INCREASED ANAEROBIC DIGESTION EFFICIENCY VIA THE USE OF THERMAL HYDROLYSIS

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree Of

Master of Science In
Environmental Science and Engineering

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June 30, 2010
Blacksburg, VA

Keywords: mesophilic, anaerobic digestion, aerobic digestion, solids removal, lipids, methane, gas production

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ABSTRACT

Waste sludge is frequently treated by anaerobic digestion to kill pathogens, generate methane gas and reduce odors so the sludge can be safely land applied. In an attempt to reduce sludge volumes and improve sludge dewatering properties, the use of thermal hydrolysis (TH), a sludge pretreatment method, has been adopted by numerous wastewater treatment plants, among them being the District of Columbia Water and Sewage Authority (DC WASA). The use of anaerobic digestion in collaboration with thermal hydrolysis has been shown to increase VS removal, COD removal and biogas production. The sludge generated also dewateres to a higher cake solids than from conventional anaerobic digestion. Unfortunately, DC WASA has found that the use of thermal hydrolysis had brought about two major issues. These are: (a) does thermal hydrolysis increase destruction of fats, oils and greases compared to conventional digestion? and (b) is the mixing method used at Virginia Tech (recirculating gas mixing) capable of stripping ammonia from the digester? Therefore the main purpose of this study is to evaluate these issues which occur with the use of the thermal hydrolysis process.

Experiments were conducted in two phases. The first phase was to assess the performance of anaerobic digesters via their biogas production with and without long chain fatty acid addition and with or without thermal hydrolysis. This research was further carried out in two stages. First
a mixture of unsaturated long chain fatty acids (hydrolyzed and unhydrolyzed) was used. The fatty acid mixture included oleic, linoleic and linolenic acids, which contain one, two and three double bonds, respectively. In the second stage, the effect of a single unsaturated fatty acid (hydrolyzed and unhydrolyzed) was analyzed. If extra gas is generated, grease addition to the digesters will be implemented. If thermal hydrolysis produces more gas, the greases will be added through the thermal hydrolysis unit rather than being added directly to the digester. The results showed that addition of long chain fatty acids greatly increased gas production and the long chain fatty acids that were thermally hydrolyzed generated more gas than the untreated long chain fatty acids, although the gain was not large.

The second phase of the study was carried out by alternating the type of recirculating gas mixing (partial and continuous) in the anaerobic bioreactor. To achieve this goal, short-term anaerobic bioreactor studies were conducted by varying the frequency of the gas. The result showed that continuous gas recirculation at the bottom of the digester was responsible for stripping ammonia from the system. It appeared that up to 500 mg/L of ammonia was being stripped from the digester operating at 20 day solids retention time. This suggests that ammonia can be stripped if a reduction of ammonia in the digester was desired.
ACKNOWLEDGEMENTS

I will like to earnestly acknowledge the priceless guidance and support that I received from my advisor, Dr. John T. Novak throughout my research duration and graduate study. I must admit that his enthusiasm towards this research interest certainly inspired me to succeed in various facets of my graduate academic career and has even allowed to me to grow professionally. Additionally, I am highly appreciative for the funding that he provided to me during my matriculation at Virginia Tech.

I would also like to thank my committee members, Dr. Gregory Boardman and Dr. Widdowson, for their inputs about the subject matter and constant guidance throughout this process. Acknowledgements should also be granted to Washington D.C Water and Sewer Authority (DCWASA) for providing financial support and funding for the aforementioned research.

Dr. Novak’s research group certainly provided invaluable assistance and positive feedback about the various aspects of my research, especially Jong Min Kim, Chris Wilson, Charan Tej Tanneru, Nirupa Maharajh, Loveenia Gulati and Anna Maria. Moreover, a very special thanks to Julie Pertruska and Jody Smiley for all the help and never ending willingness to provide advice and constructive criticism about the subject matter at hand. Last but certainly not least, I would like to thank God, my parents, girlfriend and siblings for being the driving force that I needed especially when all seem lost and for helping me believe in myself throughout my Master’s Degree matriculation.
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1 INTRODUCTION

Anaerobic processes have been widely used in wastewater treatment for over a century. Initially, these bioreactors, called anaerobic digesters, were simple concrete tanks or even lagoons in which the solids were housed in the form of a slurry, which used anaerobic microorganisms to achieve the anaerobic treatment objectives (McCarty et al., 1986). Such treatment usually used an amalgamation of physical, chemical and biological processes designed to move organic matter and solids (Kassam et al., 2003). However, due to the harmful effects of low dissolved oxygen (DO) concentrations on aquatic life, wastewater treatment engineers are mainly focused on removing pollutants that depletes the DO in receiving waters. Most oxygen demanding compounds are organic compounds, with ammonia nitrogen being a major inorganic source. However, during this wastewater treatment process various problems can occur, for instance, excess sludge production.

In an attempt to minimize such problems that may arise, the use of thermal hydrolysis (TH) has been adopted by numerous wastewater treatment plants, among them being the District of Columbia Water and Sewage Authority (DC WASA). Thermal hydrolysis utilizes a pretreatment step where sludge is processed under high temperature (150-170°C) and high pressure to hydrolyze solids making them more susceptible to anaerobic digestion and also killing pathogens. The use of anaerobic digestion in collaboration with thermal hydrolysis has shown to produce improved VS removal, COD removal and greater biogas production. Unfortunately, DC WASA has found that the use of thermal hydrolysis had brought about two major issues.
The first issue surrounds the production of gas via the use of thermally hydrolyzed fats versus unhydrolyzed fats. Past research from Wilson and Novak (2009) indicated that gas production from the thermally hydrolyzed sludge was much greater than expected based on the additional solids destruction alone. However, a high gas production could be the result of solubilization of fats in the sludge that otherwise would not be bioavailable in the digester (Wilson and Novak, 2009). Therefore, grease addition may be an especially viable procedure for a thermal hydrolysis system.

Fats, oils and greases are generally characterized as the lipid fraction in sludges. Lipids constitute important organic components of natural foods as well as a variety of synthetic compounds and emulsions found in wastewater sources (Chipasa and Medrzycka, 2006). In municipal wastewater, they amount for approximately 30-40% of the total chemical oxygen demand (Chipasa and Medrzycka, 2006). Not only are they one of the main components of wastewater, lipids are also found to be one of the most problematic ingredients. Lipids are known to cause operational problems in anaerobic digesters such as clogging, growth of filamentous bacteria and floc flotation. Floc flotation is a major concern since the flotation of biomass due to fat adhesion may cause lack of active biomass due to washout (Cammarota et al., 2001).

The aforementioned problems can severely limit the operating efficiency of digesters. Therefore a physio-chemical pre-treatment is usually adopted in an attempt to remove the lipid fraction before the anaerobic process (Cirne et al., 2007). Nonetheless, lipids are attractive substrates for both anaerobic digestion and co-digestion is sometimes used due to its ability to produce a higher
methane yield in comparison to proteins and carbohydrates. As a result, lipid-rich waste can be regarded as a huge potential renewable energy source (Hansen and Hnudsen, 1987).

A significant increase in the production of methane, from 25 to 50 m³ biogas/m³ when fish oil with a total concentration of 5% was added to a manure digester, was observed by (Angelidaki and Ahring, 1992). The operational cost of powering a wastewater plant usually can run into millions of dollars/year. Therefore, in an attempt to help reduce these costs in addition to the carbon footprint, lipids can be added to anaerobic digesters in order to enhance the production of methane which can be then used for energy production (Cavaleiro et al., 2008). Thus, this technology is a promising approach which should be further explored.

A second problem with anaerobic digesters using the thermal hydrolysis is the buildup of ammonia in the digester. Ammonia is known to be toxic to methanogenic organisms. The release of ammonia derived from the anaerobic degradation of wastewater sludge should be predictable due to the high solids loading and subsequent hydrolysis during anaerobic digestion (Wett et al., 2006). However, the ammonia concentration in units operated at Virginia Tech was not consistent with expected concentrations. Ammonia production in a 20 day SRT digester was found to be lower than a 15 day SRT digester, even though a 20 day SRT digester had slightly higher VS destruction. As a result, it was assumed that the ammonia was stripped via the mixing system.
Ammonia is a colorless gas with a characteristic pungent odor. It contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to food and fertilizers (Semmens et al., 1990). It is also known to stimulate bacterial growth at low concentrations. According to Grady et al (2006), during anaerobic processes, ammonia concentrations between 50 to 200 mg/L as N are generally within the stimulatory range. However, ammonia is quite often inhibitory at higher concentrations, and toxic if the concentration is high enough.

Ammonia may also be present in the influent wastewater, but it may be formed due to the breakdown of organic materials that contain nitrogen, that include proteins. Even at low concentrations, ammonia can be problematic to life in water environments due to the effect as a fertilizer, promoting eutrophication and is normally removed by denitrification in wastewater treatment plants (Norddahl et al., 2006). Most methods of ammonia stripping are mainly dependent on relatively large amount of energy for the operation. One such method is the use of membrane contactors which offer a superior solution for stripping ammonia because they provide a large surface area that facilitates fast separation of the ammonia from the wastewater even at low energy input per mole ammonia (Norddahl et al., 2006).
1.1 OBJECTIVES

The main purpose of this study is to evaluate the aforementioned issues which occur after the use of the thermal hydrolysis process. The performance of the anaerobic digesters was evaluated by volatile solids destruction and gas production with and without long chain fatty acid addition and with or without thermal hydrolysis. The fatty acids added were oleic, linoleic and linolenic acids, which consists of one, two and three double bonds, respectively. In the second stage, the effect of a single unsaturated fatty acid (oleic acid) with and without thermal hydrolysis was analyzed.

The second phase of the study was carried out by alternating the type of mixing (partial and continuous) in the anaerobic bioreactor. To achieve this goal, short-term anaerobic bioreactor studies were conducted by varying the frequency of the gas recirculation by the peristaltic pumps.
2 LITERATURE REVIEW

2.1 Anaerobic Treatment (Overview)

Anaerobic processes have been widely used in wastewater treatment for over a century. Initially, these bioreactors called anaerobic digesters were simple concrete tanks or even lagoons in which the solids were housed in the form of slurry, which used anaerobic microorganisms to achieve the anaerobic treatment objectives (McCarty et al., 1986). The application of biotechnology to environmental problems is increasingly receiving rigorous attention.

The use of anaerobic biodegradation to treat industrial wastewaters is a promising application of this technology. This is mainly due to the relatively low capital expenditures required in addition to the potential to create biogas which can be used as a source of energy (Masse et al., 2002). These anaerobic systems also generate less waste sludge than aerobic systems, can usually handle high organic loads, and unlike most other methods of treatment, can result in increased profits. (Kassam et al., 2003).

Anaerobic wastewater treatment systems are biological systems operating in the absence of oxygen. These systems are most suitable for the treatment of highly biodegradable and concentrated wastes (Grady et al., 1999). During the anaerobic treatment process, microorganisms break down organic molecules, and produce hydrogen, carbon dioxide and methane. Methane can comprise as much as 80% of the biogas, and the remainder consists primarily of carbon dioxide. Thus, methane, a valuable source of energy, is the primary biogas produced by anaerobic processed (Kassam et al., 2003).
2.2 Anaerobic Digestion

Anaerobic treatment by methanogenesis is often used for the stabilization of wastewater sludge and municipal solid wastes. The microbial communities that are often found in anaerobic digestion (AD) are primarily made up of prokaryotic organisms consisting of both bacteria and archaea (Grady et al., 1999). The AD of organic material basically follows four steps. These include, hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Figure 2.1 (Grady et al., 1999). AD is a complex process which requires strict anaerobic conditions (oxidation reduction potential (ORP) <=-200 mV) to proceed, and depends on the coordinated activity of a complex microbial association to transform organic material into mostly CO₂ and methane (CH₄) (Appels et al., 2008).

Despite the following steps, hydrolysis is commonly considered as rate limiting. Firstly, the hydrolysis step degrades both insoluble organic material and high molecular weight compounds which include lipids, polysaccharides, proteins and nucleic acids, into soluble organic substances e.g. amino acids and fatty acids (Fuentes et al., 2008). Afterwards, the components formed during hydrolysis are further split during acidogenesis, the second step (Appels et al., 2008).

Ammonia acids and sugars are degraded by fermentative reactions, where the organic compounds usually serve as both electron donors and acceptors (Grady et al., 1999). However, volatile fatty acids (VFA) are produced by acidogenic (or fermentative) bacteria along with ammonia (NH₃), carbon dioxide (CO₂), hydrogen sulfide (H₂S) and other by-products. Consequently, the product of the acidogenic reactions, acetic acid and hydrogen, are then used by the methanogens to produce methane gas (Grady et al., 1999).
The third stage in AD is that of acetogenesis. During this process, the higher organic acids and alcohols produced by acidogenesis are further digested by acetogens in order to produce mainly acetic acid, CO₂ and H₂. This conversion is controlled to a large extent by the partial pressure of H₂ in the mixture (Appels et al., 2008). Finally the last stage of the process of methanogenesis begins. This stage produces methane by two groups of methanogenic bacteria. The groups which are involved include aceticlastic methanogens, which splits acetate into methane and carbon dioxide. The second group of H₂-oxidizing methanogens uses hydrogen as electron donor and carbon dioxide as acceptor to produce methane (Grady et al., 1999)
Figure 2.1: Multistep Nature of Anaerobic Operations (Grady, C.P. Leslie, Daigger, Glen T., Lim, H.C., Biological Wastewater Treatment, Marcel Dekker, New York, 1999), Used under fairuse guidelines.
2.2.1 Affecting Parameters

In order for anaerobic reactors to perform at their best, they should be operated under steady state conditions. As a result, various parameters can often determine the performance of the digester. These include; pH, alkalinity, temperature, solids retention time (SRT), volatile fatty acids (VFA) and loading rates (organic and total hydraulic) (Hwang et al., 2004).

Temperature influences the growth rate and metabolism of micro-organisms and consequently the population criteria in the anaerobic reactor (Toki, 2008). During the operation of an anaerobic digester under steady-state conditions, the behaviors of various metabolic groups are in equilibrium, and as a result there is no accumulation of metabolic intermediate products in the reactor (Leitao et al., 2006). On the other hand, in periods where the system is vulnerable to a sudden temperature change, the conditions for the digestion procedure may become unstable due to varying responses of the metabolic groups of micro-organisms (Cha and Noike, 1997). This results in a buildup of volatile fatty acids and slows degradation.

It is believed that the average operating optimum temperature is within the range of (35-45°C) for mesophilic conditions and a higher temperature of (50-60°C) for thermophilic reactions (Bougrier et al., 2006). However, an increase in temperature can be beneficial to the overall process. These include increasing solubility of the organic compounds, superior biological and chemical reaction rates, and an increasing death rate of pathogens under thermophilic conditions (Cha and Noike, 1997). However, the application of high temperatures has found to have
negative effects. That is, there will be an increase of the fraction of free ammonia due to a decrease in pkₐ values, which plays an inhibitory role for the micro-organisms.

The control of pH in anaerobic treatment is also critical as the pH between 6.5-7.6 (McCarty, 2001). However, the reactor pH can be depressed by the production of organic acids as well as carbonic associated with the high concentrations of carbon dioxide gas (Leitao et al., 2006). He found that the production of gas increased by approximately 40% during slightly acidic and alkaline conditions. However, the concentration of CO₂ increased at higher pHs but its concentration decreased substantially at lower pH. The variation in the gaseous phase were the consequence of a shift in CO₂ solubility with pH (Leitao et al., 2006).

The aspects of solids retention time (SRT) has been found to be of utmost importance since it can affect the types of microorganisms that can grow in a bioreactor as well as their biological activity, thereby determining the effluent quality (Grady et al., 1996). It should also be noted that whatever SRT is selected should always exceed the minimum SRT that is associated with the microorganism related with a specific required biochemical transformation. The minimum SRT is described as the value below which a particular group of organisms is incapable of growing in a suspended growth reactor (Moen et al., 2003).

According to Cohen et al. (1982) during the homogenous anaerobic digestion processes, a balance exist between the first stage of acidogenesis of the substrate and conversion of the acid products, via methanogenic bacteria, to methane and carbon dioxide gas. This was found to be true since at high loading rates imbalances between acidogenesis and methanogenesis often lead
to the production of intermediate acidic products. The increase of volatile fatty acids (VFA) may also be described as a distinctive reactor response during periods of overloading and variations in hydraulic and organic rates (Cohen et al., 1982). Ultimately, during traumatic conditions, there may be a characteristic change in the metabolic pathway to a less complimentary route, thereby resulting in a fractional shift between VFA producers (acidogens and acetogens population) and consumers (methanogens, sulphate reducing bacteria—SRB, and nitrogen reducing bacteria—NRB). This change has also been found to change the overall characteristic of the biogas which is produced since it may have a higher carbon dioxide and hydrogen gas concentration. (Leitao et al., 2006).

### 2.2.2 Thermal Hydrolysis

Thermal hydrolysis is a process that uses heat and pressure as a substitute for biological hydrolysis. There is several advantages to this process. First, thermal hydrolysis can be accomplished in approximately 30 minutes as opposed to multiple days for biological hydrolysis. Second, pathogens are destroyed by this process. Third, and perhaps most important, thermal hydrolysis changes the physical nature of the sludge. Solids that have been thermally hydrolyzed are much less viscous so they can be easily mixed at concentrations exceeding 10% solids. This is in contrast to a practical limit of 7% for unhydrolyzed solids. This allows a more concentrated feed to take place, reducing the footprint of the digestion process.
The operation at DC WASA will concentrate combined primary and secondary solids to approximately 20% by centrifugation and then hydrolyze the solids. The resulting solids will be at 10-12% for feeding to the anaerobic digester. The drop in solids is due to condensation of steam which is used for increasing the temperature. These solids exist as a liquid as opposed to a viscous mixture. The final advantage of the thermal hydrolysis process is that the digested solids dewater readily to a cake solids of approximately 35-38%. Typical dewatered solids range from 18-25% for conventional digestion (Wilson and Novak, 2009).

2.3 Aerobic Digestion

Aerobic Digestion is a process using aerobic reactors to stabilize particular organic matter produced from primary clarification and biological treatment of wastewaters. The solids are mainly oxidized by the use of dissolved oxygen or even nitrate-N as a terminal acceptor (Grady et al., 1999). The residue that is produced is thus made up of relatively inert, humus like material which degrades relatively slowly (Zupancic and Ros, 2008).

Aerobic sludge stabilization is also found to be less costly for some municipal treatment plants compared to anaerobic stabilization and it is also produces more stabilized sludge (Matsuda et al., 1989). The process works by hydrolyzing biodegradable particulate organic matter, thereby making it soluble and converting it into ammonia, phosphate as well as biodegradable soluble organic matter (Hashimoto et al., 1982). The soluble bio-degradable organic matter is afterwards converted into CO₂ and H₂O and active biomass via heterotrophic bacteria. According to (Grady et al., 1999) the active biomass is further degraded, resulting in the production of more carbon
dioxide and water in addition to inactive biomass and nutrients via oxidation as described in figure 2.2. However, the non-biodegradable particulate organic matter in the influent is not affected by the digestion process.

![Figure 2.2: Schematic diagram of the events occurring during aerobic digestion](image)

Usually, in a well designed biochemical process, microbial growth is allowed to occur where the appropriate amount of oxygen can be supplied, thereby destroying the organic matter and allowing the treated wastewater to be discharged without having a negative effect on the environment. Generally, two cycles, namely the Carbon and Nitrogen are usually engaged so as
to deal with the various biological processes. However, in the aspect of domestic wastewater treatment process, the nitrogen cycle is of utmost importance.

Most of the nitrogen produce during the domestic treatment process is usually in the form of ammonia (NH₃) in addition to organic nitrogen. As the nitrogen cycle progresses, nitrogen is converted to NH₃ in a process called ammonification (Disse et al., 1995). NH₃ is further converted to nitrate in a process called nitrification. However, the way in which bacteria incorporate nitrogen into their cells during growth is through ammonia.

2.3.1 Affecting Parameters

The aerobic digestion of domestic wastewater is affected by numerous factors. These include SRT, temperature, pH, mixing, solid type and bioreactor configuration and oxygen uptake rate (Rajasimman and Karthikeyan, 2007). The aspects of solids retention time (SRT) has been found to be of utmost importance since it can affect the types of microorganisms that can grow in a bioreactor as well as their biological activity, thereby determining the effluent quality (Grady et al., 1996). It should therefore be noted that the lower the SRT, the higher is the amount of biodegradable organic matter wasted. Figure 2.3 illustrates the ranges of operating SRTs at which a variety of events will occur in aerobic/anoxic (Grady et al., 1996).
Another important factor that can affect the functioning of aerobic digestion is pH. Unless pH control is properly practiced, the pH will decrease during conventional aerobic digestion (CAD) if the release ammonia-N is nitrified (Alghusain and Hao, 1995). The destruction of biodegradable organic matter proceeds at low pHs, and is decreased at higher pHs. (Grady et al., 1995).

Mixing is yet another integral criterion towards the proper functioning of aerobic digesters to maintain solids in suspension. The settlement of solids will reduce the effective volume of the bioreactor, which can result in anaerobic conditions in the settled solids (Zupaneie et al., 2008). However, the provision of adequate mixing can be a challenge since a high concentration of suspended solids is usually maintained.
2.4 Ammonia

Ammonia-N is a compound of nitrogen and hydrogen with the formula NH₃. It is a colorless gas with a characteristic pungent odor. It contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to food and fertilizers (Yi et al., 2003). It is also known to stimulate bacterial growth at low concentrations. During anaerobic processes, an ammonia concentration between 50 to 200 mg/L as N is generally within the stimulatory range (Grady et al., 2006). However, ammonia is quite often inhibitory at higher concentrations, and toxic if the concentration is high enough.

Ammonia may also be present in the influent wastewater, but it may be formed due to the breakdown of organic materials that contain nitrogen, that include proteins. The production of ammonia by the breakdown of primary solids is illustrated in the following equation (Grady et al., 2006). Ammonia as a weak base that dissociates in water:

$$\text{NH}_3 + \text{H}_2\text{O} \leftrightarrow \text{NH}_4^+ + \text{OH}^-$$

At different concentrations, the both species of ammonia are known to be inhibitory. Free ammonia (NH₃) is usually more inhibitory and can cause a toxic response at concentrations of about 100 mg/L as N (McCarty, 20010). It should also be noted that the proportion of the total ammonia which is present as free ammonia increases with both pH and temperature. However, at higher pHs and temperatures, there is a decrease in ammonium ion but a subsequent increase in free ammonia.

Ammonia, even at low concentrations can be problematic to life in water environments due to the effect as a fertilizer, promoting eutrophication and is normally removed by denitrification in
wastewater treatment plants (Norddahl et al., 2006). There are many conventional ways to remove ammonia from water, however, most methods are dependent on relatively large amount of energy for the operation. One such method is the use of membrane contactors which offer a superior solution for stripping ammonia because they provide a large surface area that facilitates fast separation of the ammonia from the wastewater even at low energy input per mole ammonia (Norddahl et al., 2006).

Recent experiments have used using porous, hydrophobic hollow fiber and tubular membrane contactors for ammonia stripping from a variety of wastewater treatment effluents directly following anaerobic digestion. However, the aforementioned technology has only been reported in larger scale operation in a very few cases (Semmens et al., 1990).

2.5 Lipids

Lipids are characterized as oils, greases, fats and long-chain fatty acids and constitute important organic components of natural foods as well as a variety of synthetic compounds and emulsions found in wastewater sources (Chipasa and Medrzycka, 2006). In municipal wastewater, they amount for approximately 30-40% of the total chemical oxygen demand (Chipasa and Medrzycka, 2006). They are also known to be one of the major sources of organic matter that can be found in wastewater. Wastewater with a high concentration of lipids in the form of long chain fatty acids (LCFA) are interesting substrates for biogas production due to its high methane yield potential (Palatsi et al., 2010).
Similarly to the anaerobic process, lipids are firstly hydrolyzed to glycerol and long-chain fatty acids (LFCA). Such process has found to be catalyzed by extracellular lipases that are excreted by the acidogenesis process (Cirne et al., 2007). The LCFA are then converted into syntrophic acetogenic bacteria to hydrogen (H₂) and acetate and finally to methane (CH₄) by methanogenic archaea (Palatsi et al., 2010). The most common fatty acids in domestic wastewater and sludge are the saturated myristic, palmitic and stearic acid (C₁₈:0), and the unsaturated oleic (C₁₈:1) and linoleic acids (C₁₈:2) (Novak and Kraus, 1973). The structures of the fatty acids used in this study, which include linoleic acid (C₁₈:3) are shown in figure 2.4.
Oleic Acid

Chemical formula: $C_{18}H_{34}O_2$
fatty acid short code: C18:1
condensed formula: $H_3C-(CH_2)_7-CH=CH-(CH_2)_7-COOH$

Linoleic Acid

Chemical formula: $C_{18}H_{32}O_2$
fatty acid short code: C18:2
condensed formula: $H_3C-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-COOH$

Linolenic Acid

Chemical formula: $C_{18}H_{36}O_2$
fatty acid short code: C18:3
condensed formula: $H_3C(-CH_2-CH=CH)_3-(CH_2)_7-COOH$

Figure 2.4: Chemical structures of oleic, linoleic and linolenic acids
The degradation of LCFAs anaerobically is known to occur via the β-oxidation. However, according to (Novak and Carlson, 1970) the mechanism which results in the sequential removal of a 2-Carbon acetate groups, from a long chained carbon is shown in the figure 2.5.

Figure 2.5: Mechanism for the sequential removal of a 2-Carbon acetate groups, from the long chain carbon

According to (Novak and Carlson, 1970) the enzyme that is mainly responsible for the catalysis of the activation of fatty acids usually falls into three distinctive categories that is dependent on their lengths. It is believed that different activating enzymes are operative for long chains (14 to 20 carbons), medium chain (4 to 13 carbons) and short chains (2 to 3 carbons). Evidence has
also shown that the fatty acids chains are hydrogenated and then degraded also by the following β-oxidation pathway (Loehr and Roth, 1986).

\[
\text{Unsaturated Fatty Acid} \xrightarrow{\text{Hydrogenation}} \text{Saturated Acid} \xrightarrow{\beta-oxidation} \text{acetate}
\]

Many factors are known to influence the degradation of organic compounds. These include: molecular structure, solubility of compound in aqueous phase and various environmental factors (Denic and Weissman, 2007). However, the biodegradability of long chain fatty acids are also thought to increase with their decreasing carbon length as well as the increasing degree of unsaturation of carbon chains (Chipasa and Medrzycka, 2006). As described earlier, the degradation process of long chain fatty acids (C12 and above) is known to be identical.
2.6 Thermal Hydrolysis of Lipids

Lipids are known to be insoluble in water and therefore their biodegradability is found to be limited by their physicochemical properties. Hence they are also not susceptible to microbial uptake. However, according to (Krahe et al., 1996) the biological treatment of wastewater under thermophilic conditions (i.e. above 60°C) was found to be advantageous compared to mesophilic conditions. This is because both the diffusion coefficients and the solubility of lipids in aqueous media drastically increase with an increase in temperature (Chipasa and Medrzycka, 2006). As a result, the lipids become more soluble and therefore more accessible to microorganism and their lipolytic enzymes (Verkade et al., 1991).

According to Wilson and Novak (2009), VFA production due to lipid hydrolysis was observed to be reliant on both the hydrolysis temperature as well as the degree of saturation of the fatty acid chain. However, the oxidative stress that is imposed by the thermal hydrolysis process also increases the susceptibility of highly unsaturated fatty acids. According to (Wilson et al., 2008) both proteins and carbohydrates have distinctive gas production rates which are less than 1 m³/kg VSR, but the increase in the production of biogas in relation to VSR by thermal hydrolysis digestion, shows that there is better degradation of the liquid fraction of the feed sludge.

Data also exists that processes which receive entry inhibitory loads of LCFA usually surpass modern processes in relation to the production of methane, because the addition of the rapidly biodegradable substrate enhances the enzymatic rate for biological and acidogenesis (Kabouris et al., 2008). However, one major issue with this overall process is that, THP changes the chemical
makeup of the hydrocarbon chains which are associated with the lipid compounds. Lipids were found to be fragmented to form VFA, as well as other lower molecular weight aliphatic compounds by means of thermal hydrolysis (Wilson and Novak, 2009). Therefore, it can be concluded that since there becomes an overall increase in the bioavailability of lipid, an expected increase in biogas production is evident.
2.7 REFERENCES


Grady, C.P. Leslie, Daigger, Glen T., Lim, H.C., Biological Wastewater Treatment, Marcel Dekker, New York, 1999.


Abstract

Ammonia production in a 20 day SRT digester was found to be lower than a 15 day SRT digester, even though a 20 day SRT digester had slightly higher VS destruction. As a result, it was assumed that the ammonia was being stripped by the gas circulation mixing system. Consequently, the effects of partial and continuous mixing on ammonia stripping were investigated in Polyethylene batch fermentation conical shaped reactors at approximately 37°C. The mixing system was used to recirculate gas from the top to the bottom of the digester, thereby using an upflow gas mixing system.

For partial mixing conditions, the peristaltic pumps were turned off after an initial mixing period of one hour and remained off until next daily feeding cycle. Under continuous mixing conditions, the pump remained on for the 24 hour period. The digester was monitored as to make sure steady state conditions existed. Daily monitoring of ammonia, pH, chemical oxygen demand (COD) concentration, volatile solids (VS) % and total solids (TS) % took place once steady state was established.

Results provided evidence that ammonia was stripped during periods of continuous mixing. Therefore, the effects of mixing needs to be considered in interpreting data from the lab digestion system operated at Virginia Tech. In addition, it appears
3.1 Introduction

Ammonia-N is a compound of nitrogen and hydrogen with the formula NH₃. It is a colorless gas with a characteristic pungent odor. It contributes significantly to the nutritional needs of terrestrial organisms by serving as a precursor to food and fertilizers (Semmens et al., 1990). It is also known to stimulate bacterial growth at low concentrations. According to (Grady et al., 2006) during anaerobic processes, ammonia concentrations between 50 to 200 mg/L as N are generally within the stimulatory range. However, ammonia is quite often inhibitory at higher concentrations, and toxic if the concentration is high enough.

Ammonia may also be present in the influent wastewater, but it may be formed due to the breakdown of organic materials that contain nitrogen, including proteins. Ammonia as a weak base that dissociates in water forming alkalinity:

\[
\text{NH}_3 + \text{H}_2\text{O} \rightleftharpoons \text{NH}_4^+ + \text{OH}^-
\]

At different concentrations both species, NH₃ and NH₄⁺ known to be inhibitory. However, free ammonia (NH₃) is thought to be much more inhibitory and can cause a toxic response at concentrations of about 100 mg/L as N (Grady et al., 2006).

Ammonia, even at low concentrations can be problematic to life in water environments due to the effect as a fertilizer, promoting eutrophication. It can be removed from wastewater by denitrification in wastewater treatment plants (Norddahl et al., 2006). Most methods of ammonia stripping are dependent on relatively large amount of energy for the operation. One such method is the use of membrane contactors which offer a superior solution for stripping ammonia because
they provide a large surface area that facilitates fast separation of the ammonia from the wastewater even at low energy input per mole ammonia (Norddahl et al., 2006).

In studies of the thermal hydrolysis process for DC WASA at Virginia Tech, it was observed that ammonia levels in a 20 day SRT anaerobic digester treating thermally hydrolyzed sewage sludge were lower than levels in the 15 day SRT digester, even though a higher volatile solids reduction was occurring in the 20 day SRT digester. In an attempt to predict the ammonia concentration, DC WASA conducted modeling where they predicted that ammonia concentrations should have been much higher in the 20 day SRT digester. As a result, it was assumed that the ammonia was stripped via the mixing system. The mixing system used at Virginia Tech was to recirculate gas from the top to the bottom of the digester, thereby using an upflow gas mixing system. However, based on modeling results, it was thought that the recirculation of gas could be stripping ammonia from the liquid phase.

Therefore the aim of this study was to determine if and to what degree the gas mixing system could be stripping ammonia from the liquid phase. Since the anaerobic digesters were already thought to be inhibited by ammonia, the additional ammonia that was being stripped could be important in determining the rate of digestion and would impact the design of the digesters for DC WASA. The ammonia stripping might also provide a means by which the ammonia concentration in the digesters could be lowered, thereby reducing ammonia toxicity.
3.2 MATERIALS AND METHODS

3.2.1 Anaerobic Digester Set-up

A polyethylene fermentation reactor with a conically-shaped bottom was used as the reactor for the study. The reactor was supplied by Hobby beverage equipment Company (Temecula, California). The anaerobic digester was operated at 37°C in a temperature controlled room. Figure 3.1 depicts the schematic design of the study. The volume of the vessel was 30L and it was operated with a 20L nominal volume. The daily influent flow of 1L was sludge-feed for a period of one minute and effluent flow was also 1L in order to maintain a SRT of 20 days. The effluent sample was removed first and then feeding took place.

The digester was operated under different mixing conditions which are described below to provide for continuous mixing and alternatively limited mixing. The ammonia concentration was monitored to determine the effect of mixing on the liquid ammonia concentration in the digesters. In addition, the performance parameters, pH, COD, TS and VS were measured.
3.2.2 Sludge Samples

The reactor was initially seeded with biosolids from the Ringsend Wastewater Treatment Works (Dublin, Ireland). This plant uses thermal hydrolysis as a pretreatment step and has been in operation for several years. Dewatered raw sludge cake (at a ratio of 50% primary and 50% secondary solids) and with an approximate total solids concentration of 15-20 % was used as feed for the thermal hydrolysis unit. This sludge was collected from the District of Columbia, Water and Sewer Authority (DC WASA) Blue Plains wastewater treatment plant and subjected to thermal hydrolysis (Cambi process) by RDP Technologies, Inc. (Norristown, Pennsylvania). The overall solids concentration was decreased to 10-12% after thermal hydrolysis.
3.3 Methodology

The digester was housed in a constant temperature room at 37°C in order to maintain mesophillic conditions. A peristaltic pump (Cole Parmer-600 rpm) was used to recirculate the gas in the anaerobic digester from the head space at the top to the bottom of the digester. The pump was operated at 50% of its maximum speed. In order to maintain a constant 20 SRT, 1 L of the sludge was wasted and 1 L of the feed was sludge-fed to the digester each day for a period of one minute. For partial mixing conditions, the peristaltic pumps were operated for the first hour after feeding and then turned off until the next feeding cycle 23 hours later. Under continuous mixing conditions, the pump remained on for the 24 hour period to provide continuous mixing.

All the prior studies for DC WASA over the past 4 years were conducted using continuous mixing. Until the discrepancy between the modeling results and the measured results for ammonia was found, ammonia stripping was not considered to be a factor in the results. The digester was monitored as to make sure steady state conditions existed. Therefore, daily monitoring of ammonia, pH and chemical oxygen demand (COD) concentration, volatile solids (VS) and total solids (TS) according to Standard Methods (APHA, 1998) was conducted.

3.3.1 Statistical Data

In order to determine whether or not the recorded data was statistically different, a student’s T-test was performed. The t-test assesses whether the means of two groups are statistically different from each other. The statistics t-test allows us to determine a probability value (p-value) that indicates how likely we could have gotten these results by chance. Conventionally, if there is a
less than 5% chance i.e. \((p<0.05)\) of getting the observed differences by chance, we reject the null hypothesis and say we found a statistically significant difference between the two groups. If the data shows that there was a greater than 5% chance i.e. \((p>0.05)\) of getting observed differences, then we can assume that results are statistically similar. The \textit{R-Project for Statistical Computing} was used to carry out this test. One the other hand, the errors bars are expressed as the percentage of possible error amounts for each data point. They help indicate whether there are significant differences between two variables that are being compared and represent \((\pm)\) the standard deviation from the mean.

### 3.4 Results

Analysis was performed only after it was determined that the digesters were at steady state. When little variation occurred in ammonia concentration, pH, chemical demand (COD) concentration, volatile solids % and total solids, steady state was assumed. Thus, the performance of the anaerobic digester was analyzed at steady state in an attempt to produce the best results. The average values for pH, COD, TS, VS and the effects of mixing on ammonia concentration are shown in figures 3.2, 3.3, 3.4 and 3.5, respectively.
3.4.1 pH

The control of pH in anaerobic treatment is also critical as the desired pH is usually between the narrow ranges of 6.5-7.8 (Mc Carty, 2001). However, reactor pH can be depressed as the production of organic acids as well as carbonic acid associated with the high concentrations of carbon dioxide gas. Leitao et al., (2006) found via an investigation, that the production of gas increased by approximately 40% during slightly acidic and alkaline conditions. The concentration of CO₂ increased at higher pHs but its concentration decreased substantially at a higher pH. The variations in the gaseous phase were the consequence of a shift in CO₂ solubility with pH (Leitao et al., 2006).

The pH taken for the anaerobic digester in figure 3.2 shows a slightly lower pH during continuous mixing than during partial mixing. The pH was 7.80 ± 0.04 and 7.85± 0.03 (x ± μ) during continuous mixing and partial mixing respectively. It should also be noted that gas circulation in the anaerobic digesters helps in the maintenance of a constant pH without major changes. The main reason for the lower pH during continuous mixing was attributed to the stripping of ammonia into the gas collecting tipping meter. This can also be supported by the following equation:

\[
\text{Stripped} \\
\text{NH}_4^+ \rightarrow \text{NH}_3 + \text{H}^+ \\
\]

The release of the H⁺ ions during ammonia stripping is mainly responsible for the decreased pH.
3.4.2 Chemical Oxygen Demand (COD)

As described by (Grady et al., 1999), the production of methane gas usually decreases the COD demand of waste streams. It also provides the mechanism for stabilization of biodegradable organic matter that is present. However, it should also be noted that since there is a direct correlation between COD stabilization in anaerobic processes and methane production, methane production can be determined from the COD removed in the process.

As stated, COD data was also collected to monitor the steady state of the digester. Figure 3.3 shows that the average total COD concentration for the feed was 110,369 mg/L ± 4991 (x ± μ) whereas the average concentration of the effluent sample was 48619 mg/L ± 4393 (x ± μ). The average COD removal was approximately 56%, which was consistent with the results of (Tanneru, 2009)
3.4.3 Solids %

Solids analysis was also done to determine the efficiency of digesters since the hauling cost for disposing solids can be exceedingly high. Volatile solids percentages were measured as a standard method weight loss from the percentage of solids after the ignition at (550°C). The total solids percentages were measured at 150°C and performed throughout the operation of the digester. From figure 3.4, it can be seen that the average VS% of the feed sludge was roughly 6% for the feed samples during the overall operation of the digester, while effluent samples had an average VS% of 3%. As a result, there was an average volatile solids reduction (VSR) of approximately 50%.
Additionally, VS was determined throughout the overall operation of the digester and figure 3.5, shows that an average feed TS% of 7.5% for the feed samples and the effluent was 4.5%. As a result, there was an average volatile solids reduction (VSR) of approximately 40%.

Figure 3.4: Average VS% for feed and effluent samples
3.4.4 Effects of Mixing on Ammonia concentration

Ammonia, even at low concentrations can be toxic to life in water environments due to its effect as a fertilizer, promoting eutrophication and is normally removed by denitrification in wastewater treatment plants (Norddahl et al., 2006). There are many conventional ways to remove ammonia from water; however, most methods are dependent on relatively large amount of energy for the operation. Conversely, one solution is to strip the ammonia from these sources for subsequent optimal uses.

For the digesters, a lower ammonia concentration should reduce toxicity. Therefore, if ammonia is stripped from the digesters by gas mixing, better VS and COD removal should occur.
As shown in the figure 3.6, ammonia concentrations were compared between a Cambi 150°C/15 day SRT and a Cambi 150°C/20 day SRT digesters. This showed that ammonia concentration was lower during the Cambi 150°C/20 day SRT digester (2200mg/L) as compared to the Cambi 150°C/15 day SRT digester (2508 mg/L), even though the 20 day SRT digester had slightly higher VS destruction (Tanneru, et al, 2009).

Therefore in order to evaluate the reason behind the lower ammonia for the 20 day SRT system, the digester was operated using two types of mixing. Figure 3.7 shows that the concentration of ammonia was lower during periods of continuous mixing when compared to periods of partial mixing. The assumed steady-state periods are also shown.
This figure provided evidence that ammonia was stripped during periods of continuous mixing as the gas recirculation by the peristaltic pumps was varied. The data also indicate that during the steady-state period, the continuous mixing provided an average ammonia value of 2400 mg/L, while for the one hour per day mixing condition, the concentration was 2900 mg/L.

### 3.4.5 COD Reduction

Figure 3.4 shows that the average COD reduction, recorded during the similar period of steady state of ammonia analysis. The average COD reduction during continuous mixing was \(60\% \pm 0.017\) whereas the COD reduction during partial mixing was \(55\% \pm 0.022\). This confirmed the notion that a lower ammonia concentration will reduce ammonia toxicity and since ammonia was
stripped from the digesters by gas mixing, thereby accounting for a higher COD reduction. Statistical analysis also proved that there was a significant difference between the two sets of data since a p-value of 0.041 was recorded. Since P < 0.05, the data were significantly different.

Figure 3.8: Average COD Reduction during steady state

3.4.6 VS Reduction

Figure 3.9 describes the average VS reduction % observed during the steady state period similar to the ammonia analysis done via continuous and partial mixing. An average VS reduction of 54% was recorded during continuous mixing and 47% was recorded during the period of partial mixing. Statistical analysis also proved that there was a significant difference between the two sets of data since a p-value of 0.019 was recorded.
This results showed a direct correlation to the lower ammonia concentration which was also recorded during the period of continuous mixing, thereby confirming the effect of ammonia on solids reduction. These results are similar to the COD data and show that the effect of continuous recirculation of gas is to strip ammonia which thereby improves solids reduction. It is thought that this is due to removal of ammonia inhibition due to both ammonia stripping and a lower pH.

Figure 3.9: Average VS Reduction during steady state

### 3.4.7 Discussion

The operation of digesters using continuous mixing was effective for dispersing the contents of the reactor. However, a consequence of the gas recirculation was to strip ammonia from solution. Although stripping of ammonia might be an effective method for removing the inhibitory ammonia, it caused the data from the experimental reactor setup to be somewhat problematic in
that the concentration was lower than might be found in a full-scale digester system. Since the concentration of ammonia was thought to be inhibitory to the digesters, the actual concentration that would be found for various digestion configurations and pretreatment methods is important.

In this study, it was found that the ammonia concentration could be underestimated by approximately 500 mg/L. As indicated in Figures 3.8 and 3.9, this difference also had an impact on COD and solids reduction in the digesters. When the ammonia was lower, the solids and COD reduction was greater. This indicates that the performance of the digesters operated with gas recirculation would likely overestimate the removal of solids and COD. This is especially important for the system that used thermal hydrolysis because the ammonia was higher and the inhibition greater than for conventional digesters where the feed solids and potential for ammonia would be lower.

As a result, the performance of the TH system that is proposed for use by DC WASA must be evaluated in terms of the potential for a higher ammonia and lower degradation rates than were measured by Tanneru (2009) in his study. In addition, these data suggest that gas stripping might be a potential method for use in the digestion mixing system to lower ammonia levels.

3.5 Conclusions

Anaerobic digestion degrades protein and this result in the accumulation of ammonia while degrading organic matter. Therefore the research was conducted so as to determine the effects of
mixing in an attempt to decrease the overall ammonia concentration. The main conclusion that can be drawn from this study is as follows:

- Continuous mixing results in lower ammonia in the digesters

- Ammonia concentration appears to be approximately 500mg/L lower during continuous mixing as compared to during partial mixing. This is important for the operation of digesters because ammonia inhibits digestion. Therefore gas mixing might be preferred for TH systems with a high solid loading.

- The lower ammonia resulting from ammonia stripping resulted in better COD and solids removal from the system. The COD and solids removal from an operating digester with a different mixing system would likely be poorer than measured using the gas mixing system in our laboratories.
3.6 References


MANUSCRIPT 2: SHORT-TERM ANAEROBIC STUDIES AS AN INDICATOR OF THE EFFECTS OF LIPIDS ON GAS PRODUCTION DURING ANAEROBIC DIGESTION

Abstract

Lipids are known to be attractive substrates for both anaerobic digestion and co-digestion, since lipids have the ability to produce a higher methane yield in comparison to proteins and carbohydrates. Therefore, the benefit of adding lipids to a digester in order to enhance methane production is therefore a promising approach which should be better explored. Thus, the effects of the thermally hydrolyzed and unhydrolyzed fatty acids, linoleic (C18:2), oleic (C18:1) and linolenic (C18:3) on gas production during anaerobic digestion was investigated at 37°C. Polyethylene conical shaped reactors were used as the anaerobic digesters.

During the first stage, a mixture of the long chain unsaturated fatty acids, oleic, linoleic and linolenic acids were analyzed. In the second stage, the effects of a single unsaturated fatty acid, i.e. oleic acid, was analyzed. The results showed that addition of long chain fatty acids greatly increased gas production and the long chain fatty acids that were thermally hydrolyzed generated more gas than the untreated long chain fatty acids, although the increase was small. In the presence of a mixture of fatty acids, the volume of biogas produced while using fats that were hydrolyzed was 17.7 ft³, whereas 16.3 ft³ was produced during the use of unhydrolyzed fats.
For the oleic acid, 19.2 ft$^3$ and 18.5 ft$^3$ of biogas were produced with addition of hydrolyzed oleic acid and unhydrolyzed oleic acid, respectively.
4.1 Introduction

In municipal wastewater, the lipid fraction accounts for approximately 30-40% of the total chemical oxygen demand (Chipasa and Medrzycka, 2006). Lipids are attractive substrates for both anaerobic digestion and co-digestion due to their ability to produce a higher methane yield in comparison to proteins and carbohydrates. As a result, lipid-rich waste can be regarded as a huge potential energy source (Hansen and Knudsen, 1987).

A significant increase in the production of methane, from 25 to 50 m³ biogas/m³ cattle waste, when fish oil with a total concentration of 5% was added to a manure digester (Angelidaki and Ahring, 1992). The operational cost of powering a wastewater plant usually can run into millions of dollars/year. Therefore, in an attempt to help reduce these operational costs in addition to the carbon footprint, lipids can be added to anaerobic digesters in order to enhance the production of methane which can be then used. This technology is therefore a promising approach which should be further explored.

Similar to other organics in the anaerobic process, lipids are first hydrolyzed to glycerol and long-chain fatty acids (LCFA). Such process has found to be catalyzed by extracellular lipases that are excreted by the acidogenesis process (Cirne et al., 2007). The LCFA are then converted by syntrophic acetogenic bacteria to hydrogen (H₂) and acetate and finally to methane (CH₄) by methanogenic archaea (Palatsi et al., 2010). The most common fatty acids in domestic wastewater and sludge are mainly the saturated myristic, palmitic and stearic acid (C18:0), and the unsaturated oleic (C18:1) and linoleic acids (C18:2) (Novak and Kraus, 1973). Thus, the aim
of this study is to assess the effects of hydrolyzed and unhydrolyzed lipids on gas production. Ultimately, a mixture of single, double and triple bonded unsaturated fatty acids versus the use of only one single bonded fatty acid was examined.

4.2 MATERIALS AND METHODS

4.2.1 Anaerobic Digester Set-up

Three similar polyethylene batch fermentation reactors with a conically- shaped bottoms were used as the anaerobic digesters for the study. They were all supplied by the Hobby beverage equipment company located in Temecula, California. The anaerobic digesters were all operated at 37°C and as a continuously stirred tank reactor (CSTR). The following figure 4.1 depicts the experimental design of the study.
4.2.2 Sludge Samples

The reactor was initially seeded with biosolids from the Pepper’s Ferry Wastewater Treatment Plant (Radford, VA) and mixed primary and waste activated sludge from the DCWASA Blue Plains wastewater treatment plant. However, after the digesters were set-up, they were fed sludge collected from the DCWASA treatment plant that was made up of a ratio of 50% primary and 50% secondary solids by weight. Sludge samples were shipped weekly by DC WASA in cooler with a cold pack and stored in a refrigerator at 4°C until used.
4.3 Methodology

The volume of each digester was operated with a 10L nominal volume and in order to maintain a constant 20 days SRT, 500ml of the sludge was wasted and 500ml of the feed was slug-fed to the digester each day for a period of one minute. Three peristaltic pumps (Cole Parmer-600 rpm) were used to recirculate the gas in each anaerobic digester from the head space to the bottom of the digester. It should also be noted that the sludge fed to the digesters was maintained at 3% total solids and 10% lipids by total mass were fed to each digester, to allow the daily amount of fat added to the reactor to equal 1.5 grams. That is:

\[
3\% \text{ TS} = 30000 \text{mg/L}
\]

Therefore: \(3\% \text{TS} @ 10\% \text{ Fats} = \)

\[
\frac{1 \times 30000 \text{mg} \times 0.5 \text{L}}{10 \text{L} \times \text{d}} = 1.5 \text{g fats/day}
\]

From the above calculation, in order to account for the total volume of 500ml of total feed, 1.5g of fats was mixed with 450g of sludge and 48.5g of distilled water.

The first digester was fed with an equal amounts of a combination of 10% thermally hydrolyzed fatty acids, namely (linoleic, linolenic and oleic) and sludge collected from the DCWASA plant. The second digester was fed a mixture of 10% raw or unhydrolyzed fats (linoleic, linolenic, oleic) and sludge collected from the DC WASA plant, while the last digester was used as the control, i.e. it was fed only pure sludge. This accounted for the total 500ml of feed, assuming that 1g of water is equivalent to 1ml. The effects of only oleic acid on the digesters were also determined using the same amount of added lipids.
The digesters were monitored for steady state based on pH, COD reduction, VS reduction and TS reduction, according to Standard Methods for the examination of Water and Waste Water (APHA, 1998). Daily biogas production of the anaerobic digesters was measured by connecting each digester to a wet tip gas meter, and the values and associated error bars reported are indicative of the average standard deviation of sampling.

4.4 Results and Discussion

Analysis was performed only after it was determined that the digesters were at steady state. Steady state conditions were assumed after a period of forty days and were determined by daily monitoring of pH, chemical oxygen demand (COD) concentration, volatile solids (VS) % and total solids (TS) %. Therefore, by the use of such analyses, the efficiency of each digester was compared.

4.4.1 pH

The control of pH in anaerobic treatment is also critical as the desired pH is usually between the ranges of 6.5-7.6 (McCarty, 2001). Conversely, reactor pH can be depressed due to the production of organic acids as well as carbonic acid associated with the high concentrations of carbon dioxide gas. However, the production of gas increased by approximately 40% during slightly acidic conditions (Leitao et al., 2006). Moreover, a lower pH will result in inhibition of methanogens and a high pH can result in the buildup of unionized ammonia (Grady et al., 1999).
Figure 4.2 shows the overall pH of the digesters during this study. The pH of the digester receiving hydrolyzed fats, unhydrolyzed and no fats (control) were 7.15±0.04, 7.18±0.07 and 7.22±0.05 (x ± µ), respectively. The pHs of each digester was operated within the expected ranges of 6.5-7.6; therefore, no inhibitory effect due to variations in pH was thought to occur. It was speculated that the pH of the digesters with the fats was lower because more CO₂ was generated by the additional degradation of fatty acids. The equation for the effect of CO₂ on the pH is shown in the equation below.

\[ \text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^- \]
4.4.2 Chemical Oxygen Demand (COD)

As described by (Grady et al., 1999), the production of methane gas decreases the COD demand of waste streams. It also provides the mechanism for stabilization of biodegradable organic matter. However, it should also be noted that since there is a direct correlation between COD stabilization in anaerobic processes and methane production, methane can be determined from the COD removed in the process. The data describing the efficiency of each digester in terms of COD reduction is shown in Figure 4.3.

Figure 4.3 (a) and (b) shows the average COD concentrations using a mixture of fatty acids and oleic acid, respectively. When the digesters were fed with a mixture of fatty acids, the COD concentration in the feed samples which included the fatty acids were much higher than the raw sludge sample. Also, the overall COD concentrations of the effluent samples were higher than when they were only fed oleic acids.
The use of a mixture of fatty acids was started in mid-January for a period of about three months, while the use of oleic acid was started in mid-April for a period of one month. This may have been the main reason for such differences in the COD concentrations since the digesters had more time to be acclimatized during the use of a mixture of fatty acids. The most common fatty acids in domestic wastewater and sludge are the saturated myristic, palmitic and stearic acid.
(C18:0), and the unsaturated oleic (C18:1) and linoleic acids (C18:2), therefore, such differences may be attributed to the fact that the biodegradability of long chain fatty acids increases with a decreasing carbon chain lengths and increasing degree of unsaturation of carbon chains (Chipasa and Medrzycka, 2006). This is the most likely due to differences in solubility. The least soluble fatty acids are the unsaturated. Therefore, for this study, oleic acid is the least soluble of the three that were used.

4.4.3 COD Reduction

Figure 4.4 (a) and (b) shows that the average COD reduction for the digester with a mixture of hydrolyzed fats was 67% compared to 55% when fed with hydrolyzed oleic acid. A COD reduction of 66% was recorded when the digester was fed with a mixture of raw fats and 52% was recorded during the use of only raw oleic acid. This is thought to be due to differences in the fatty acid solubility. For oleic acid, thermal hydrolysis would result in the degradation of oleic acid into smaller molecules so it is expected that their hydrolysis would be beneficial for degradation compared to the unhydrolyzed oleic acid. Since two of the three fatty acids are relatively soluble, thermal hydrolysis will not be effective by using a mixture of these. The control showed a lower COD reduction compared to either of the fats amended digesters, with or without hydrolysis.
Analysis of the COD reduction data during the use of a mixture of fatty acids showed that there was a significant difference between the two sets of data from the use of hydrolyzed fats and raw fats digesters, since a p-value of less than 0.05 was recorded. Therefore the difference in the data
was statistically significant. Additionally, a p-value of $1.491 \times 10^{-8}$ was recorded between the data sets of hydrolyzed fats and the digester with no fats. Therefore the difference between these sets of data was also statistically significant. Furthermore, a p-value of $2.3 \times 10^{-6}$ was recorded between the data sets of raw fats and the digester with no fats. Therefore the difference between these sets of data was also statistically significant. Hence, all three differed significantly with the hydrolyzed fats showing a greater COD reduction than the hydrolyzed fats and both showed a higher COD reduction than the control.

Likewise, analysis of the COD reduction during the feeding of oleic acid only showed that there was a significant difference between the two sets of data from the use of hydrolyzed fats and raw fats digesters, since a p-value of $3.27 \times 10^{-6}$ was found. Hence the differences in the data were statistically significant. Additionally, a p-value of $1.491 \times 10^{-8}$ was recorded between the data sets of hydrolyzed fats and the digester with no fats. Therefore the difference between these sets of data was also statistically significant. Furthermore, a p-value of $2.31 \times 10^{-6}$ was recorded between the data sets of raw fats and the digester with no fats. As a result, the differences between these sets of data were also statistically significant.

The statistical analysis also indicates that the three sets of data for both the mixture of fats and only oleic acid differed with the hydrolyzed fats having the greatest COD removal and the control having the least COD removal. The data also showed that for the oleic acid feed, thermal hydrolysis was more important than for mixed fats. This was attributed to the differences in solubility of fatty acids.
4.4.4 Solids Reduction

The variation of TSR data is shown in the figure 4.5 (a) and (b). There was a higher TSR % among the digesters that were fed either hydrolyzed or raw oleic acid only when fed with a mixture of fatty acids. The average TSR for the digesters fed with a mixture of hydrolyzed fats and a mixture of raw fats were both 40%. However, a TSR % of 51% and 47% were recorded when the digesters were fed by hydrolyzed oleic acid and raw oleic acid respectively, both of which were much higher than the TS reduction of the control.

Analysis of the TSR % during the use of a mixture of fatty acids, confirmed that there was no significant difference between the two sets of data from the use of hydrolyzed fats and raw fats digesters, since a p-value of greater than 0.05 was found. Therefore the data was statistically similar. However, a p-value of 1.38x10^{-5} was recorded between the data sets of hydrolyzed fats and the digester with no fats. As a result, the differences between these sets of data were also statistically significant. Furthermore, a p-value of 2.85x10^{-7} was recorded between the data sets of raw fats and the digester with no fats. Therefore the difference between these sets of data was also statistically significant.

Likewise, analysis of the TSR% during the use of only oleic acid, showed that there was no significant difference between the two sets of data from the use of hydrolyzed fats and raw fats digesters, since the p-value was greater than 0.05. Therefore the data was statistically similar. Additionally, a p-value of 0.0061 was recorded between the data sets of hydrolyzed fats and the digester with no fats. Hence the difference between these sets of data was also statistically
significant. Furthermore, a p-value of greater than 0.05 was recorded between the data sets of raw fats and the digester with no fats. Consequently, these sets of data were statistically similar.

Figure 4.5 (a) and (b): Average TSR % during the use of (a) mixture of fatty acids and (b) oleic acid only
Variations were also seen among the VSR data, as shown in the following figure 4.6 (a) and (b). There was also a higher VSR % among the two digesters that were fed with hydrolyzed and raw fats when compared to the control. The average VS reduction for the digesters fed with a mixture of hydrolyzed fats and a mixture of raw fats were both 50%. However, a TSR % of 62% and 58% were recorded when the digesters were fed by hydrolyzed oleic acid and raw oleic acid, respectively; both of which were much higher than the VS reduction of the control, which was 47% and 58% during the use of a mixture of fatty acids and oleic acid respectively.

Analysis of the VSR % during the use of a mixture of fatty acids, confirmed that there was no significant difference between the two sets of data from the use of hydrolyzed fats and raw fats digesters, since a p-value of greater than 0.05 was recorded. Therefore the data was statistically similar. However, a p-value of 0.012 was recorded between the data sets of hydrolyzed fats and the digester with no fats. As a result, the differences between these sets of data were also statistically significant. Furthermore, a p-value of 0.011 was recorded between the data sets of raw fats and the digester with no fats. Consequently the difference between these sets of data was also statistically significant.

Likewise, analysis of the VSR % during the use of only oleic acid, showed that there was a significant difference between the two sets of data from the use of hydrolyzed fats and raw fats digesters, since a p-value of 0.032 was recorded. Hence the differences in the data were statistically significant. Additionally, a p-value of 0.023 was recorded between the data sets of hydrolyzed fats and the digester with no fats. Therefore the difference between these sets of data was also statistically significant. However, a p-value of 1 was recorded between the data sets of
raw fats and the digester with no fats. Consequently, these sets of data were statistically similar.

As a result, the differences between these two sets of data were also statistically significant.

Figure 4.6 (a) and (b): Average VSR % during the use of (a) mixture of fatty acids and (b) oleic acid only
The explanation behind the higher variations in solids reduction especially seen with the use of lipids, is mainly due to the fact that the lipids acted as an additional high energy substrate for microbial growth. This resulted in an increase concentration of microorganism in the treatment system which accounted for an increased degradation. There was also a further increase by the use of hydrolyzed fats since hydrolysis has been found by Wilson (2009) to break long chain fatty acids into smaller and more soluble units, thereby enhancing their degradation.

### 4.4.5 Correlation between Gas Production and VS Destruction

One of the major benefits to anaerobic digestion is the production of biogas which is can be eventually used as a fuel supply. However, this is mainly accomplished by the stabilization of biodegradable particulate organic matter. Such performance is often quantified by the percent of VS destruction. According to (Grady et al., 1999), at a SRT of 15-20 days, 80 to 90% of the influent biodegradable particulate organic matter will be converted to methane gas. Such values correspond to VS destruction of about 60% which were contained in the primary solids and 30 to 50% of the VS found in waste activated sludge (WAS). However, about 70% of the organic matter in municipal primary solids, measured as either COD or VS, is biodegradable in an anaerobic environment (Grady et al., 1999). A typical value of specific gas production is $15\text{ft}^3/\text{lb}$ of VS destroyed.

Figure 4.7 (a) and (b) shows that the average biogas produced per lb of VS destroyed slightly varied. However, a higher volume of biogas was produced while lipids were added to the digesters. During the use of a mixture of fats, the volume of biogas produced while using fats
that were hydrolyzed was 17.7 ft³/lb (1.1 L/gm) whereas 16.3 ft³/lb (1.02 L/gm) was produced during the use of pure fats. A normalized volume of 19.2 ft³/lb (1.19 L/gm) and 18.5 ft³ (1.15 L/gm) were produced during the use of hydrolyzed oleic acid and raw oleic acid, respectively. Clearly, these values were much higher than during the use of no lipids (control).

Analysis of the amount of biogas produced as VS was destroyed during the use of a mixture of fatty acids and only oleic acid was also performed. During the use of a mixture of fatty acids, the data confirmed that there was significant difference between the two sets of data from the use of hydrolyzed fats and raw fats digesters, since a p-value of 0.0075 was recorded. Therefore the data was statistically significant. Additionally, a p-value of 1.046x10⁻⁵ was recorded between the data sets of hydrolyzed fats and the digester with no fats. As a result, the differences between these sets of data were also statistically significant. Also, a p-value of 8.43x10⁻⁷ was recorded between the data sets of raw fats and the digester with no fats. Therefore the difference between these sets of data was also statistically significant.

Likewise, analysis of the amount of biogas produced as VS were destroyed during the use of oleic acid only showed that there was a significant difference between the two sets of data from the use of hydrolyzed fats and raw fats digester, since a p-value of 0.0075 was recorded. Hence the differences in the data were also statistically significant. Additionally, a p-value of 1.05x10⁻⁵ was recorded between the data sets of hydrolyzed fats and the digester with no fats. Therefore the difference between these sets of data was also statistically significant. Also, a p-value of 8.35x10⁻⁸ was recorded between the data sets of raw fats and the digester with no fats.
Consequently, these sets of data were statistically similar. As a result, the differences between these sets of data were also statistically significant.

Figure 4.7: Average biogas produced per pound of VS destroyed during the use of (a) a mixture of fatty acids and (b) oleic acid only
4.5 Conclusions

The results of this study demonstrate that the addition of lipids enhances biogas production. The data also indicate that thermal hydrolysis is beneficial for the degradation of fats, especially when the fats are minimally soluble. Therefore, thermal hydrolysis was most beneficial when oleic acid was fed to the digesters. When a mixture of oleic, linoleic and linolenic were fed, the benefits of thermal hydrolysis were limited. However, in all cases, the addition of fatty acids, with or without thermal hydrolysis was beneficial with regard to both COD and volatile solids reduction and also for gas production.

Gas production for the addition of fatty acids was much greater than the control and much greater than typical values from the literature. In the case of the addition of the mixture of fatty acids, the specific gas production was 17.7 ft³/lb (1.1 L/gm) for hydrolyzed fats and 16.3 ft³/lb (1.02 L/gm) for unhydrolyzed fats. A normalized volume of 19.2 ft³/lb (1.19L/gm) and 18.5 ft³ (1.15 L/gm) were produced during the use of hydrolyzed oleic acid and unhydrolyzed oleic acid, respectively.
4.6 References


