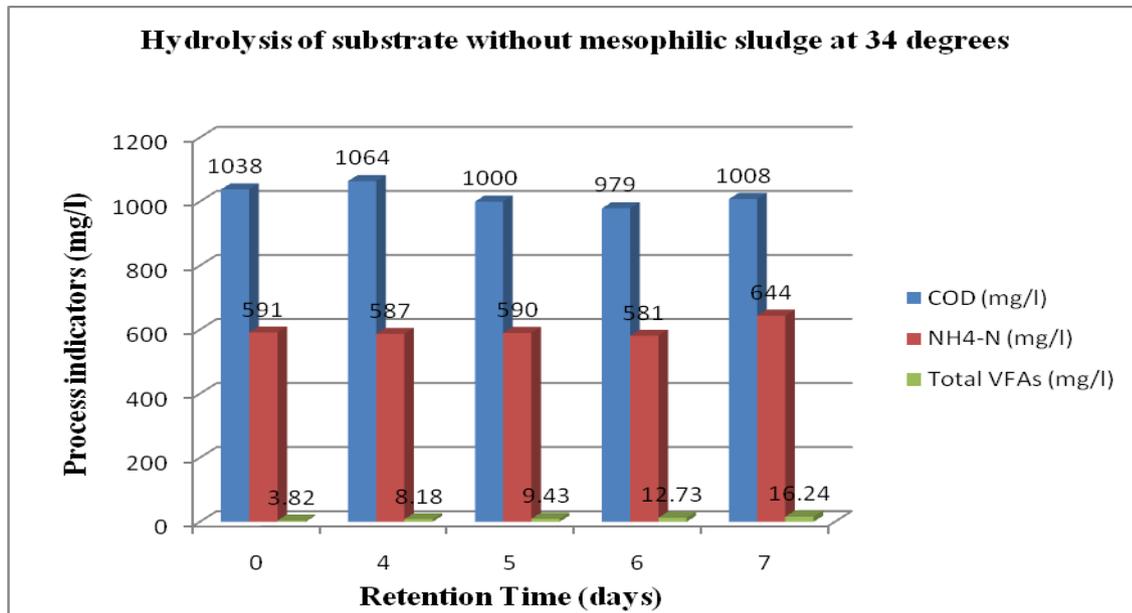


Figure 4: Hydrolysis of the original substrate at 34°C without addition of mesophilic sludge.

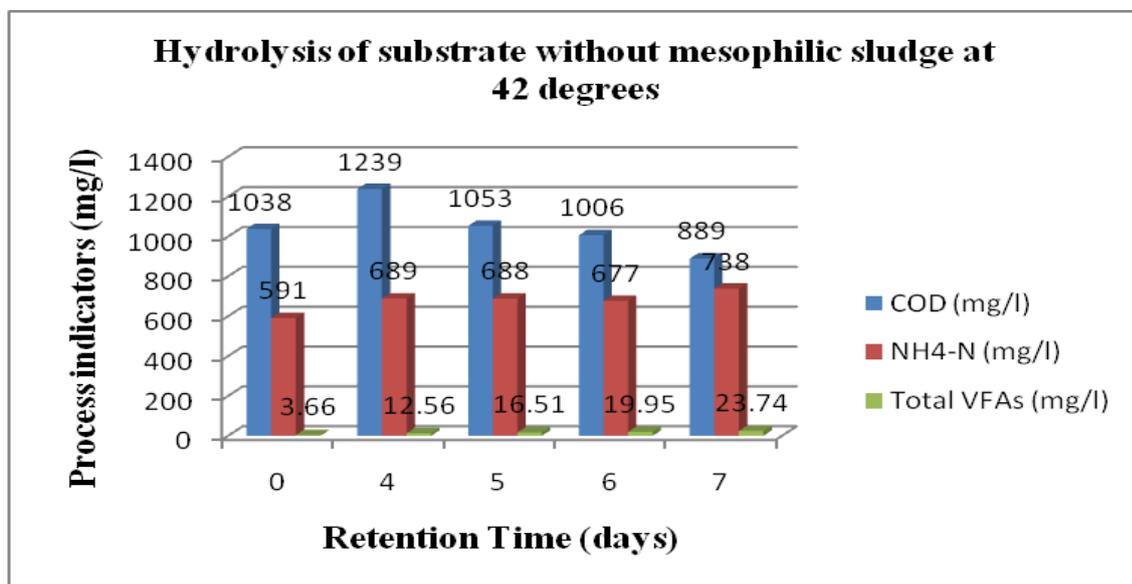


Figure 5: Hydrolysis of the original substrate at 42°C without addition of mesophilic sludge.

From both figures, it is observed that the concentration of soluble chemical oxygen demand (sCOD) first increases then generally reduces as the time for the substrate hydrolysis increases. This observation is supported by the works of (Sundin, 2008). This increase in COD removal, indicating the progress of feedstock degradation, also shown in an increased concentration of the total volatile fatty acids as the hydrolysis and degradation of the substrate is going on along the time from day zero through day 7 (Sundin, 2008). In the same way, the concentration of NH₄-N is increasing indicating the degradation of proteins present in the substrate. The degradation product of proteins can either exist as free ammonia- NH₃ or ammonium ion- NH₄⁺ depending on the pH. The insignificant change in the concentration of ammonium ions is also reflected by the minimal changes (4.0-4.5) in the pH level of the entire process as it is shown in Figure 6.

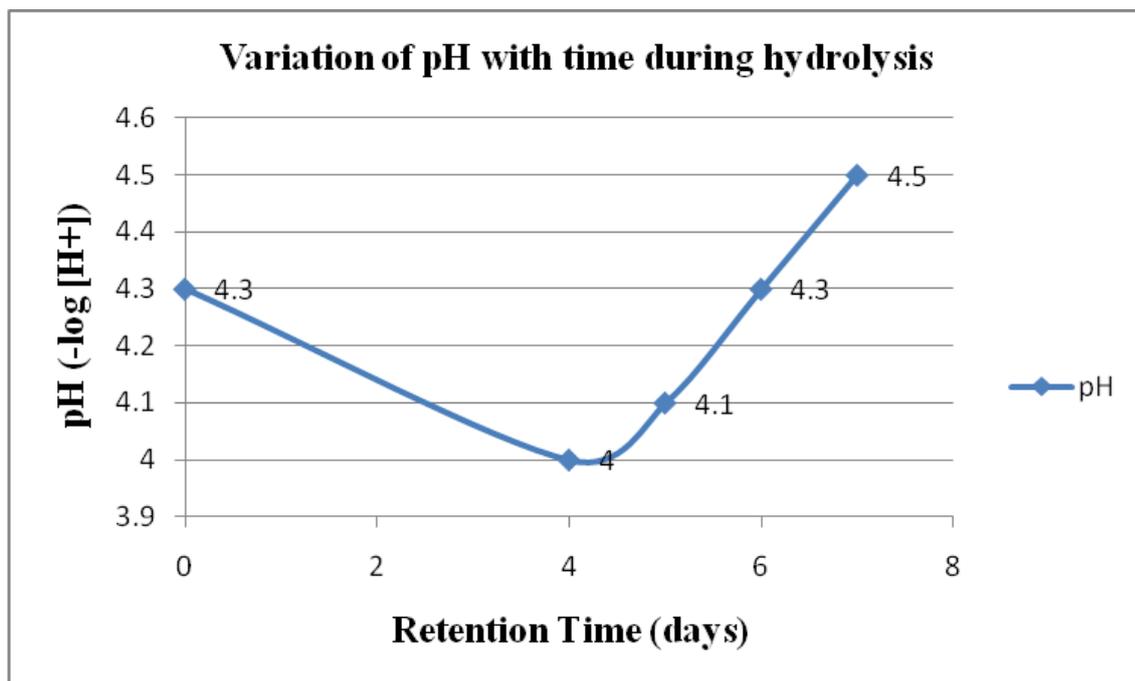


Figure 6: Sample graph of pH variation during the hydrolysis of normal substrate at 34°C with mesophilic sludge

However, the effect of temperature can be seen as the concentrations of the degradation products, *i.e.* COD, VFA and NH₄-N, are higher when the substrate was hydrolysed at higher temperature, *i.e.* at 42°C (Figure 4 vs Figure 5).

5.2. Effect of Mesophilic sludge

Sludge obtained from a waste water treatment plant was added to the substrate as inoculum to enhance the degradation process. The effect of the inoculum is shown by comparing Figure 7 (substrate hydrolysis at 34°C without the addition of mesophilic sludge) and Figure 8 (substrate hydrolysis at 34°C but with the addition of mesophilic sludge).

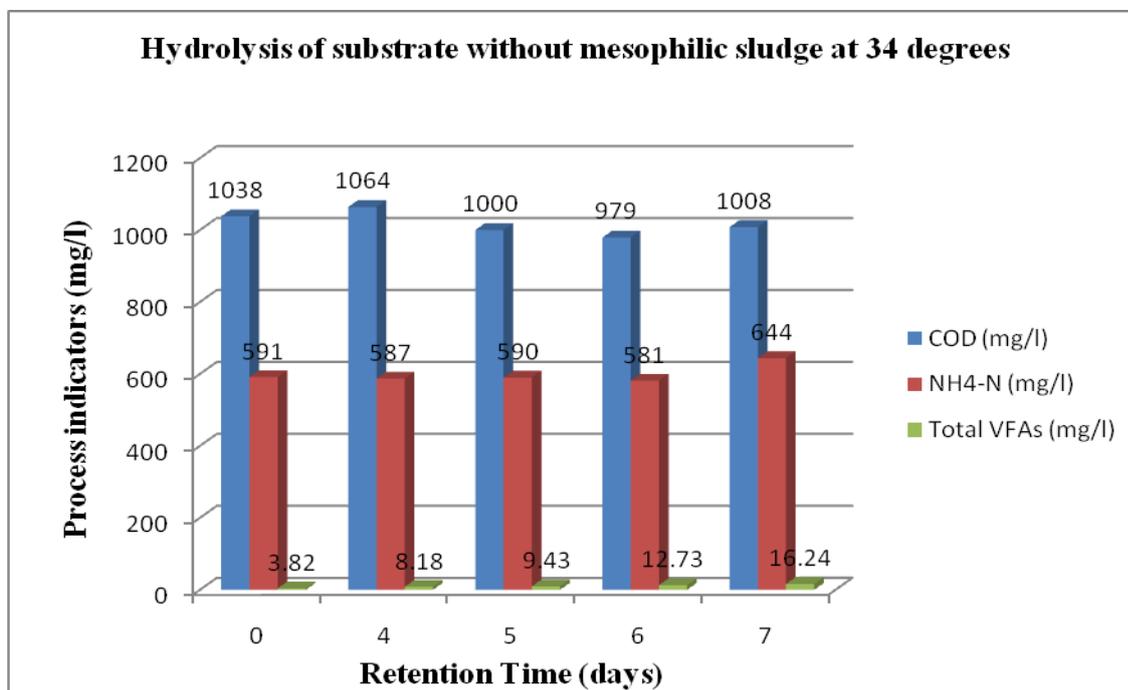


Figure7: Substrate hydrolysis at 34°C without the addition of mesophilic sludge.

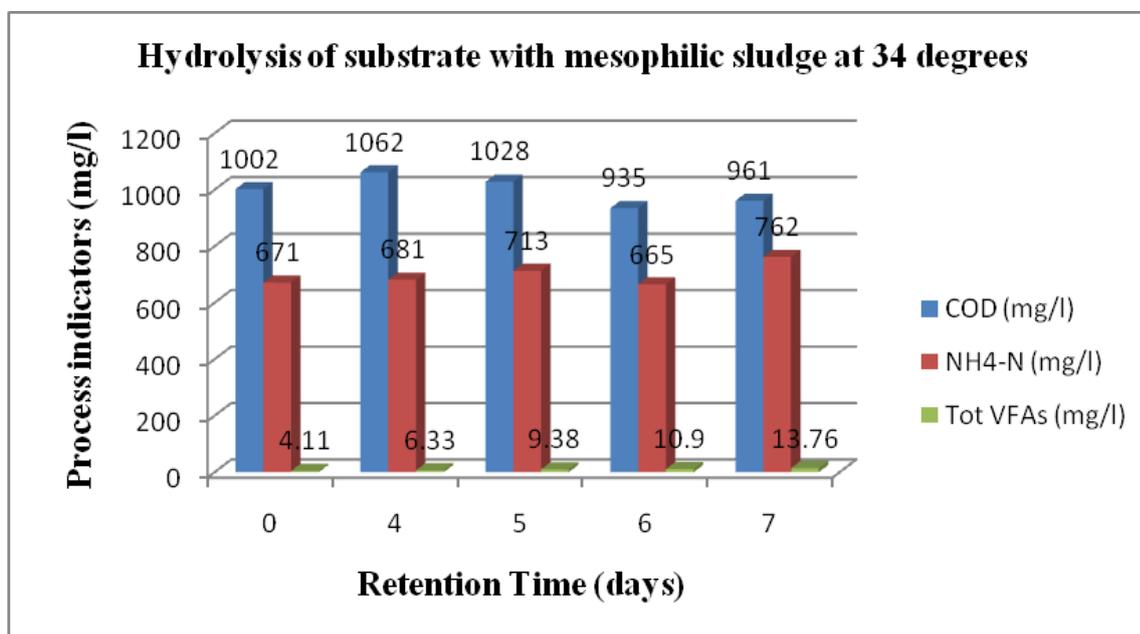


Figure 8: Substrate hydrolysis at 34°C with the addition of mesophilic sludge.

By comparing Figure 7 and Figure 8, it can be found that the rate of COD degradation from day 5 to and through day 7, which indicates the extent of degradation of solubilized COD, is faster when mesophilic sludge as inoculums was present in the samples (Figure 8). However, there was a less and insignificant difference in the amount of total volatile fatty acids as well as $\text{NH}_4\text{-N}$ produced when the operations were carried out with mesophilic sludge compared with that without mesophilic sludge addition. The effect of the addition of mesophilic sludge in terms of the amount of volatile acid produced was only observed when the substrate C/N ratio was increased as it is shown on Figure 9 compared with Figure 10 below.

5.3. Effect of increasing the C/N ratio of the substrate

The C/N ratio of the substrate was increased while keeping the VS content the same by adding special shredded paper material which is rich in carbon content. By so doing, we provided micro organisms with more optimal conditions supporting their action of hydrolysis on the substrate. This increase in the C/N ratio limits the likely hood of ammonia production which would have negative consequences of inhibiting the following anaerobic digestion process. A clear illustration of this effect is observed by comparing Figure 9 (concentrations of volatile fatty acids measured during the pre-hydrolysis of the original substrate mixture) and Figure 10 (concentrations of volatile fatty acids measured during the pre-hydrolysis of the original substrate with increased C/N ratio). From Figure 9, it can be concluded that, an imbalance was observed in the composition of substrate, and this low C/N ratio was not a suitable environment resulting in higher amounts of VFAs during the pre-hydrolysis process. Increasing the temperature resulted in a further increase of the concentration of VFAs.

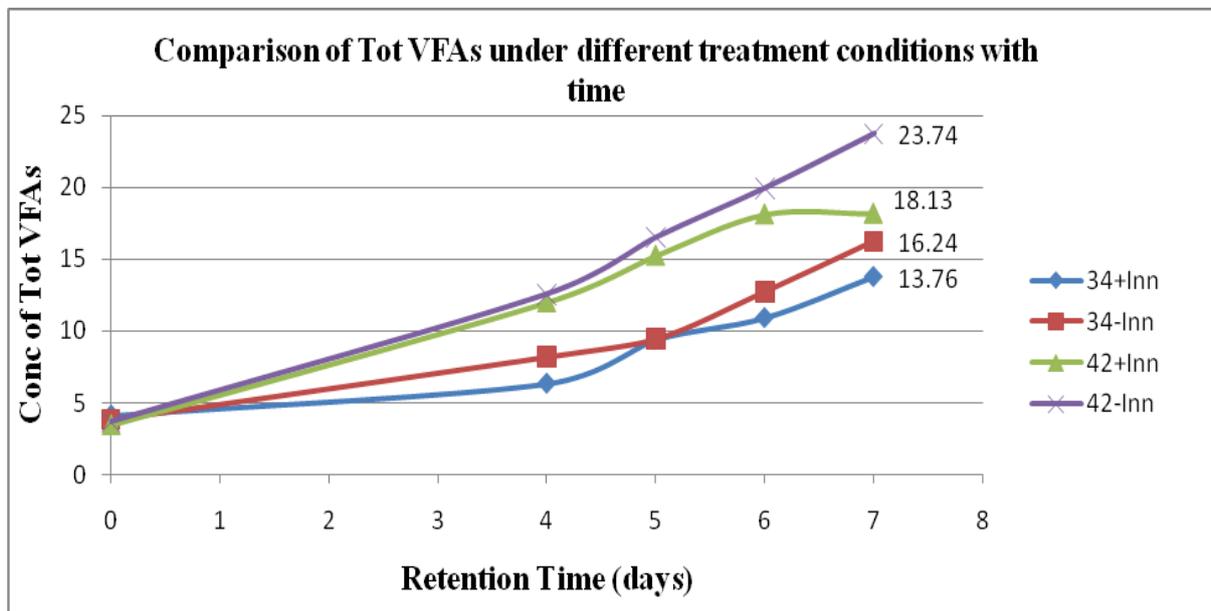


Figure 9: Concentrations of volatile fatty acids measured during the prehydrolysis of the original substrate mixture (i.e. lower C/N ratio), compared under different treatment conditions of different temperatures (34 and 42°C) and with (+Inn) or without (-Inn) addition of mesophilic sludge.

By looking at the trends of the various process indicators during anaerobic digestion, one would suggest that there exist a correlation between COD removal and the amount of the total volatile fatty acids produced. As the degradation of the substrate increases, the amount of COD reduces (Ueno Y. et al; 2007). Simultaneously, the amount of total fatty acids produced increases. This correlation can be very well seen on Figure 11 when the addition of paper provided an increased C/N ratio optimizing microbial conditions.

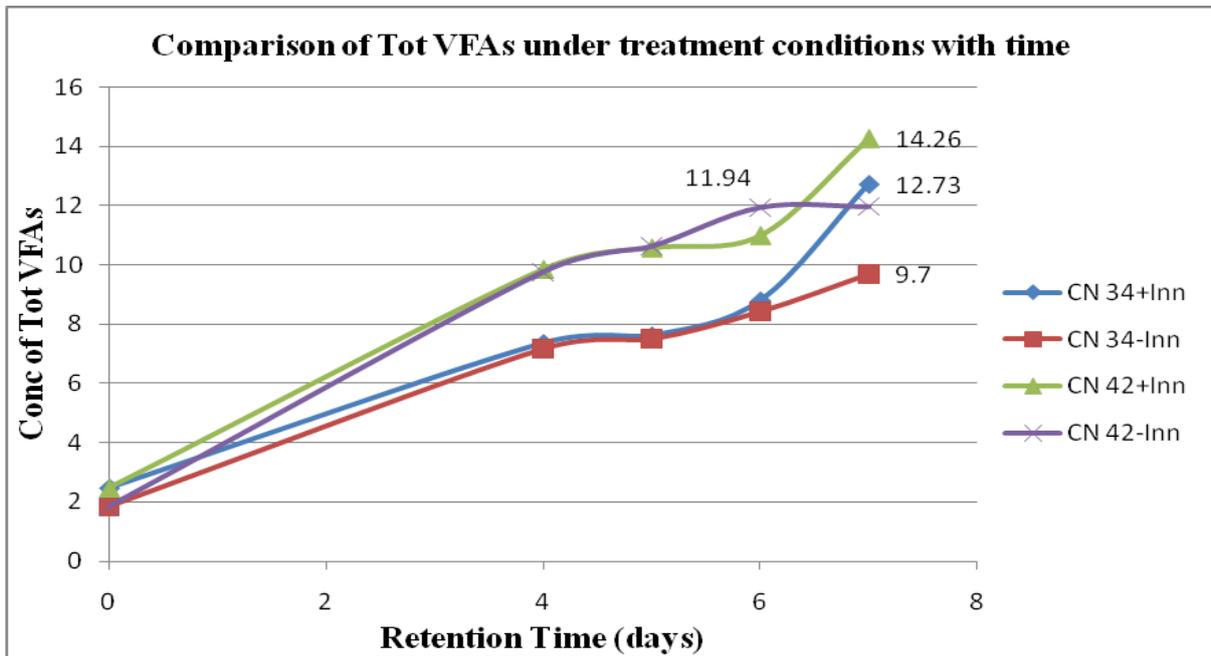


Figure 10: Concentrations of volatile fatty acids measured during the prehydrolysis of substrate with an increased C/N ratio, compared under different treatment conditions of different temperatures (34°C and 42°C) and with (+Inn) or without (-Inn) addition of mesophilic sludge.

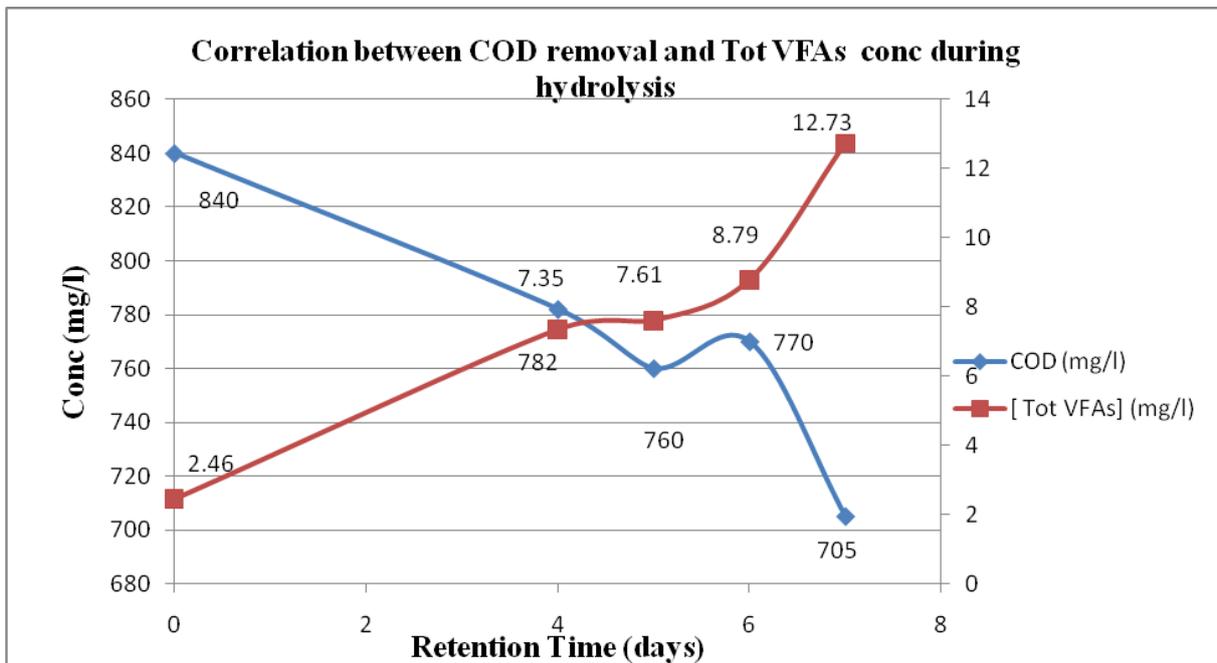


Figure 11: Correlation between COD removal and amount of total volatile fatty acids produced during prehydrolysis of substrate with increased C/N ratio at 34°C with addition of mesophilic sludge.

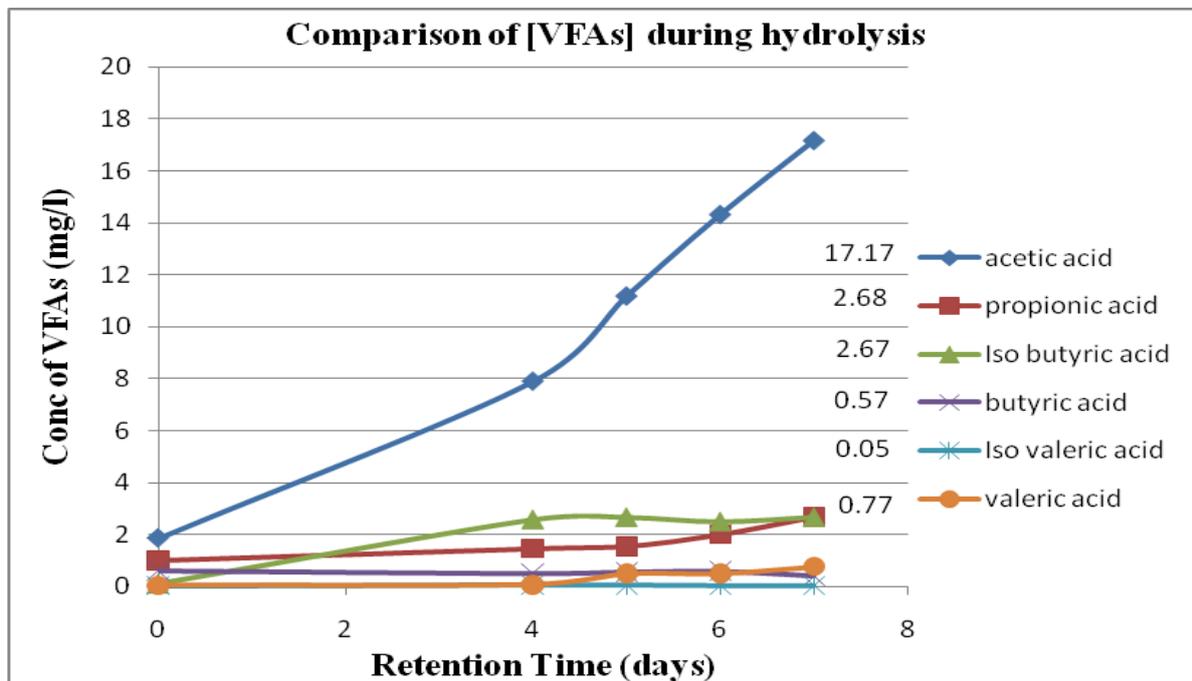


Figure 12: Proportions of various volatile fatty acids produced during the hydrolysis of substrate with low C/N ratio and without mesophilic sludge at 42°C

Figure 12 gives example on how the concentration of different volatile fatty acids vary during the prehydrolysis process. It is noticed that the production of various volatile fatty acids during the pre-hydrolysis of the substrate is different. Acetic acid; precursor of methane production and the less toxic of all is the most prominent one. While the concentrations of propionic acid, iso- butyric acid and valeric acid were lower, the least concentration was observed in iso-valeric acid.

6.0 Discussion

The successes of AD in terms of effective degradation of substrate, system stability and both quality and quantity of biogas produced during the process, strongly depend on hydrolysis/fermentation phase and its intermediary products. This study focused on evaluating the process parameters during a prehydrolysis step by hydrolyzing two substrates with different C/N ratios under different conditions.

When the substrate was hydrolysed at a temperature of 34°C and 42°C without the addition of mesophilic sludge, it was noticed that the solubilization was faster at 42°C than at 34°C. The dissolved COD removal was about four times more at 42°C; with only 3.9% COD removal at 34°C compared to 14.4% at 42°C. This indicates that the activity of different enzymes produced by various microorganisms involved in the degradation process was higher at 42°C.

At this temperature and after day 7 of the hydrolysis, the concentration of total volatile fatty acids was 23.74mg/l compared to 16.24mg/l at 34°C, among them with acetic acid being the most predominant (17.7mg/l) one. The same temperature condition favored the digestion that was carried out after the addition of mesophilic sludge as inoculum to the substrate. Though most literature suggest 35°C - 37°C to be the most suitable temperature for mesophilic digestion (Yadvika et al., 2004), 42°C showed to improve the prehydrolysis rate in this study. Gijzen et al., (1987) suggest that an important drawback of the degradation of solid organic waste is its slow rate and incomplete degradation. To combat this drawback, rumen microorganisms were accustomed to the digestion medium to increase and improve the conversion of the substrate. In the same light and in this study, the effect of adding mesophilic sludge as inoculum to improve the degradation process of the substrate was determined in terms of COD removal and in the amount of total volatile fatty acids produced. Operations at 34°C with mesophilic sludge had a COD removal of 4.1% with 13.76mg/l of total volatile fatty acid compared to 3.9% COD removal with 16.24mg/l of total volatile fatty acids after day 7 for operations at the same temperature but without mesophilic sludge. The insignificant difference in the COD removal and the amount of total volatile fatty acids produced can make us to conclude that the effect of mesophilic sludge was not significant. This means that the nature of the microorganisms present in the sludge, obtained from a waste water treatment plant was not suitable to improve the degradation of the original substrate mixture used in the study. This also means that the degradation observed was performed by the act of microorganisms basically presented in the waste mixture.

On the other hand, the substrate mixture was a protein-rich mixture with high nitrogen content, which could cause energy deficient in the form of carbon for the microorganisms. However, when the C/N ratio was increased by adding paper material, the carbon content of the substrate was increased thereby increasing the energy supply to the microorganisms. Moreover, treating this substrate mixture under the same conditions of temperatures at 34°C and 42°C, with and without the addition of mesophilic sludge, the results now showed a pronounced effect of mesophilic sludge addition, so that the degradation of substrate increased. There was 16% COD removal when the substrate was treated at 34°C with mesophilic sludge compared to 10% reduction during the hydrolysis of samples without mesophilic sludge. At 42°C, the substrate with mesophilic sludge had 10.2% COD reduction compared to 7.8% reduction observed with the substrate without mesophilic sludge. The COD removal increased simultaneously with an increase in the amount of produced total volatile

fatty acids, thereby satisfying the correlation between COD removal and the amount of total volatile fatty acids produced.

7.0 Conclusions

From this work, we conclude that solubilization of the substrate (with increased C/N ratio) occurred at 42°C. A predominant quantity of acetic acid (17.7mg/l), which will further be degraded through methanogenesis to methane, was produced. At this temperature, the hydrolysis was faster and there was a relative high degree of COD release together with a removal of 14.4%.

The effect of mesophilic sludge (microbial action) observed by the increase in the production of VFAs was only noticed when the C/N ratio of the original substrate was increased. Whilst a higher value of VFAs was observed at 42°C both in the case of without or with mesophilic sludge addition. Increasing the C/N ratio resulted in lower VFAs at all conditions. The optimum time for the hydrolysis was 4 days and this suggest that a longer retention time for the substrate will cause some of the carbon to be lost as carbon dioxide during the aerobic degradation. At this optimum retention time, the proportion of propionic acid to acetic acid was least, indicating that there was less acid accumulation which otherwise would affect the methane production process negatively.

There was no significant effect on substrate solubilization after the addition of mesophilic sludge.

Though our analysis did not investigate the importance of aerating the substrate during pre-hydrolysis, recent literature suggest that aeration of substrate during hydrolysis will greatly enhance the solubilization of the substrate and also create a mix culture of microorganisms which favors the solubilization.

8.0 Future work

On the basis of the results of this work, we recommend that the anaerobic digestion process at Sobacken would preferably be run in a two phase digestion scheme. In phase I, hydrolysis and fermentation operate in one tank and in phase II, acetogenesis and methanogenesis operating in another tank. See figure 2.

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Appendix

Appendix I. Calculations of substrate's TS and VS

The percentage of TS and VS in the substrate of the first batch of experiment was calculated using equation 5 and 6 respectively as follows:-

The weight of three samples A, B and C was recorded using 24 hours oven dried crucibles. These samples weighed; 37.2715, 37.3738 and 37.13 respectively. This weight was then averaged as shown below.

$$\begin{aligned} \text{Average weight} &= \frac{37.2715 + 37.3738 + 37.12}{3} \\ &= 37.2551 \end{aligned}$$

$$\begin{aligned} \text{Average weight after} &= \frac{3.0526 + 3.0413 + 3.0639}{3} \\ \text{drying at } 105\text{ }^{\circ}\text{C} &= 3.0526 \end{aligned}$$

$$\% \text{ TS} = \frac{3.0526}{37.2551} \times 100$$

$$\text{TS} = \underline{8.195\%}$$

$$\begin{aligned} \text{Average weight after} &= \frac{0.8598 + 0.8734 + 0.8612}{3} \\ \text{ignition at } 550\text{ }^{\circ}\text{C} &= 0.8648 \end{aligned}$$

$$\% \text{ GF}^* = \frac{3.5523 - 0.8648}{3.5523} \times 100$$

$$\% \text{ GF}^* = 75.650$$

$$\text{VS (\% of TS)} = 100 - \text{GR}, \text{ or } \text{VS} = \text{LoI (GF}^*) \times \% \text{TS}$$

$$\text{VS} = 0.7565 \times 8.195$$

$$\text{VS} = 6.1995\%$$

$$\underline{\underline{\% \text{ VS} \simeq 6.2}}$$

Appendix II: Calculation of shredded paper VS

During the second batch of experiments, the carbon-nitrogen (C/N) ratio of the substrate was increased by adding a special paper material

$$\begin{aligned} \text{Average weight of wet Slurry} &= \frac{39.4515 + 39.0882 + 44.3619}{3} \\ &= 31.1090\text{g} \end{aligned} \quad \begin{aligned} \text{Average weight after ignition at } 550\text{ }^\circ\text{C} &= \frac{0.3967 + 0.4307 + 0.5929}{3} \\ &= 0.4734\text{g} \end{aligned}$$

$$\begin{aligned} \text{Average weight after drying at } 105\text{ }^\circ\text{C} &= \frac{3.909 + 3.935 + 4.383}{3} \\ &= 4.0756 \end{aligned}$$

$$\% \text{ TS} = \frac{4.0756}{31.1090} \times 100$$

$$\text{TS} = 13.101\%$$

$$\text{Weight of 200ml of sample} = 220.62\text{g}$$

$$\begin{aligned} \text{Amount of VS in sample} &= 0.089\% \text{ is } 11.57 + 6.2 \\ \Rightarrow \text{Amount of VS in 220.62g of sample} &= \frac{0.089 \times 220.62}{100} = 8.885 \approx 0.089\% \\ &= 19.635\text{g} \end{aligned}$$

$$\begin{aligned} \text{Amount of paper added to the sample} &= 1/3 \text{ of sample weight} \\ &= 1/3 \times 19.635 \end{aligned}$$

$$\text{Amount of paper added to the sample} = 6.545\text{g}$$

$$\begin{aligned} \Rightarrow \text{Amount of sample (Gram VS) added} &= 19.635\text{g} - 6.545\text{g} \\ &\approx 13.1\text{g} \end{aligned}$$

$$\text{Calculating the amount of sample which contains 13.1g VS} \Rightarrow \frac{13.1\text{g}}{0.089} = 147.19\text{g}$$

Calculating the amount of paper added to the sample with respect to its VS content

But paper has 82% of VS content which corresponds to 6.545g

$$\begin{aligned} \Rightarrow \text{Amount of paper added to the sample to yield 82\% VS} &= \frac{6.545\text{g}}{0.82} \\ &= 7.98\text{g} \end{aligned}$$

$$\Rightarrow 7.98\text{g of paper should be added to the sample}$$

Therefore 200ml of substrate contained: 147g sample, 7.98g paper.

Batch one-experiments: Operation of samples with only substrate's carbon-nitrogen (C/N) ratio at 34°C and 42°C; with and without mesophilic sludge.

Appendix III: Operation at 34°C with mesophilic sludge

HRT (days)	pH	COD (mg/l)	NH ₄ -N(mg/l)	VOLATILE FATTY ACIDS (mg/l)						
				Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Total VFAs
0	4.3	1002	671	1,84	1,31	0,12	0,73	0,05	0,06	4,11
4	4.0	1062	681	4,31	0,80	0,72	0,46	0,02	0,02	6,33
5	4.1	1028	713	5,71	1,90	1,06	0,45	0,04	0,22	9,38
6	4.3	935	665	7,53	1,52	0,90	0,46	0,04	0,45	10,9
7	4.5	961	762	10,33	1,62	0,82	0,42	0,04	0,53	13,76

Appendix IV: Operation at 34°C without mesophilic sludge

HRT(days)	pH	COD (mg/l)	NH ₄ -N(mg/l)	VOLATILE FATTY ACIDS (mg/l)						
				Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Total VFAs
0	4.3	1038	591	1,87	0,98	0,11	0,80	0,03	0,03	3,82
4	4.0	1064	587	5,50	1,33	0,71	0,53	0,05	0,06	8,18
5	4.1	1000	590	6,71	1,10	0,87	0,56	0,06	0,13	9,43
6	4.3	979	581	9,13	1,65	0,97	0,51	0,05	0,42	12,73
7	4.3	1008	644	12,15	2,14	0,96	0,46	0,05	0,48	16,24

Appendix V: Operation at 42°C with mesophilic sludge

HRT(days)	pH	COD (mg/l)	NH4-N (mg/l)	VOLATILE FATTY ACIDS (mg/l)						
				Acetic	Prop ionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Total VFAs
0	4.7	1002	671	1,81	0,86	0,11	0,56	0,04	0,05	3,43
4	4.1	1099	725	7,15	1,33	2,81	0,51	0,04	0,16	12
5	4.2	1084	742	10,50	1,34	2,45	0,56	0,04	0,36	15,25
6	4.3	1085	754	13,11	1,62	2,35	0,60	0,04	0,41	18,13
7	4.5	1090	750	15,90	1,75	2,45	0,57	0,04	0,48	21,19

Appendix VI: Operation at 42°C without mesophilic sludge

HRT(days)	pH	COD (mg/l)	NH4-N (mg/l)	VOLATILE FATTY ACIDS (mg/l)						
				Acetic	Prop ionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Total VFAs
0	4.7	1038	591	1,86	1,01	0,10	0,62	0,03	0,04	3,66
4	4.1	1239	689	7,90	1,46	2,57	0,51	0,05	0,07	12,56
5	4.2	1053	688	11,18	1,55	2,66	0,57	0,05	0,50	16,51
6	4.3	1006	677	14,32	2,00	2,49	0,60	0,04	0,50	19,95
7	4.6	889	738	17,17	2,68	2,67	0,41	0,04	0,77	23,74

Batch two-experiments: Operation of samples with substrate's carbon-nitrogen (C/N) ratio increased (paper added), at 34°C and 42°C with and without mesophilic sludge.

Appendix VII: Operation at 34°C, substrate's C/N ratio increased with the addition of mesophilic sludge

HRT (days)	pH	COD (mg/l)	NH4-N (mg/l)	VOLATILE FATTY ACIDS						
				Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Total VFAs
0	4.9	840	479	1,21	0,67	0,10	0,40	0,04	0,04	2,46
4	4.3	782	627	5,40	1,10	0,22	0,43	0,06	0,14	7,35
5	4.4	760	586	5,80	1,06	0,13	0,33	0,03	0,26	7,61
6	4.4	770	600	6,07	1,48	0,56	0,41	0,04	0,23	8,79
7	4.6	705	597	10,57	1,17	0,22	0,37	0,03	0,37	12,73

Appendix VIII: Operation at 34°C, substrate's C/N ratio increased but without mesophilic sludge

HRT (days)	pH	COD (mg/l)	NH4-N(mg/l)	VOLATILE FATTY ACIDS						
				Acetic	Propionic	Iso-butyric	Butyric	Iso-valeric	Valeric	Total VFAs
0	4.9	802	423	0,69	0,55	0,08	0,47	0,02	0,02	1,83
4	4.2	897	604	5,25	1,06	0,35	0,39	0,05	0,07	7,17
5	4.3	730	596	5,31	1,32	0,30	0,34	0,06	0,17	7,5
6	4.4	838	592	6,20	1,40	0,33	0,22	0,04	0,24	8,43
7	4.6	801	603	7,42	1,34	0,34	0,24	0,04	0,32	9,7

Appendix IX: Operation at 42°C, substrate's C/N ratio increased with the addition of mesophilic sludge

HRT (days)	pH	COD (mg/l)	NH4-N (mg/l)	VOLATILE FATTY ACIDS						
				Acetic	Prop ionic	Iso-butyrlic	Butyrlic	Iso-valeric	Valeric	Total VFAs
0	4.9	841	479	1,21	0,68	0,10	0,40	0,04	0,04	2,47
4	4.3	809	560	7,95	1,16	0,27	0,27	0,03	0,17	9,85
5	4.4	769	580	8,86	1,01	0,20	0,29	0,02	0,19	10,57
6	4.4	769	586	8,92	1,16	0,28	0,36	0,03	0,24	10,99
7	4.4	755	841	12,10	1,36	0,34	0,28	0,023	0,16	14,26

Appendix X: Operation at 42°C, substrate's C/N ratio increased but without mesophilic sludge

HRT (days)	pH	COD (mg/l)	NH4-N (mg/l)	VOLATILE FATTY ACIDS						
				Acetic	Prop ionic	Iso-butyrlic	Butyrlic	Iso-valeric	Valeric	Total VFAs
0	4.9	802	423	0,69	0,55	0,08	0,47	0,02	0,02	1,83
4	4.2	898	600	7,81	1,11	0,26	0,38	0,02	0,18	9,76
5	4.3	899	606	8,75	1,09	0,22	0,33	0,04	0,20	10,63
6	4.4	868	584	9,80	1,23	0,35	0,31	0,04	0,21	11,94
7	4.5	828	719	5,50	1,41	0,40	0,30	0,03	0,23	7,87