Thermal hydrolysis of LCFAs and Influence of pH on Acid-Phase Co-Digestion of FOG

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Abstract

Two different sludge pretreatments were investigated in an attempt to improve the management and performance of processes for the co-digestion of biosolids with fats, oils, and grease (FOG). The mechanisms of long chain fatty acids (LCFA) degradation in thermal hydrolysis pretreatment and the influence of pH on LCFA degradation in two-phase co-digestion systems were studied.

LCFA thermal hydrolysis was investigated at different temperatures (90-250 °C) and reaction times (30 minutes and 8 hours). Approximately 1% of saturated fatty acids were degraded to shorter chain fatty acids at 140 and 160 °C (8-hr thermal hydrolysis). Only 1% or less of unsaturated fatty acids were degraded from 90 to 160 °C (8-hr thermal hydrolysis). Little degradation (< 1%) of both saturated and unsaturated LCFAs was observed at a 30-min reaction time. Both groups of LCFAs were stable up to 250 °C (30-min hydrolysis). The use of chemical-thermal treatments was also investigated. Only unsaturated LCFAs, C18:1 and C18:2, were degraded when thermally hydrolyzed with hydrogen peroxide coupled with activated carbon or copper sulfate.

Semi-continuous, acid-phase digesters (APDs) under different pH conditions were studied in order to understand the effects of pH on FOG degradation. Increases in soluble chemical oxygen demand (SCOD) were observed in all APDs. However, the APDs with pH adjustment appeared to perform better than the controls in terms of solubilizing organic compounds. Approximately 38% and 29% of total COD (TCOD) was solubilized, and maximum volatile fatty acid (VFA) concentrations of 10,700 and 7,500 mg/L TCOD were achieved at pH 6 and 7, respectively; It is useful to note that the feed sludge had a VFA concentration of 2,700 mg/L COD. Higher pH (6.0-7.0) showed less accumulation of LCFA materials and more soluble LCFAs in the APDs. This indicates that the lower pH in the APDs was most likely the cause of precipitation and accumulation of LCFAs due to saturation of unsaturated LCFAs.

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Chapter 1: Introduction

In recent years, great interest has developed in increasing biogas production from anaerobic digesters to maximize the energy that can be recovered at Water Resource Recovery Facilities (WRRFs). Co-digestion of fats, oils, and grease (FOG) has become more popular in practice and for research studies. Addition of FOG increases biogas production; however, addition of the main components in FOG, long chain fatty acids (LCFAs), can cause inhibition to methanogens in anaerobic digestion systems. Moreover, LCFAs have been shown to cause sludge flotation, foaming, and "grease balls," which resist degradation, cause operational problems, and eventually lead to digester failure. Pretreatment of FOG before anaerobic digestion is believed to mitigate these problems. However, limited studies have been done on mechanisms of LCFA degradation in thermal hydrolysis and acid-phase pretreatments.

The following literature review describes studies that were done to investigate the effects of thermal pretreatment and two-phase digestion (TPD) on degradation mechanisms of lipids and long chain fatty acids. Many investigators have studied the effects of thermal hydrolysis as a pretreatment process. The treatment has proved to be effective for VS removal, COD removal, cell wall lysis, and biogas production. The major conclusions were that thermal pretreatment can induce cell wall rupture and improve solubilization of proteins, carbohydrates, and lipids (Wilson, 2009; Bougrier et al., 2008). To enhance solubilization of nutrients, chemical-thermal pretreatments were used in many studies (Penaud et al., 1999; Valo et al., 2004; Liu, 2003). The degradation of lipids has been studied for many years. Unsaturated lipids are readily degraded to shorter chain fatty acids and other by-products, such as alkanes, alkenes, esters, and diacylglycerols, by thermal hydrolysis (Nawar, 1984). However, limited studies have been done on the thermal hydrolysis of LCFAs.

Another popular pretreatment method is the anaerobic two-phase system. The effects and operational parameters of the two-phase, co-digestion systems are later explained in the literature review. The main conclusion was that acid-phase digesters (APD) help saturate the unsaturated fatty acids, induce cell lysis, and increase soluble organic compounds. The main advantage of APDs is that unsaturated fatty acids, which inhibit methanogens, are saturated prior to being fed into gas-phase digesters (Komatsu et al., 1991). However, saturated fatty acids are found to be a cause of LCFA accumulation in the APDs. In addition, an LCFA accumulation on top of the

liquid layers provides no benefits to the wastewater treatment plants and can cause operational problems.

The first manuscript presented, Thermal Degradation of Long Chain Fatty Acids, investigates the effects of thermal hydrolysis pretreatment on LCFA degradation. The study revealed that thermal hydrolysis duration, temperature, and the degree of saturation affect the degradation of LCFAs. During a 30-min thermal hydrolysis pretreatment, the degradation of both saturated and unsaturated LCFAs was minimal. However, only small increases in degradation were detected at an 8-hr reaction time. Both saturated and unsaturated LCFAs were stable and resisted degradation up to 160 °C. The catalyst experiments conducted showed that hydrogen peroxide coupled with the catalysts, such as activated carbon or copper sulfate, degraded unsaturated fatty acids via an oxidation process. However, the saturated fatty acids were stable under all conditions considered. In addition, the thermal hydrolysis of FOG was investigated. The findings supported the earlier experiments on LCFA stability during thermal hydrolysis treatments.

The second manuscript, Influence of pH and FOG Degradation in Acid-Phase Co-Digestion, presents the effects of pH on LCFA degradation and accumulation, and overall performance of the two-phase systems. The APDs served a role in saturating the unsaturated fatty acids. However, LCFAs accumulated onto biomass and floated to the top of the liquid layers in the APDs. The pH of the APDs did not affect degradation of LCFAs; however, pH had a great effect on LCFA accumulation and some effect on the overall performance of the TPD system. Higher pH (6.0-7.0) showed less saturated LCFA accumulation in the APDs. The best performance in terms of solubilization and biogas production was found at pH of 6.

Substantial contributions of this research have been made in the field of anaerobic co-digestion pretreatment processes. Thermal hydrolysis pretreatment of FOG needs to be carefully considered. The study revealed that LCFAs were not degraded under typical thermal hydrolysis conditions. Moreover, degradation of complex molecules, such as lipids, could result in LCFA accumulation in thermal hydrolysis reactors. This research also helps developed the existing knowledge on the influence of pH in acid-phase digesters. Acid-phase co-digestion as a pretreatment process seemed to work more effectively in terms of saturating LCFAs. However,

an optimum pH in APDs is required to maximize the hydrolysis rate and mitigate LCFA accumulation issues.

Chapter 2: Literature Review

Anaerobic Digestion Pretreatment Process

Anaerobic digestion has been shown to have many advantages over aerobic processes (McCarty, 1964). The anaerobic digestion process consists of hydrolysis, acidogenesis, acetogenesis, and methanogenesis steps to break down biodegradable compounds and produce methane. The hydrolysis of cell walls and membrane rupture of substances can produce biopolymeric proteins, lipids, carbohydrate, and other nutritional compounds which can be hydrolyzed by the extracellular enzymes. Sludge solubilization and degradation require an extensive time period. Normally, a period of 3 to 8 days at 35- 37 °C is required for microorganisms and enzymes to break large-molecule compounds down to the smaller ones. Hydrolysis has proven to be the rate-limiting step in anaerobic digestion processes (Eastman and Ferguson, 1981). To enhance and accelerate the hydrolysis step, various advanced pretreatment processes have been studied such as thermal hydrolysis, mechanical grinding, chemical, and enzymatic pretreatments. Thermal hydrolysis pretreatment has proven to be one of the most advantageous pretreatment options (Hospido et al., 2005; Haug et al., 1983).

Thermal Hydrolysis

Thermal Hydrolysis is a waste-sludge pretreatment process that uses high pressure (5-8 bar) and high temperature (130 - 180 °C). The heat and pressure help increase viscosity of organic compounds. Then, the process is followed by the rapid release of reactor pressure to create forces that destroy microbial cell walls. Decreasing viscosity, splitting cell walls and fracturing complex molecules helps make these compounds more available for biological degradation. Many researchers have illustrated that thermal hydrolysis pretreatment can help increase methane production, enhance organics degradation, improve waste sludge dewaterability, increase volatile solid destruction, and decrease digester sludge retention time and lower digester volume (Haug et al., 1978; Pilli et al., 2015; Jolis, 2008).

In most studies, temperature has been shown as one of the most important parameters of thermal hydrolysis pretreatment. Pinnekamp (1989) found that thermal pretreatment gave an economic advantage over anaerobic digestion processes without thermal pretreatment in terms of stability and gas production at 135 – 180 °C. Haug et al. (1978) also reported that at 175 °C, thermal pretreatment can increase methane production up to 60-70% compared with a control; however, inhibitory materials are created when the pretreatment temperature is at 175 °C or above. In another study, the researchers found that high-temperature pretreatments at 170 °C were more efficient than low-temperature pretreatments with alkaline conditions (Valo et al., 2004).

Gavala et al. (2003) found that the effects of pre-treatment duration and temperature mostly depend on the composition of sludge. Typical primary sludge consists of carbohydrates (55%), proteins (18%), and lipids (10%) (Miron et al., 2000). Typical secondary sludge, which is mainly bacteria and aggregation of bio-polymers, consists of proteins (36%), carbohydrates (20%), and lipids (7%) (Miron et al., 2000). In a study on thermal hydrolysis of macromolecular components, such as proteins, lipids, and polysaccharides, the researchers found that thermal pretreatment can destroy cell walls and make proteins accessible for biodegradation (Mller, 2001). Another study found that proteins converted to ammonia, smaller molecular weight peptides, and VFAs at a temperature range from 130 °C to 220 °C (Wilson, 2009). Li et al. (2013) found that thermal hydrolysis and ultrasonic pretreatments of fats, oils, and grease (FOG) could increase methane production up to approximately 10% than the one without thermal treatments. It is important to note that the study primarily focused on methane production from anaerobic digesters. However, there is little to none that studied the degradation of long chain fatty acids (LCFAs) in the thermal pretreatment process.

Thermal Hydrolysis of LCFAs

A great interest has developed in recent years to increase methane production from anaerobic digesters to recover energy at water resource recovery facilities (WRRFs). One way to accomplish this is to add high energy products, especially fats, oils, and grease (FOG) to digesters. Fats, oils, and grease are generated during cooking and food processing. Once FOG enters the collection system, it can accumulate on pipe walls forming hardened deposits through

physical/chemical reactions. FOG will typically be transported and disposed in either landfills or incinerators. This material can also be delivered to the WRRFs by commercial haulers. The facility may be able to recover a tipping fee for its disposal, but the floating portion of this material generally accumulates in the primary scum treatment processes of the WRRFs and provides no additional benefits. The production of excess recoverable biogas from this material would bring turn this material into a value added product for wastewater treatment.

Addition of the high energy density FOG can help to increase both the quantity and quality of biogas generated in digesters. However, one of the major handling problems in co-digestion processes is that many long chain fatty acids (LCFAs), the molecule that stores the majority of the energy in FOG, are converted from unsaturated to saturated fatty acids during the early stages of anaerobic digestion. The insoluble and hydrophobic saturated LFCAs will coalesce and form "grease balls" that can resist degradation and accumulate in a layer on top of the liquid in the digester (Varin, 2013; Pereira et al., 2005). It would therefore be very useful to have a pretreatment operation implemented to reliably improve LCFA degradation, reduce the handling problems, and prevent other issues caused by LCFAs. Thermal hydrolysis is believed to be a potential solution for facilitating FOG decomposition through thermal degradation of the LCFAs.

Thermal hydrolysis of triglycerides to long chain fatty acids was investigated in earlier studies (O'Rourke, 1968; Crossley et al., 1962). The effects of heat treatment on lipids and fatty acids can be divided into two categories: oxidation and thermal studies. Oxidation studies explain oxidative mechanisms under a flow of oxygen or air with different degrees of heating while the thermal studies did not distinguish between oxidative and non-oxidative mechanisms. The thermal studies mainly focused on degradation products and mechanisms including hydrolysis. Lascaray (1949) concluded that the splitting reactions of oils homogenously occurred in the oil phase, in the presence of moisture, and activated by hydrogen ions. The hydrolysis of fats increases with temperature and the presence of catalyst reagents. The mechanism of triglyceride degradation has been studied by Crossley et al (1962). The triglycerides decompose at high temperatures (240 °C-260 °C) in the absence of oxygen and at 190 °C in the presence of oxygen (Crossley et al., 1962). Nawar's study illustrated that when heating random glycerides at 200 °C for 3 hours; shorter chain fatty acids were released in a greater abundance than long chain

fatty acids (Nawar, 1969). Many researchers have investigated the thermal hydrolysis of vegetable oil and grease waste in order to produce biofuel (Biller et al., 2011; Shin et al., 2012). Shin et al. (2012) observed that the primary mechanisms during thermal hydrolysis (300-370 °C) of fatty acids (C18:0, C18:1, and C18:2) were isomerization and pyrolysis. However, the study showed that LCFAs were stable up to 300 °C. In a more recent study, Wilson et al. (2009) investigated thermal hydrolysis (170 °C) of macromolecular components in wastewater sludge and found that the hydrolysis of unsaturated lipids produced more VFA compared to the saturated ones. The study found that unsaturated lipids (e.g. glyceryl trilonolenate) produced approximately 470 mg/L as HAc, while the saturated lipids (e.g. glyceryl tristearate) produced only 60 mg/L as HAc. Even though thermal hydrolysis of lipids has been investigated extensively, the hydrolysis of LCFAs were not clearly understood and required additional investigation.

Thermal Pretreatment of LCFAs (catalysts)

Municipal wastewater treatment plants generate sludge daily. Advanced biological, mechanical, and chemical processes have been proposed to reduce sludge production. Many of these are pretreatments prior to anaerobic digestion to increase solids destruction and increase methane production. These methods include thermal hydrolysis, chemical oxidation (H_2O_2, O_3, etc.), mechanical disintegration, enzymatic pretreatments, etc. Chemical-thermal pretreatments were reported to induce cell lysis and increase solubilisation of sludge as well as reduce excess sludge production and improve dewaterability (Liu, 2003; Neyens et al., 2003a; Rocher et al., 1999). Improving dewaterability requires disruption of the sludge cell structure. Chemical-thermal hydrolysis is recognized to have a great potential to help reduce sludge production.

Many investigators found that acid-thermal hydrolysis can reduce the dry solid contents and enhance sludge dewaterability at pH 3 with a temperature range of 120 – 155 °C (Neyens et al., 2003b). Rocher et al. (1999) reported that acid-thermal hydrolysis treatment will induce cell lysis and help release dissolved organic carbon (DOC) considerably well at pH 1. In the same study, the authors observed that the quantity of acid required for treatment was larger than the alkaline required for the same level of released DOC (Rocher et al., 1999).

Most investigators reported that alkaline-thermal treatment is able to increase dewaterability, dry solid content, and the amount of DOC released (Neyens et al., 2003a; Rocher et al., 1999). Valo et al. (2004) found that alkaline-thermal hydrolysis helped increase COD and VS solubilization which resulted in 71% COD degradation and a 54% increase in biogas production at a pH of 10 and 170 °C. Many authors suggested that, at pH 10, the alkaline-thermal hydrolysis could rupture cell structure, improve dewaterability, and reduce sludge production (Neyens et al., 2003a; Rocher et al., 2001).

Hydrogen peroxide is known as a strong oxidant used to treat various inorganic and organic pollutants in environmental applications. The Fenton reaction is a catalytic process which generates hydroxyl radicals from the decomposition of hydrogen peroxide at low pH with Fe^{2+} as a catalyst. Hydroxyl radicals generated from reactions can react with organic substances which induce decomposition process via oxidation. In some studies, researchers investigated various types of catalytic materials for hydrogen peroxide decomposition (Lücking et al., 1998; Ono et al., 1977; Chiou, 1983; Quinlan and Gutteridge, 1988). Ono et al. (1977) found that activated carbon can produce free radicals during the decomposition of hydrogen peroxide. In another study, the authors reported that hydrogen peroxide treatment after acid or alkaline treatment was also able to increase sludge solubilisation (Kim et al., 2009). Decomposition of hydrogen peroxide with catalysts or hydrogen peroxide alone has proved to disintegrate and decomposed organic substances in the sludge (Chiou, 1983; Tokumura et al., 2007; Neyens et al., 2003c)

Catalytic thermal pretreatment of lipids and long chain fatty acids (LCFAs) has been recently investigated due to growing interests in bio-fuel generation. With high temperature and pressure, lipids can be decomposed to LCFAs and hydrocarbons even though Biller and Ross (2011) reported that the hydrocarbon portion is small. Watanabe et al. (2006) found that stearic acid (C18:0) is stable up to 300 °C unless catalysts such as alkali hydroxide (NaOH and KOH) or metal oxides (CeO_2, Y_2O_3 and ZrO_2) are present. Many researchers found that triglyceride hydrolysis can produce fatty acids (Holliday et al., 1997; King et al., 1999). Alelenzi et al. (2009) found that fatty acids also acted as catalysts in the hydrolysis reaction of triglycerides. In the same study, the authors also found that high energy was required in order to start hydrolysis reactions. Aldehydes, volatile fatty acids, and esterified compounds can be produced without the

interaction with ·H or ·OH (Frankel, 2014). In a qualitative study, the investigators found that oleate (C18:1) and linoleate (C18:2) can be hydrolyzed at 180 °C and produced aldehydes, methyl ketones, alcohols, alkanes, and furan (Berdeaux et al., 2012). However, it is very important to note that these experiments were done to mainly investigate the decarboxylation of fatty acids at high temperatures (270-350 °C) compared to typical thermal hydrolysis pretreatment temperatures of municipal wastewater (130-180 °C). Limited studies have been done on long chain fatty acids degradation, especially, in municipal wastewater treatment applications.

Two-Phase Digestion

Wastewater treatment plants (WWTPs) have been using anaerobic sludge digestion processes for over a century. The anaerobic digestion processes have been shown to have advantages over aerobic process. The treatment process produces methane gas and significantly decreases biosolids in the effluent (Grady Jr et al., 2011; McCarty and Smith, 1986). Anaerobic digestion consists of two distinct groups of microorganisms, acid-forming and methane-forming. The first group of microorganisms plays an important role in hydrolysis and acidogenesis of organic substances. The second group, methane-forming microorganisms, mainly plays a role in methanogenesis. These two groups are different in terms of environmental conditions, growth kinetics, and nutritional needs. Providing optimum environments for both groups of microorganisms can enhance anaerobic digestion process efficiency, stability, and control. Pohland and Ghosh (1971) first introduced physical phase separation for each group by dividing an anaerobic digester into two digesters with different operational conditions . The first reactor which is called an acid-phase digester (APD), fermenter, or hydrolysis reactor plays a role in hydrolysis and acidogenesis. The second reactor which is called a gas-phase or methane reactor is similar to the conventional single-phase digester.

Acid phase

Many studies have been done on two-phase anaerobic digestion in order to illustrate and separate bacterial groups in the APDs (Demirel and Yenigün, 2002). Ng et al. (1999) found that the dominant microorganisms in an APD were rod-shaped bacteria. The shape and size of bacteria were confirmed to change with respect to hydraulic retention time and temperature in an APD (Penaud et al., 1997; Cha and Noike, 1997). Zhang and Noike (1991) compared one-phase and two-phase digestion processes under the same conditions and found that bacterial population levels of two-phase and single-phase were in the same order population levels.

In an APD, available organic carbon is readily converted to volatile organic acids. Hydraulic retention time (HRT) is found to be an important parameter to control the efficiency and stability of a two-phase system (Massey and Pohland, 1978). Hydrolysis and acidogenesis take place simultaneously in the APD, with acetic and propionic acids as the main volatile products. However, hydrolysis is found to be a rate-limiting step in the conversion of organic substances. Eastman and Ferguson (1981) found that the carbohydrate and nitrogenous compounds degraded at 70% and 55%, respectively. However, no degradation of lipids was found. The soluble VFAs, alkaline, and super saturated carbon dioxide that are produced in the process are the cause of lower pH in the APD.

Effects of pH on acid-phase digestion

Optimum conditions of an APD depends on various parameters such as hydraulic retention time (HRT), pH, temperatures, and types of feed stocks. Due to the hydrolysis and acidogenesis steps occurring simultaneously, the optimum HRT was reported to be between 0.5 – 3 days depending on the types of wastewater (Demirel and Yenigün, 2002). Kozuchowska et al. (1995) found that the optimum temperature for treating coffee waste was 45 °C. In the same study, the authors suggested that the mesophilic conditions (35 °C and 45 °C) provided more stable acid composition compared to the thermophilic ones (55 °C and 60 °C) (Kozuchowska and Evison, 1995). Many investigators have reported various optimum pH conditions for the APD ranging from 5.2 – 7.9 (Demirel and Yenigün, 2002). Zoetemeyer et al. (1982) studied the influence of pH on acidogenic dissimilation of glucose over a range of 4.7 to 7.9 and found that

the optimum pH range was between 5.7 and 6.0. In another study, the authors found that pH between 4.2 and 5.2 in the APD did not affect the VFA production and COD solubilization; however, the higher pH conditions (5.9 – 6.2) showed maximum carbohydrate hydrolysis and VFA productions (Elefsiniotis and Oldham, 1994). Horiuchi et al. (1999) reported that microbial populations changed in the APD due to the pH shift from 6.0 to 8.0. Their study illustrated the shift of end products from butyric to acetic and proprionic acids as the pH increased. More recently, Wu et al. (2009) studied the effect of pH on anaerobic fermentation of primary sludge at room temperature over a pH range of 3.0 to 11.0 and found that alkaline conditions (pH of 8.0 to 10.0) can increase soluble chemical oxygen demand (SCOD) especially soluble protein and carbohydrate. A recent study from Varin et al. (2013) suggested that higher pH levels helped mitigate accumulated LCFA materials in the APD. Even though various optimum pH conditions were reported, alkaline pH can noticeably provide benefits to APD processes.

Fat, Oils, and Grease (FOG)

Biological mechanism of LCFA degradation

Fat, oil, and grease (FOG) are high energy compounds which are generated from restaurants, households, and cooking industries. FOG has a beneficial potential for incineration, biodiesel production, and anaerobic digestion. Addition of FOG into anaerobic digestion process has proven to help increase biogas production up to 30-80% in full-scale wastewater treatment plants (Muller et al., 2010; Bailey, 2007). Main components in FOG are triglyceride esters, which are reported to rapidly hydrolyze to fatty acids (Heukelekian and Mueller, 1958). Novak and Carlson's study (1970a) on the kinetics of long chain fatty acids' anaerobic degradation found that β-oxidation is the primary degradation mechanism of LCFAs. The study also revealed that unsaturated fatty acids degraded more quickly compared to saturated fatty acids. In the same study, the authors suggested that unsaturated fatty acids need to convert to saturated ones before the B-oxidation process takes place (Novak and Carlson, 1970a). In the B-oxidation process, a long chain fatty acid degrades by n-2 carbons produce an acetate molecule, four electrons, and four hydrogen ions (Weng and Jeris, 1976; Jeris and McCarty, 1965).

$$CH_3(CH_2)_nCOOH + 2H_2O \rightarrow CH_3(CH_2)_{n-2}COOH + CH_3COOH + 4e^- + 4H^+$$

Then the electrons need to be moved from electron carriers such as FADH and NADH to appropriate electron acceptors such as H^+. The hydrogen gas is formed in the process and ready for hydrogentrophic methanogenic use (Lalman and Bagley, 2000).

$$4e^- + 4H^+ \rightarrow 2H_2$$

More recently, some investigators found that unsaturated fatty acids does not need to be saturated prior to β-oxidation (Lalman and Bagley, 2000). Intermediate products such as palmitic (C16:0) and myristic acids (C14:0) were produced during the degradation of oleic (C18:1) and linoleic (C18:2) acids; the authors also found that no stearic acids (C18:0) were produced in the process (Lalman and Bagley, 2000). Due to the saturated fatty acid degradation occurring at a much slower rate, the absence of stearic acid (C18:0) indicate that stearic acid (C18:0) is not an intermediate product of oleic (C18:1) and linoleic (C18:2) acids degradation. The study revealed that stearic acid (C18:0) degraded less than 50% after 50 days at 21 °C in cultures acclimated to glucose (Lalman and Bagley, 2001). Lalman and Bagley (2001) concluded that oleic (C18:1) and linoleic (C18:2) acids are more energetically favorable to convert to shorter chain fatty acids than stearic acid (C18:0). A more recent study confirmed that palmitic acid (C16:0) is an intermediate product and stearic acid (C18:0) is not likely to be an intermediate product of oleic (C18:1) and linoleic (C18:2) acids degradation (Pereira et al., 2002).

LCFA inhibition of anaerobic digestion process

The inhibitory effect of long chain fatty acids (LCFAs) on the anaerobic digestion process was mentioned in early 1960s by McCarty et al. (1964); the authors concluded that continuous feeding and avoiding overload of organic materials such as LCFAs could mitigate the problems. Hanaki et al. (1981) found that LCFAs hydrolyzed from milk cause inhibitory effects, and $CaCl_2$ could not reduce the effect once it occurred. In the same study, the investigators found that lipid concentration about 10% of organic concentration could cause a severe inhibition (Hanaki et al., 1981). The authors revealed that the LCFA inhibition of acetogens and acetotrophic methanogens induced a lag period in methane production. It is important to note that an accumulation of LCFAs was found in the solid phase within 24 hours in the batch experiments (Hanaki et al., 1981). The LCFA inhibition occurred due to physical interactions

between the LCFAs and the membrane of the microorganisms were investigated in other studies (Hotchkiss, 1946; Demeyer and Henderickx, 1967). Rinzema et al. (1994) reported that LCFA inhibition is related to LCFA concentrations and not to a LCFA:biomass ratio. The study illustrated that capric acid (decanoic acid) has a severe inhibitory effect of acetotrophic methanogens at concentrations of 1,154 to 1,550 mg/L (Rinzema et al., 1994). In recent studies on inhibitory effects of LCFAs, the investigators found that linoleic and oleic acids inhibited aceticlastic methanogenesis at a concentration of 30 mg/L or more, while stearic acid concentrations up to 100 mg/L did not inhibit the process (Lalman and Bagley, 2001). However, some authors reported that hydrogenotrophic methanogens in the system only slightly inhibited by the unsaturated fatty acids (Lalman and Bagley, 2000; 2001). Further study found that mixtures of LCFAs inhibited butyric acid degradation compared to the effects of individual LCFA (Lalman and Bagley, 2002). In an extensive study on LCFA inhibition, the researchers suggested that transport (diffusion) limitations of LCFAs cause the inhibitory effects on acetoclastic methanogens (Pereira et al., 2004). Hydrogen utilizing methanogens can survive, while acetogenic methanogens face the effect of transport limitations because the acetate is too large to pass through LCFA layers (Pereira et al., 2004). Hydrogen utilizing methanogens were not inhibited because small molecules such as H_2 can easily be mineralized to methane and passed through the LCFA layers, which adsorb onto biomass.

LCFA accumulation and adsorption

FOG can be quickly degraded to LCFAs, which accumulate in anaerobic digesters, cause sludge floatation and, eventually, the digesters' failure due to LCFA inhibition or sludge washout. The accumulation can occur within 24 hours (Hanaki et al., 1981). An early study on physicochemical effects of LCFAs on bacterial cells illustrated that LCFA toxicity occurs due to physical interactions with cell walls causing problems with nutritional adsorption and transport (Galbraith and Miller, 1973). In a more recent study, the authors found that LCFAs associated with sludge biomass by precipitation with divalent ions such as calcium or magnesium, adsorption onto bacterial cells, or entrapment on to biomass (Pereira et al., 2005). The authors also found that LCFA toxicity due to LCFA accumulation onto biomass is not permanent and its effect is reversible (Pereira et al., 2004). Palmitic acid (C16:0) was found to be the most

important LCFA which accumulates in anaerobic digestion systems (Pereira et al., 2002; Bishnoi, 2012; Kabouris et al., 2009). Pereira et al. (2002) concluded that, in the presence of oleic acid, further B-oxidation of palmitic acid was inhibited and palmitic acid began to accumulated in the digesters. In a more recent study, the investigators found that the higher pH of acid-phase reactors can help mitigate the LCFA accumulation issues (Varin, 2013). The mechanisms of LCFA accumulation in the digestion systems have been studied extensively recent years; however, there are some operational parameters, such as pH, that have not been investigated and clearly understood.

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Chapter 3: Thermal Degradation of Long Chain Fatty Acids

Abstract

The thermal hydrolysis of pure saturated fatty acids (C16:0 and C18:0) and unsaturated fatty acids (C16:1, C18:1, and C18:2) was investigated at 90 °C to 160 °C for 30-min and 8-hr durations. Hydrolysis efficiencies were calculated based on mass yield (i.e. mg/g parent compound), which accounted for all C2-C24.

Very little degradation (less than 1%) of LCFAs was observed from 30-min thermal hydrolysis. At 140 and 160 °C for 8 hours, only 1% of C16:0 and C18:0 were degraded to volatile fatty acids (VFAs) and medium chain fatty acids (C8:0-C12:0). Saturated fatty acids degraded uniformly to C2 to C14. The result indicate that thermal treatment does not break readily alkane carbon bonds. Saturated fatty acids tended to convert to alkanes (1.5-2.0% of total fatty acids) instead of fatty acids at the longer thermal treatment (8 hours).

At an 8-hr thermal hydrolysis duration, the temperature range from 90 to 160 °C did not significantly affect unsaturated LCFA degradation. About 1% or less of the unsaturated LCFAs were degraded to C2 to C14 by-products; higher amounts of C6, C7, and C8 than other intermediates were produced. The unsaturated by-products seen were due to β-scission at allylic or vinylic positions. Thermal hydrolysis of LCFAs with digested sludge was also investigated. The amount of VFAs and LCFAs in primary and secondary sludge at 140 and 160 °C was approximately 30-60% higher than at 90-120 °C. The increase in fatty acids was thought to be from lipid degradation in the sludge mixture.

Thermal hydrolysis of fatty acids with different catalysts (high acidity, high alkalinity, metals, and hydrogen peroxide) was also investigated. While saturated LCFAs were stable under all catalytic conditions, unsaturated LCFAs were nearly completely degraded to carbon dioxide (CO_2) via an oxidation process when hydrolyzed with hydrogen peroxide and activated carbon or copper sulfate.

Introduction

Interest in anaerobic co-digestion processes has been growing due to its ability to produce more biogas than conventional digestion systems. Addition of high strength waste, especially fats, oils, and grease (FOG), to anaerobic digesters has been shown to increase methane production (Pinnekamp, 1989; Long et al., 2012; Kim, 2010). However, long chain fatty acids (LCFAs), the main components in FOG, can cause operational problems such as methanogenic inhibition, sludge flotation, clogging, and scum formation (Rinzema et al., 1994; Galbraith and Miller, 1973; Hwu et al., 1998). An early study revealed that β-oxidation is the primary degradation mechanism of LCFAs (Novak and Carlson, 1970b). In the same study, researchers

also found that unsaturated fatty acids quickly become saturated in anaerobic digesters (Novak and Carlson, 1970b). Many studies confirmed that unsaturated fatty acids convert to saturated fatty acids during the early stages of degradation (Lalman and Bagley, 2000). More recent studies showed that oleic acid (C18:1) degraded to palmitic (C16:0) and myristic (C14:0) acids, without undergoing β-oxidation (Lalman and Bagley, 2001). Varin (2013) reported that the insoluble and hydrophobic LCFAs, especially palmitic (C16:0) and stearic (C18:0) acids, formed "grease balls" and accumulate in a layer on top of the digester liquid. These accumulated and aggregated LCFAs and sludge provided no benefits and can cause operational problems for Water Resource Recovery Facilities (WRRFs). Due to abilities to fracture and solubilize complex molecules, thermal hydrolysis pretreatment was believed to be a potential solution for these problems.

Temperature is recognized as an important parameter in thermal hydrolysis pretreatment. The optimum temperature range of thermal hydrolysis was reported to be between 160 to 180 °C; higher temperatures do not improve and even could decrease sludge biodegradability due to the formation of refractory organics (Pinnekamp, 1989; Stuckey and McCarty, 1978; Bougrier et al., 2007). Thermal hydrolysis pretreatment was proven to increase soluble chemical oxygen demand (SCOD) and the production of volatile fatty acids (VFA). The effect of temperature on the solubilization of large molecules, such as proteins, carbohydrates, lipids, COD, etc., has been studied by many authors (Bougrier et al., 2008; Haug et al., 1978).

The mechanisms of lipid degradation to LCFAs were investigated in early studies (O'Rourke, 1968; Crossley et al., 1962). The study concluded that lipids can be thermally degraded to LCFAs and high amounts of VFAs. Wilson (2009) studied thermal hydrolysis of macromolecules, such as proteins, carbohydrates, and lipids, and found that unsaturated lipids, such as glyceryl trilonolenate, were hydrolyzed and produced significant amounts of VFAs as compared to the saturated lipids. Extensive studies have been done on the degradation of lipids to LCFAs; however, only a limited number have focused on LCFA degradation during the thermal hydrolysis of municipal sludge.

Thermal decomposition of lipids and LCFAs is known to require the production of radicals to trigger the reactions. The initial step may take place by decomposition of hydroperoxides via heat or catalytic reactions (Nawar, 1984). Sufficient radicals can create chain

reactions by abstracting hydrogen from complex molecules. Decomposition products and the mechanisms are complicated. Not only can C-C bond scission take place, but numerous other decomposition reactions can simultaneously occur. Many authors have found that LCFAs can be degraded under catalytic and thermal conditions (Nawar, 1969; Youssef et al., 2011; Fu et al., 2010). However, it is very important to note that these experiments were investigated under higher pressure and temperatures (200-500 °C) than for the thermal hydrolysis pretreatment of municipal sewage sludge.

Chemical-thermal pretreatments have been reported to induce cell lysis, increase sludge solubilisation, decrease total solids, and improve dewaterability (Liu, 2003; Rocher et al., 1999; Neyens et al., 2003c). Rocher (1999) reported that acid-thermal hydrolysis treatment induced cell lysis and released dissolved organic carbon (DOC) well at pH 1. In the same study, the authors found that alkaline-thermal treatments produced the same results with less amount of alkaline required as compared to the acid-thermal treatments. Hydrogen peroxide is known as a strong oxidant and used to treat various inorganic and organic pollutants. The decomposition of hydrogen peroxide, with or without catalysts, was shown to disintegrate and decompose organic substances in municipal sludge (Chiou, 1983; Tokumura et al., 2007). Most studies were performed with aggregated chemical compounds without consideration for the effects of the treatments on individual compounds.

In this study, thermal hydrolysis of LCFAs was investigated at 90 – 160 °C under various catalytic conditions (acid, alkaline, metals, and hydrogen peroxide thermal treatments). The purpose of this study was to understand how LCFAs degrade under different thermal hydrolysis temperatures and catalytic conditions, and to discuss the potential problems of FOG addition into thermal hydrolysis reactors.

Materials and methods

Samples and reagents

Thermal hydrolysis experiments were performed with individual, pure LCFAs. The behavior of both saturated fatty acids (C16:0 and C18:0) and unsaturated fatty acids (C16:1,

C18:1, and C18:2) was investigated (Table 1). Primary and secondary sludge used in this study were obtained from the Christiansburg wastewater treatment plant in Virginia, USA.

Table 1 LCFA compounds

Compounds	Supplier	Purity
Palmitic Acid (C16:0) CAS no. 57-10-3	MP Biomedicals, LLC	≥95%
Palmitoleic Acid (C16:1) CAS no. 373-49-9	Acros Organics	≥99%
Stearic Acid (C18:0) CAS no. 57-11-4	MP Biomedicals, LLC	≥90%
Oleic Acid (C18:1) CAS no. 112-80-1	MP Biomedicals, LLC	≥99%
Linoleic Acid (C18:2) CAS 60-33-3	MP Biomedicals, LLC	≥65%

Thermal Hydrolysis Experiments

Individual LCFAs at 30,000 mg/L COD (300 mg of a LCFA in 10 mL de-ionized water) were prepared to investigate the effects of thermal pretreatment on LCFA degradation. Thermal hydrolysis experiments were carried out in Parr No. 4745 acid digestion bombs at typical hydrolysis temperatures (90 – 150 °C) and Parr No. 4749 bombs for higher temperatures (160 – 250 °C). Heat was applied by means of a Fisher Scientific 220 V muffle furnace. Thermal treatments were applied for 30 minutes and 8 hours. The thermal hydrolysis duration started after the warm-up period when the temperature in the furnace reached the desired level. After incubation in the furnace, the samples were cooled in a water bath to room temperature before being analyzed. The corresponding pressures for the selected hydrolysis temperatures were as follows: 90 °C (91 kPa), 120 °C (207 kPa), 140 °C (357 kPa), and 160 °C (616 kPa). In the second phase of this study, additional experiments for thermal hydrolysis pretreatment were carried out by mixing 30,000 mg/L COD of LCFAs (0.1 mL) with 10,000 mg/L COD of primary sludge (8.8 mL).

Thermal hydrolysis with catalyst experiments

Additional experiments, done in the same fashion as previously described, were completed with the presence of catalysts. The experiments were separately performed under different catalytic conditions which consisted of thermal hydrolysis coupled with alkaline, acid, metals, activated carbon, and hydrogen peroxide treatments (Table 2). Calcium chloride ($CaCl_2$, 4 g/L) was obtained from Fisher Scientific Inc. Sodium hydroxide (NaOH, 50%, Mallinckrodt Chemical Works) was used for an alkaline pretreatment condition (pH 13). Hydrochloric acid (HCl, Fisher Scientific inc.) was used to adjust pH for an acid-thermal condition (pH 2.5). The activated carbon (5 g/L) used was coal-based granular activated carbon obtained from Calgon Carbon Corporation. Copper sulfate ($CuSO_2$, 5 g/L, Fisher Scientific Co.) was obtained from Thermo Fisher Scientific Co. Hydrogen peroxide (H_2O_2, 11.9 g/L, 35% aqueous solution) was purchased from the Johnson Metthey Company.

Table 2 Catalyst experimental matrix

LCFA	Catalyst Experiments					
C16:0 C18:0 C18:1 C18:2	Acid-thermal treatment (H2SO4, pH 2.5)	CaCl2 treatment (4 g/L)	Alkaline-thermal treatment (NaOH, pH 13)	H2O2 (11.9 g/L) treatment coupled with activated carbon (5 g/L)	H2O2 (11.9 g/L) treatment coupled with CuSO4 (5 g/L)	H2O2 (11.9 g/L) treatment

Sample Analysis

Volatile fatty acids (VFA) analysis (direct inject method) was performed using a Shimadzu gas chromatograph (GC; Model HP 5890) with flame ionization detector (FID). A Nukol^{TM} column (15m x 53mm capillary column with 0.5 µm film thickness) was used. Helium gas was used as the carrier gas at a flow rate of 16 ml/min along with hydrogen (45 ml/min), air (450 ml/min), and nitrogen (30 ml/min). The temperature was started at 80 °C and gradually increased to 140 °C over 10 minutes.

LCFAs were converted to Fatty Acid Methyl Esters prior to analysis with a gas chromatograph (Model HP 6890) with FID). LCFAs were extracted by adding 2 mL of a hexane:MTBE (1:1) solution and 10 µL of sulfuric acid to 10 mL samples in a glass tube and vigorously shaking the tubes for 45 seconds. The process was repeated three times to maximize the amount of LCFAs extracted. Solvents were then evaporated from the samples under a nitrogen gas stream. A 3-mL transesterification fluid (methanol:chloroform:hydrochloric acid, 10:1:1) was added to the extracted LCFA samples and heated to 90 °C for 30 minutes. Then, 2 mL of extract solution containing a 4:1 mix of hexane and chloroform was used to extract methyl esters for chromatography analysis. Fatty acid methyl esters were analyzed on a GC (Hewlett Packard Model 6890) with FID, using a capillary column (100m x 0.25mm ID x 0.2 µm film thickness) with hydrogen as the carrier gas (35 ml/min). The temperature was held at 170 °C for 2.5 minutes, increased to 250 °C at a rate of 4 °C/min, and then held at 260 °C for 2 minutes.

Alkanes were analyzed by means of a GC-MS system (Model Focus GC series, Thermal Scientific Co.). Extractions were carried out using hexane as a solvent. A Restek Corp. column (30m x 25mm Rxi-5Sil MS column with 0.5 µm film thickness) was used with helium as the carrier gas (1 ml/min). The temperature was held at 40 °C for 3 minutes, increased to 300 °C at a rate of 10 °C/min, and held at 300 °C for 2 minutes. The injector temperature was 250 °C in split mode with a split ratio of 10:1.

Results and Discussion

Thermal hydrolysis of organic substances can be evaluated based on both the transformation of the parent molecules to form lower molecular compounds and the formation of soluble monomeric substances. In this study, hydrolysis efficiencies were calculated based on mass yield (i.e. mg/g parent compound). The hydrolysis products accounted for all fatty acids, C2-C24. Other potential hydrolysis products, such as ethanol, lactic acid, and alkenes, were found to be at negligible levels, except n-alkane by-products.

Effect of thermal hydrolysis on LCFA hydrolysis (30-min duration)

LCFA degradation depended primarily on thermal hydrolysis temperature, reaction time, and degree of saturation of the LCFAs. Typical thermal hydrolysis pretreatment durations were in the area of 30 to 60 minutes (Li and Noike, 1992). In this study, very little to no degradation of both saturated and unsaturated LCFAs was detected with a reaction time of 30 minutes. Small amounts of acetic acid were detected as degradation components for all LCFAs, while oleic acid (C18:1) also produced noticeable amounts of heptanoic acids (C7) (Figure 1C). Measurable heptanoic acid (C7) from oleic acid (C18:1) hydrolysis indicates that free radicals from weak pi-bond cleavage enhance beta scission (March, 1968). Caproic acid (C5) was observed to form at higher concentrations than other VFAs during thermal hydrolysis of linoleic acid (C18:2) (Figure 1E). The findings from this study confirm other studies that showed stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids were stable under high temperatures (up to 160 °C) at a hydrolysis duration of 30 minutes (Shin et al., 2012).

The thermal hydrolysis duration was increased to 8 hours to see if this would improve degradation. VFA production increased significantly compared to the 30-min hydrolysis duration (Figure 1B, 1D, and 1F). Components of the degradation products were similar to that formed from shorter exposure times. At 160 °C, the amount of VFA produced after 8 hours was greater than after 30 minutes; production of C18:0, C18:1, and C18:2 was, respectively, 1,307%, 610%, 325% greater than the 30-min thermal hydrolysis yields. The VFA production of C18:0, C18:1, and C18:2, at 160 C, was approximately 87, 52, and 26 mg/L COD, respectively. The most VFA produced was from stearic acid (C18:0) with a concentration of 87 mg/L COD. However, the degradation of LCFAs was minimal considering the initial LCFA concentration was approximately 30,000 mg/L COD.

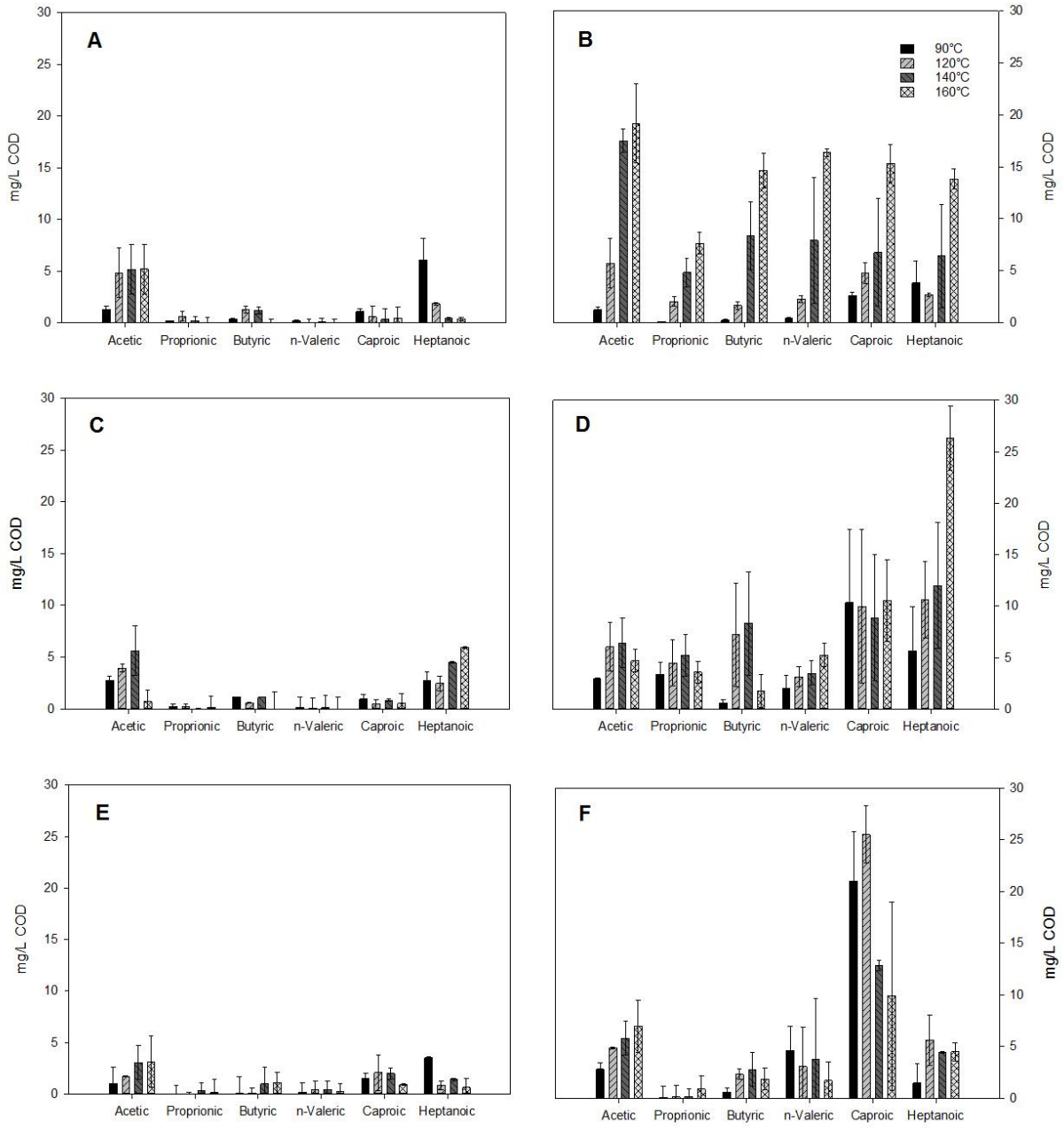

Figure 1 VFA productions at different thermal hydrolysis durations and temperatures: (A) C18:0, 30 minutes, (B) C18:0, 8 hours, (C) C18:1, 30 minutes, (D) C18:1, 8 hours, (E) C18:2, 30 minutes (F) C18:2, 8 hours

Effects of thermal hydrolysis on LCFA degradation (8-hr duration)

After establishing that longer durations of thermal hydrolysis marginally improved LCFA degradation and VFA production, different temperatures and pressures were tested at 8 hours. The production of VFAs from stearic acid (C18:0) at 160 °C contributed an approximately 87 mg/L COD, which was about 0.3% of total fatty acids. The distribution among C2-C7 compounds was similar, with concentrations of 15 mg/L COD for each acid at 160 °C, except propionic acid at 8 mg/L. The component of VFA produced from oleic acid (C18:1) was different with approximately 50% of total C2-C7 fatty acids being heptanoic acid (C7). While oleic acid (C18:1) produced noticeable amounts of C7. Linoleic acid (C18:2) formed high amounts of caproic acid (C6), which contributed approximately 38% of total C2-C7 fatty acids. At 160 °C and 8 hours, it is important to note that measurable amounts of caprylic acid (C8) were also produced from both oleic (C18:1) and linoleic (C18:2) at concentrations of 35 mg/L COD and 55 mg/L COD, respectively.

In this study, saturated LCFAs (C16:0 and C18:0) were non-selectively hydrolyzed to shorter chain fatty acids (C2-C16). This indicated that β-scission was one of the main degradation mechanisms which occurred at non-specific C-C bonds. More degradation was observed at higher temperatures (140-160 °C) than lower temperatures (90-120 °C) (Table 3). At 140 and 160 °C, the increase of C2-C15:0 was observed which indicates that palmitic acid (C16:0) was degraded more at higher temperatures. However, no more than approximately 1% of LCFAs were hydrolyzed under all conditions tested (Table 3).

Table 3 Percent hydrolysis of each LCFA after 8-hr thermal hydrolysis pretreatment

Acid	% Hydrolysis			
	90 °C	120 °C	140 °C	160 °C
C16:0	0.16(±0.12)	0.20(±0.15)	0.82(±0.12)	1.01(±0.08)
C16:1	0.78(±0.13)	0.97(±0.14)	0.75(±0.14)	0.78(±0.11)
C18:0	0.43(±0.11)	0.49±(0.16)	0.73(±0.19)	0.73(±0.06)
C18:1	0.26(±0.10)	0.32±(0.04)	0.34(±0.03)	0.31(±0.00)
C18:2	0.64(±0.30)	1.03±(0.44)	0.85(±0.35)	0.94(±0.41)

The unsaturated fatty acids (C16:1, C18:1 and C18:2) did not show a correlation in degradation products with temperatures (90 – 160 °C). It is important to note that the degradation products of unsaturated fatty acids were observed at hydrolysis temperatures as low as 90 °C. Caproic (C6), heptanoic (C7), and a high amount of caprylic acid (C8) acids were produced as degradation products of palmitoleic acid (C16:1). Similarly, Caproic (C6), heptanoic (C7), and caprylic (C8) acids were produced from oleic acid (C18:1) thermal hydrolysis. Heptanoic acid (C7) increased significantly at hydrolysis temperatures above 140 °C (Figure 1). The increase of C7 with temperature indicated that the β-scission at the allylic position was the main degradation mechanism of oleic acid (C18:1) (Nawar, 1969; Shin et al., 2012). Surprisingly, a high amount of caprylic acid (C8) was seen after an 8-hr thermal treatment (Figure 2). Caprylic acid (C8) is unlikely to be formed because the vinylic bond is much stronger than the allylic bond (Nawar, 1969). Linoleic acid (C18:2) produced high amounts of caproic acid (C6), which indicated that β-scission of linoleic acid (C18:2) also occurred at a C-C bond next to the allylic position. The degradation patterns of each unsaturated LCFA were different from one another, perhaps due to differences in the position of the double-bond in each fatty acid.

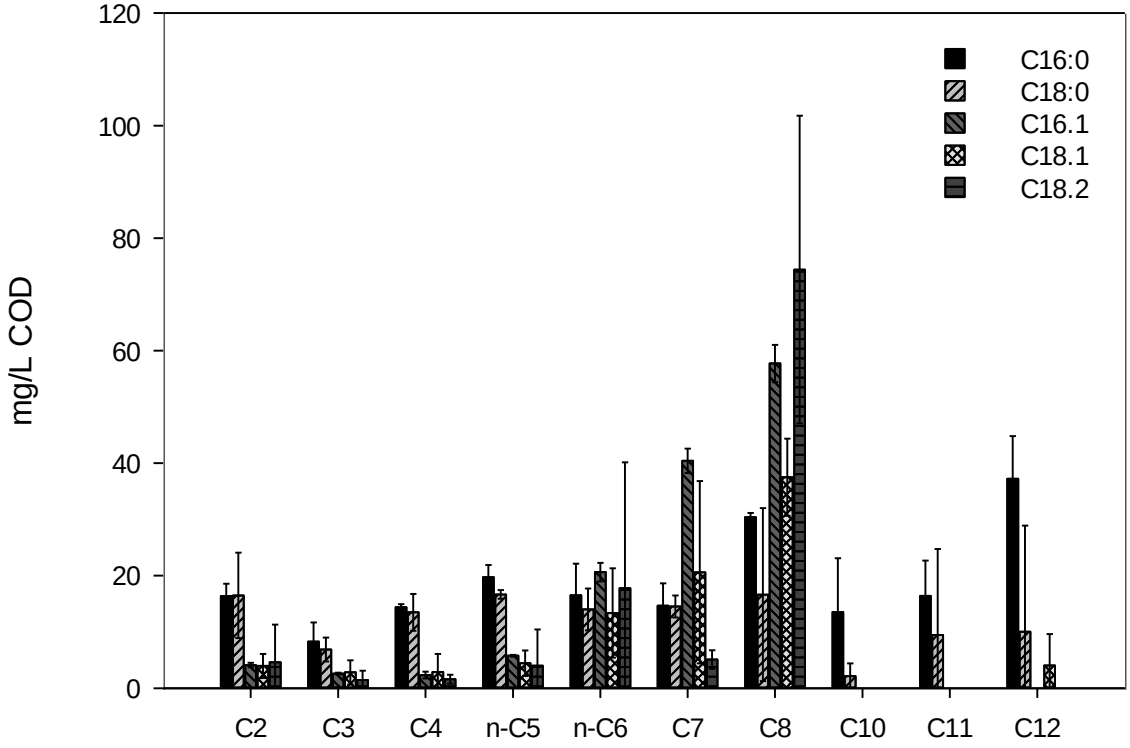

Figure 2 VFA and medium chain fatty acid productions from thermal hydrolysis pretreatment at 160 °C (8-hr duration)

The decomposition products from thermal hydrolysis of LCFAs can be predicted based on this study. Although decomposition products can be accurately measured, the degradation pathways are complex. The degree of saturation, temperature, reaction time, and the stability of decomposition products themselves evidently exert major influences on the decomposition pathways (Fu et al., 2011).

In many studies, authors have reported that saturated fatty acids are more stable than unsaturated ones (Nawar, 1984; Shin et al., 2012). However, saturated fatty acids can also undergo oxidation and generate complex decomposition products. In this study, alkane products were detected from the degradation of saturated fatty acids (C16:0 and C18:0). The n-alkanes were detected at low temperatures (90-160 °C). The findings in this study confirm those reported by Fu et al. (2011), who reported that that saturated fatty acids, such as palmitic (C16:0) and stearic (C18:0), have a relatively high decarboxylation rate as compared to unsaturated fatty

acids. High amounts of long chain alkanes were produced, especially C14-16 alkanes from saturated fatty acids (C16:0 and C18:0) after an 8-hour thermal pretreatment in deionized water (Table 4). Palmitic (C16:0) and stearic (C18:0) acids produced C14 alkanes up to 430 and 197 mg/L COD, respectively. Even though relatively high amounts of alkanes were present, very little shorter chain fatty acids were produced. This indicates that decarboxylation was the main degradation mechanism for saturated LCFAs and β-scission occurred at a much slower rate.

Unsaturated fatty acids are required to undergo hydrogenation to form saturated fatty acids, before decarboxylation can take place (Fu et al., 2011). In this study, however, the results showed that there was no increase in C18:0 production when unsaturated fatty acids (C18:1 and C18:2) were thermally hydrolyzed. This confirmed that no hydrogenation occurred because the stearic acid (C18:0) production was not observed and very little to no n-alkanes were produced after an 8-hr treatment. A possible explanation for this is that there was little hydrogen or other reductants present in the reactors. Nevertheless, unsaturated fatty acids tended to decompose to shorter chain fatty acids instead of converting to alkenes or alkanes.

Table 4 n-alkane productions from at 160 °C and 8 hours (mg/L COD)

n-Alkane	Temperature	Duration	C12	C14	C15	C16
C16:0	160	30m	0.89	3.88	3.13	0.00
C18:0	160	30m	1.58	2.68	2.79	1.01
C18:1	160	30m	0.91	1.27	1.99	1.19
C18:2	160	30m	1.32	1.70	1.37	0.00
C16:0	160	8hr	68.02	430.23	295.39	25.58
C18:0	160	8hr	76.20	196.77	133.36	104.60
C18:1	160	8hr	1.93	2.70	3.54	1.56
C18:2	160	8hr	0.74	1.38	1.37	0.37

Change in viscosity and deformation of saturated LCFAs was observed after 30-min and 8-hr thermal pretreatments. Saturated LCFAs, both C16:0 and C18:0, formed solid substances, which attached onto the vessels at the top of the liquid layer. The solid substances were later found to be the starting compounds. Unsaturated LCFAs (C16:1, C18:1, and C18:2) changed

viscosity and color at higher reaction time (8 hours). After an 8-hr thermal hydrolysis, a thick brown layer of unsaturated LCFAs floated on top of the other liquid. Similar to the saturated LCFAs, the brown liquid was found to be the starting compounds. Oxidation, polymerization, and other chemical changes can cause a color change when oils are heated (Tan et al., 1985). Changes in color can also be explained by the presence of unsaturated carbonyl compounds (Tsaknis et al., 2002). These compounds have the ability to absorb energy of the magnitude of visible light (Tsaknis et al., 2002). The increase in viscosity and darker color of unsaturated fatty acids were similar to the fat/oil under frying conditions at 180 °C (Rani et al., 2010). The increase in viscosity can be affected by its degradation products, such as dimers, trimers, alcohols, and hydrocarbons (Rani et al., 2010). Changes in the viscosity after thermal hydrolysis may cause a reduction in the rate of degradation in anaerobic digesters. Moreover, substances that accumulated on the walls of thermal hydrolysis reactors will be a nuisance.

In the second phase of this study, 100 mg (0.1 mL of 30,000 mg/L COD) of LCFAs and 8.8 gm (8.8 mL of 10,000 mg/L COD) of primary sludge were mixed before heating in a furnace to investigate the effects of sludge on LCFA degradation. After thermal hydrolysis at both 30-min and 8-hr durations, the VFA and LCFA productions of pure LCFAs were subtracted from the mixture of LCFAs and sludge. The LCFA hydrolysis yields were similar to those seen in the first phase. VFA production due to primary sludge hydrolysis was investigated. Significant acetic acid production was found at all temperatures tested (Figure 3). In this experiment, the observations indicate that VFA production from LCFAs was minimal. Therefore, the increase in VFA production during primary sludge hydrolysis was not thought to be from LCFAs. The thermal hydrolysis of primary sludge alone produced high amounts of palmitic acid (C16:0) at 140 and 160 °C.

A greater concern than accumulation of VFA and saturated LCFAs, such as C16:0, is the increase of unsaturated LCFAs, especially C18:1 (Figure 3). Some amounts of C18:1, up to 70 mg/L, were observed when the hydrolysis temperature was increased to 160 °C. Oleic acid (C18:1) is known to cause inhibitory effects on methanogenesis. In a previous study, oleic acid (C18:1) at a concentration of 30 mg/L was found to inhibit aceticlastic methanogens, whereas stearic acid (C18:0) concentrations up to 100 mg/L did not inhibit the process (Lalman and Bagley, 2001). However, the processes receiving the threshold inhibitory loads of LCFAs were

found to outperform the conventional ones in terms of methane production due to high levels of microbial activity in a co-digestion process (Tezel et al., 2008).

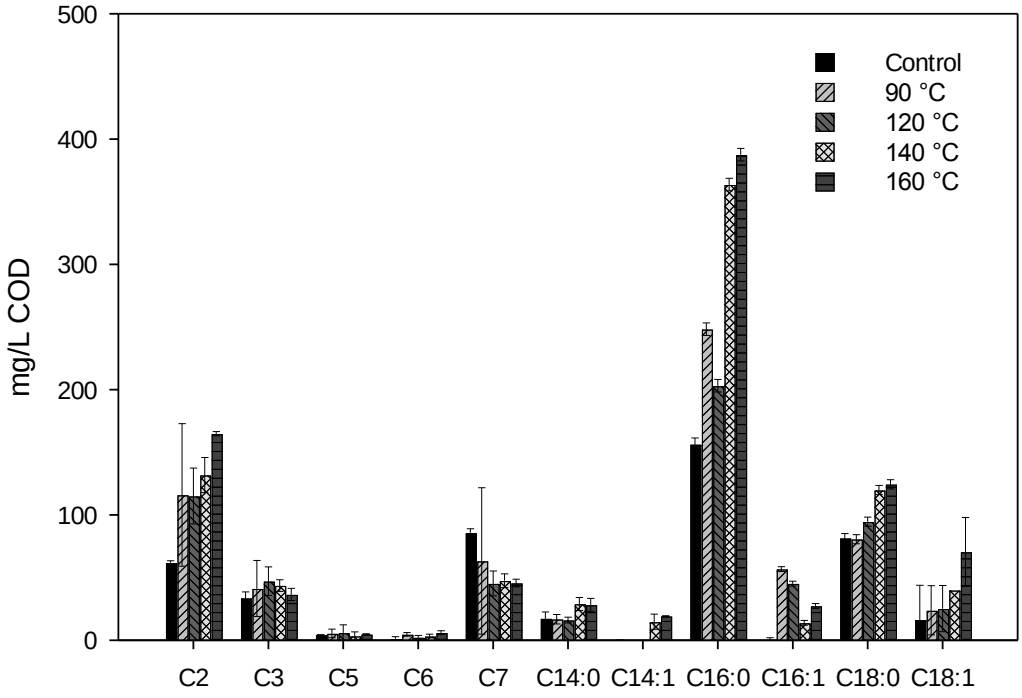

Figure 3 Thermal hydrolysis of primary sludge at different temperatures (30-min duration)

Lipid hydrolysis

Grease trap waste (GTW) from the HRSD Nansemond wastewater treatment plant (Suffolk, VA) was used to investigate the thermal degradation mechanisms of lipids and LCFAs. The VFA production increased with increases in hydrolysis temperatures. Heptanoic acid (C7) production was significant in comparison with other VFAs. At 140 and 160 °C, great amounts of heptanoic acids (C7) were increased up to approximately 100% and 200% of the original concentrations, respectively. As previously stated, the production of heptanoic acid (C7) was assumed to be from degradation of C18:1. The overall increase in percent hydrolysis at 90, 120,

140, and 160 °C was approximately 18%, 12%, 48%, and 218% of the original concentrations, respectively.

The production of small amounts of VFAs and medium chain fatty acids confirms the findings that LCFAs did not degrade well under thermal pretreatment at a temperature range of 90 – 160 °C (30-min duration). However, the increase in both saturated and unsaturated LCFAs illustrates that lipids or longer chain fatty acids degraded to LCFAs during thermal pretreatment, but LCFAs degraded to a lesser extent. At 160 °C, significant amounts of C16:0, C18:0, C18:1, and C18:2 were detected (Figure 4). The increase of LCFAs suggests two possibilities: (1) lipids were hydrolyzed, and (2) grease balls were solubilized during the treatment. Lesser amounts of unsaturated fatty acids were observed than the saturated types. This indicates that grease balls probably solubilized at a faster rate compared to lipid hydrolysis. A large number of possible decomposition reactions could occur during thermal hydrolysis as previously described. However, the stability of both saturated and unsaturated LCFAs indicates that the predominant degradation in thermal hydrolysis was not of LCFAs, but lipids.

An increase in VFA production was observed corresponding to higher temperatures. The accumulation of both saturated and unsaturated LCFAs was found after a 30-min thermal hydrolysis pretreatment (Figure 4). Significant amounts of saturated fatty acids, especially C16:0, accumulated after thermal hydrolysis treatment. At 160 °C, the accumulation of C16:0 and C18:0 acids were greatly increased, up to approximately two times the original concentrations. Saturated fatty acids are believed to be the main cause of maintenance problems. However, a greater concern is the accumulation of unsaturated LCFAs after thermal hydrolysis treatment. Low concentrations of unsaturated LCFA, as low as 10-30 mg/L, were reported to inhibit methanogenesis while saturated LCFAs as high as 100 mg/L showed no sign of inhibition (Lalman and Bagley, 2000). An increase in unsaturated fatty acids (C18:1 and C18:2) can later promote inhibition problems in anaerobic digesters when the hydrolysis temperature are above 140 °C.

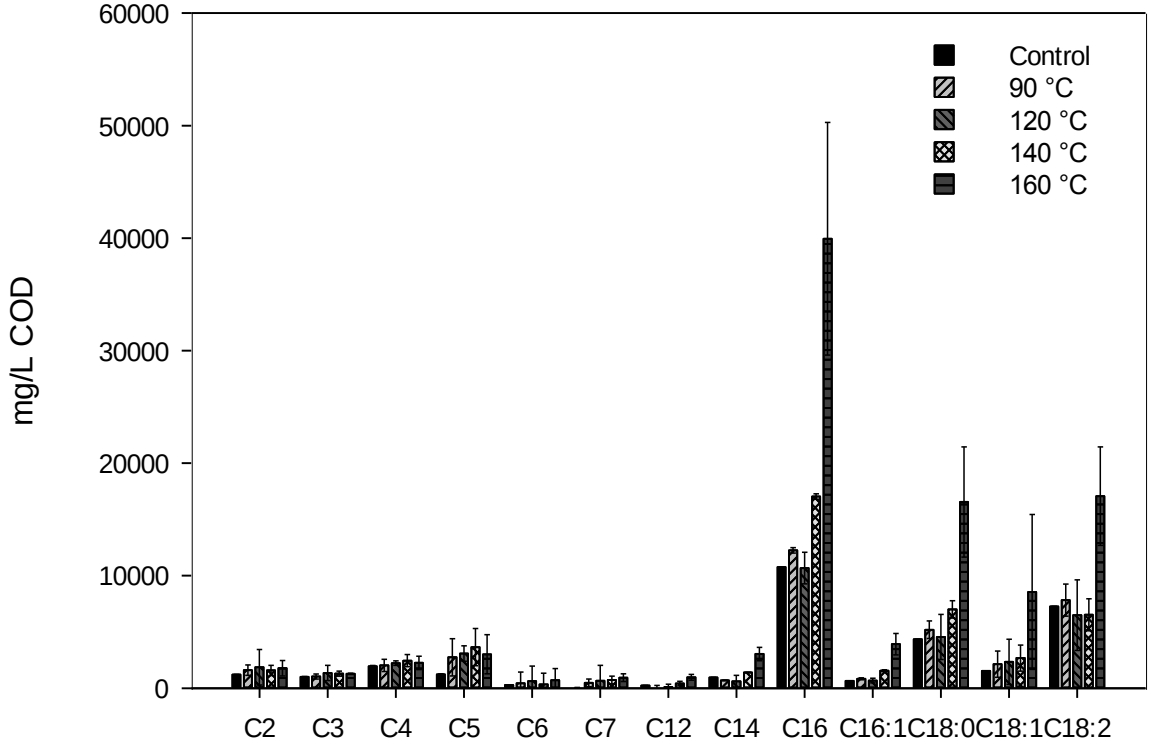

Figure 4 Thermal hydrolysis products of GTW (30-min duration)

Catalyst experiments

Alkaline, acid, and hydrogen pretreatments were investigated to determine if the degradation of LCFAs could be enhanced. Acid-thermal hydrolysis at high temperatures has been shown to induce cell lysis and release dissolved organic carbon (Rocher et al., 1999). However, in this study, acid-thermal treatments did not improve LCFA degradation (Figure 5). Alkaline-thermal treatments have been shown to increase VS solubilization and induce cell-lysis better than acid-thermal treatments (Valo et al., 2004; Rocher et al., 1999). Valo et al. (2004) found that increase in COD solubilization up to 83% was detected at 170 °C (30 minutes) with pH 12. In this study, alkaline treatments were performed by first incubating the LCFAs at pH 13 (24-hr, 25 °C) and then thermally treating the samples at 160 °C for 30 minutes. No degradation of saturated and unsaturated LCFAs was observed. Both saturated and unsaturated LCFAs formed semi-solid substances via saponification.

Addition of hydrogen peroxide alone resulted in a small increase in degradation of both saturated and unsaturated LCFAs, as compared to the controls. A small production of VFAs (C2-C7) was observed from treatments of both saturated and unsaturated fatty acids. However, when LCFAs were hydrolyzed with hydrogen peroxide and activated carbon, the fatty acids produced noticeable amounts of VFAs. C16:0 and C18:0 produced acetic acid (C2) as a main decomposition product (0.80% and 0.60% of total fatty acids, respectively). Saturated fatty acids were moderately stable, even with hydrogen peroxide-thermal treatment coupled with activated carbon. However, unsaturated fatty acids (C18:1 and C18:2) underwent almost complete oxidation to carbon dioxide (CO_2) (Figure 5). GC analysis indicated that hydrogen peroxide (11.9 g/L) coupled with activated carbon (5 g/L) treatment oxidized the unsaturated fatty acids, C18:1 and C18:2 to carbon dioxide (CO_2) as shown in Figure 5. Nevertheless, the oxidation process occurred non-selectively. Small amounts of fatty acids (C2 – C18:2) remained after 30 minutes of thermal hydrolysis. The remaining fatty acids, C18:1 and C18:2, were 0.30% and 0.02% of initial concentrations of the compounds, respectively. The hydrogen peroxide coupled with copper sulfate treatment did not perform as well as peroxide-carbon treatment. Significant VFA products were observed, especially caproic acid (C6) from linoleic acid (C18:2) degradation during peroxide-copper sulfate treatments. The remaining fatty acids, C18:1 and C18:2, were 1.15% and 0.18% of initial concentrations of the compounds, respectively.

The decomposition reaction between unsaturated fatty acids and molecular oxygen via free radical mechanisms is well-established. The initial step requires a few radicals to start the decomposition processes. Both activated carbon and copper sulfate can produce free radicals during the decomposition of hydrogen peroxide. Sufficient free radicals can start the chain reaction by abstracting hydrogen atoms at allylic positions next to the double bonds of unsaturated fatty acids, followed by the attack at these locations to form peroxy radicals, ROO·. Then, the radicals abstract hydrogen from other molecules to form hydroperoxides, which are the main products in lipid autoxidation (Nawar, 1984). The hydroperoxides start the chain reaction, which creates numerous complex breakdowns of the substances. Unsaturated fatty acids oxidized by hydrogen peroxide treatment are converted to CO_2 and H_2O with small amounts of fatty acids remaining. This can explain the significant degradation of unsaturated fatty acids observed in this study. This study also confirms an earlier study by Valo et al. (2004) that hydrogen peroxide treatment tended to oxidize the compounds instead of degrading them. This treatment would not

be beneficial to the co-digestion process due to the loss of valuable, high-energy fatty acids through the oxidation process.

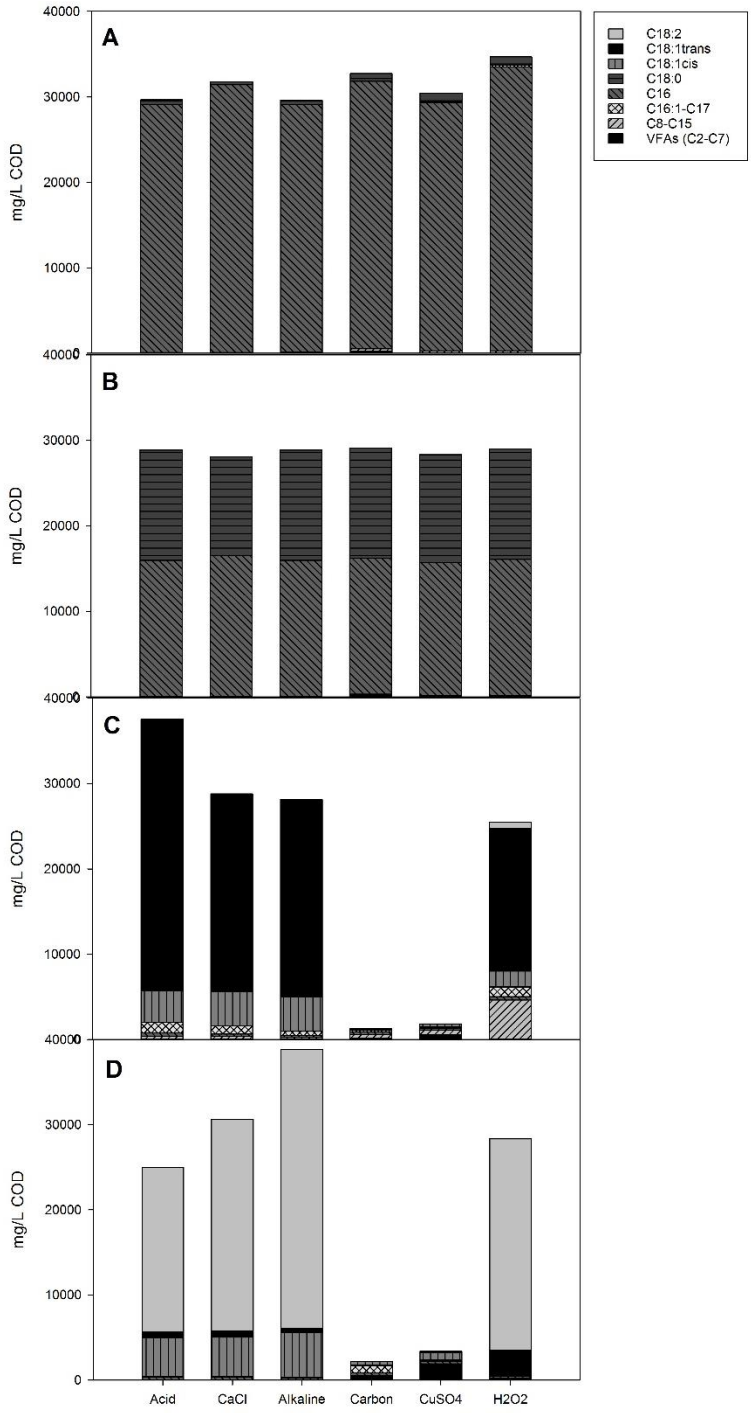

Figure 5 Chemical-thermal experiments (H_2SO_4, pH 2; $CaCl_2$, 4 g/L; NaOH, pH 13; H_2O_2 (11.9 g/L) with activated carbon, 5 g/L; H_2O_2 with $CuSO_4$, 5 g/L; H2O2 (11.9 g/L)) at 160 °C (30-min duration): (A) C16:0, (B) C18:0, (C) C18:1, and (D) C18:2

LCFA and Maintenance Issues

The findings of this study illustrate that LCFAs do not degrade under typical thermal hydrolysis temperatures and retention times. However, the increase in saturated fatty acids from FOG degradation was significant, up to two fold the original concentrations (Figure 4). Another concern is that LCFAs will accumulate in thermal hydrolysis reactors, which will increase operating and maintenance costs. A thermal hydrolysis scale was received from a Cambi reactor. The scale was typically accumulated, especially in a co-digestion reactor. The scale that deposited in the thermal units of this study was found to consist of 11.5% LCFAs and 88.5% of unknown organic compounds. The saturated LCFAs, C16:0 and C18:0, accounted for 46% and 31% of the total LCFAs, respectively (Figure 6).

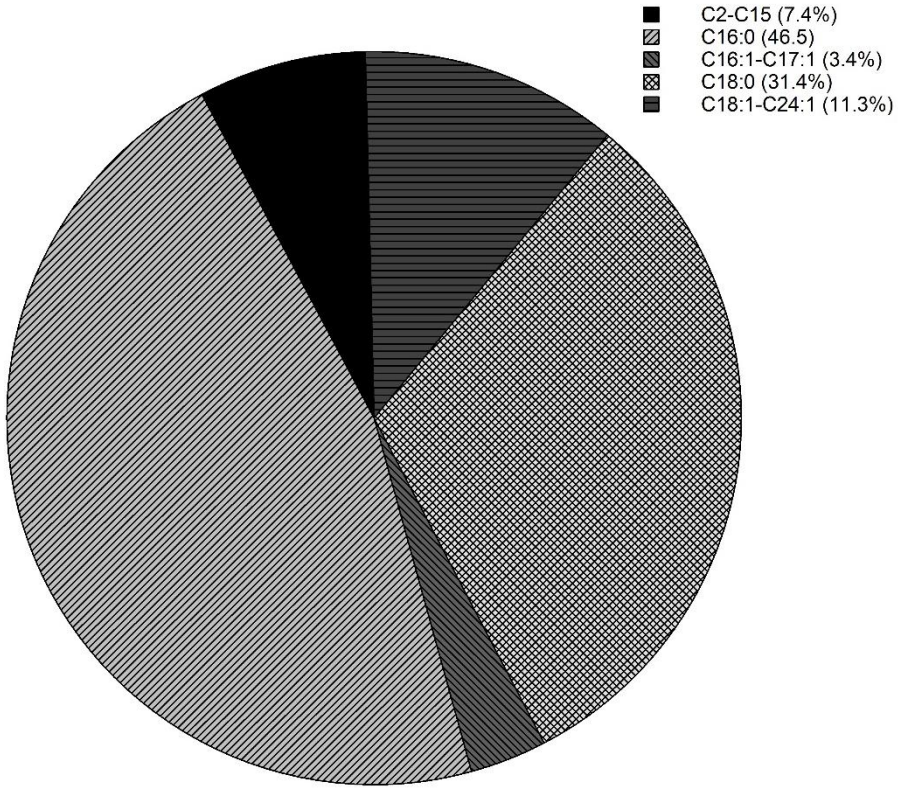

Figure 6 LCFA compositions from thermal hydrolysis reactor scum

The findings of this research indicate that thermal hydrolysis pretreatment does not effectively break down LCFAs to lower molecular compounds. However, the study confirms that thermal hydrolysis could break down more complex molecules, such as lipids or triglycerides,

and increase solubilization of grease balls. The implications of this work for wastewater treatment are that the addition of FOG into thermal hydrolysis reactors needs to be carefully considered. An increases in the degradation of more complex molecules and less degradation of LCFAs will result in LCFA accumulation, which can later inhibit methanogenesis in the anaerobic digesters.

Conclusion

Thermal hydrolysis of both pure saturated and unsaturated LCFAs was investigated at different temperatures (90-250 °C) and durations (30 minutes and 8 hours). In addition, chemical-thermal pretreatments were also investigated. The conclusions from this study are:

1. Thermal hydrolysis of LCFAs at 90-160 °C was not effective at both typical (30 minutes) and extensive (8 hours) thermal treatment durations.
2. Little degradation (less than 1%) of LCFAs was seen in 30-min, thermal hydrolysis pretreatments.
3. Approximately 1% or less of the saturated fatty acids, C16:0 and C18:0, was degraded to shorter chain fatty acids at 140 and 160 °C (8-hr contact). The saturated fatty acids produced more n-alkanes than shorter chain, fatty acids.
4. The degree of saturation had some effect on the thermal hydrolysis of unsaturated fatty acids. Linoleic acid (C18:2) was found to degrade more than C18:1. However, hydrolysis was less than 1% at 160 °C.
5. Chemical-thermal pretreatments were found to be ineffective in treating saturated LCFAs. However, hydrogen peroxide coupled with activated carbon or copper sulfate almost completely degraded unsaturated LCFAs via oxidation to carbon dioxide (CO_2).
6. A significant increase in C16:0, C18:0, C18:1, and C18:2 was found when FOG was thermally treated at 160 °C (30 minutes). This supports the finding that both saturated and unsaturated LCFAs were stable under typical thermal hydrolysis pretreatments.

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Chapter 4: Influence of pH and FOG Degradation in Acid-Phase Co-Digestion

Abstract

Semi-continuous, acid-phase (2-d SRT) co-digestion reactors under different pH conditions (control, 4.5, 5.5, 6.0, 6.5, and 7.0) were studied in order to understand the effects of pH and FOG degradation patterns in acid-phase reactors. Increases in soluble chemical oxygen demand (SCOD) were observed in all acid-phase reactors. Approximately 23-38% of the total COD (TCOD) was solubilized and maximum volatile fatty acid (VFA) concentrations of 10,700 mg/L were achieved at pH 6. Little total gas and methane were produced in all acid-phase digesters. Unsaturated long chain fatty acids (LCFAs), especially C18:1, were almost completely degraded while saturated LCFAs, especially C18:0, were produced. Non-degraded LCFAs, mainly C16:0, accumulated on top of the liquid layer. The pH was found to have a significant impact on a LCFA accumulation in the APDs. Higher pH levels in the APDs correlate to less accumulation of LCFA materials.

Gas-phase (20-d SRT) reactors (approximate pH 7.1) receiving the effluents from the acid-phase reactors were also monitored. The greatest methane production of 0.39 L methane/day-L reactor was observed in the gas-phase reactors that received inputs from the acid-phase digesters at pH 6.

Introduction

In recent years, great interest has developed in the co-digestion of municipal sewage sludge and high strength wastes such as fats, oils, and grease (FOG). Addition of these high energy compounds to anaerobic digestion has been shown to help decrease biosolids in the effluent and increase biogas production.

Two-phase digestion (TPD), an advanced anaerobic treatment process consisting of an acid-phase digester (APD) and a gas-phase digester (GPD), is growing popular due to its ability to shorten solids retention time (SRT), increase methane production, and digest fats, oils, and grease (FOG) efficiently.

Lipids and triglyceride esters in FOG were reported to be rapidly hydrolyzed to fatty acids when fed to anaerobic digesters (Heukelekian and Mueller, 1958). However, long chain fatty acids (LCFAs), the main components in FOG, were found to cause operational problems, including inhibition of methanogens, sludge flotation, clogging, and scum formation (Rinzema

et al., 1994; Galbraith and Miller, 1973; Hwu et al., 1998). In an early study, investigators found that β-oxidation was the primary degradation mechanism of LCFAs (Novak and Carlson, 1970b). In the same study, authors reported that unsaturated fatty acids quickly become saturated in anaerobic digestion (Novak and Carlson, 1970b). Unsaturated fatty acids were later confirmed to convert to saturated fatty acids during the early stages of anaerobic digestion (Lalman and Bagley, 2000). More recently, authors reported that oleic acid (C18:1) was degraded to palmitic (C16:0) and myristic (C14:0) acids without undergoing β-oxidation (Lalman and Bagley, 2001). The insoluble and hydrophobic LCFAs, especially palmitic (C16:0) and stearic (C18:0) acids, formed "grease balls" and accumulated in a layer on top of the digester liquid (Varin, 2013). These accumulated and aggregated LCFAs and sludge provide no benefits to Water Resource Recovery Facilities (WRRFs).

Most investigators reported that palmitic acid (C16:0) was the main LCFA that caused accumulation in the anaerobic digestion systems (Pereira et al., 2002; Bishnoi, 2012; Kabouris et al., 2009). High amounts of palmitic acid (C16:0) were reported in other studies to be adsorbed onto biomass in enhanced granular sludge bed (EGSB) systems. The authors also found that LCFA inhibition was reversible once LCFAs that adsorbed onto biomass were metabolized (Pereira et al., 2005; Palatsi et al., 2012).

Even though a high saturation rate of unsaturated LCFAs is the main cause of LCFA accumulation, there are only few studies on the effects of operational parameters to mitigate these problems. Miron et al. (2000)suggested that the high partial pressure of hydrogen gas could cause saturation of unsaturated LCFAs and accumulation of saturated LCFAs. Varin et al. (2013) also found that higher pH levels result in less accumulated LCFA materials. Many authors reported that various optimum pH conditions for the APD ranges from 5.2 – 7.9, but none mentioned LCFA accumulation (Demirel and Yenigün, 2002). Different strategies have also been investigated in order to prevent LCFA inhibition and accumulation. Addition of easily-degradable substrates, such as glucose or cysteine, in the early stages of inhibition (Kuang et al., 2006), addition of adsorbents, such as bentonite, (Angelidaki et al., 1990), dilution of the reactor's content with inoculum (Palatsi et al., 2009), and saponification (Battimelli et al., 2010) were proposed to help with recovery and prevent LCFA inhibition.

Despite many efforts to prevent LCFA inhibition and accumulation, some fundamental parameters, such as pH, have not been fully investigated. In this study, the effects of pH on LCFA degradation in acid-phase reactors and overall operational performance were investigated.

Materials and Methods

Feed Characteristics

Municipal sludge was obtained from the Christiansburg Wastewater Treatment Plant (Christiansburg, VA). Seed sludge was taken from the anaerobic digester at the plant. Primary and secondary sludge mixtures at a ratio of 70% to 30% were stored at 4 °C until used to feed the digesters. FOG was shipped from the HRSD Nansemond wastewater treatment plant in Suffolk, VA. Large suspended particles were removed to mitigate clogging problems in the reactors. FOG was mixed a paddle mixer for 5-10 seconds to ensure homogeneity before feeding. The feed components were as follows:

Table 5 Feed Characteristics

Component	Feed Sludge	FOG
COD (g/L)	33.6 (±1.55)[a]	133.9 (±5.29)
Overall Feed as % COD	80 (±1.22)	20 (±0.27)
Total Solids (g/L)	24.7 (±0.11)	42.8 (±0.06)
Volatile Solids (g/L)	22.4 (±0.09)	40.8 (±0.36)
LCFA (g/L COD)	2.81(±0.96)	93.9 (±35.9)

[a] Bracketed values show 95% confidence interval

Two-Phase Co-digestion

Four sets of sequential acid-phase and gas-phase digesters were constructed for this study. High-density polyethylene batch fermentation reactors supplied by the Hobby Beverage Equipment Company (Temecula, California) were used for two-phase reactors and sealed airtight

for anaerobic conditions. Peristaltic pumps were used to continuously recirculate gas in these conical-bottom vessels to mix the reactors and suspended gritty material. All digesters were operated at 37 °C.

Semi-continuous, acid-phase digesters (APD) were operated at a 2-day SRT. Feeding and wasting was done manually, twice a day. The vessels had a volume of 25 L, but were operated at 4 L. The APDs were operated at different pH conditions in order to study the effects of pH (Table 6). Two separate operating period were conducted under identical operating conditions except for the pH in each digesters. A schematic representation of the two-phase co-digestion systems during the first operating period is shown in Figure 7. The mixture of primary and secondary sludge (70:30) and fat, oils, and grease (FOG) were mixed before feeding into the systems with a FOG loading comprising 20% of the total COD (TCOD). All digesters were operated for 15 days to ensure steady state before sampling began. Sampling occurred three times a week prior to feeding the sludge each day. The experiments were separated into two operating periods. For the first operating period, four acid-phase digesters where operated at different conditions where the control received no FOG addition, one APD received 20% FOG without pH buffering (pH 4.5), and the other two APDs received 20% FOG with K_2HPO_4 buffer (pH 6.0 and pH 7.0). The APDs were cleaned to investigate an accumulation of grease balls before the second operating period began. In the second operating period, the four acid-phase digesters were operated in the same way where all APDs received FOG addition, but at different pH levels. One APD was operated without pH adjustment, whereas the other three were operated at pH levels of 5.5, 6.0, and 6.5.

Four gas-phase digesters (GPD) receiving effluents from the acid-phase reactors were operated at a 20-day SRT with an active volume of 15 L. GPDs were fed with 375 mL of APD effluents twice a day. All reactors were operated for 20 days to ensure steady state before sampling began.

Table 6 Operational parameters

FOG Addition (% of total COD)	Steady-state pH	Buffer Addition (K_2HPO_4) (g-day-1)
0	4.5	0
20	4.4	0
20	5.5	26
20	6.0	32
20	6.5	40
20	7.0	46

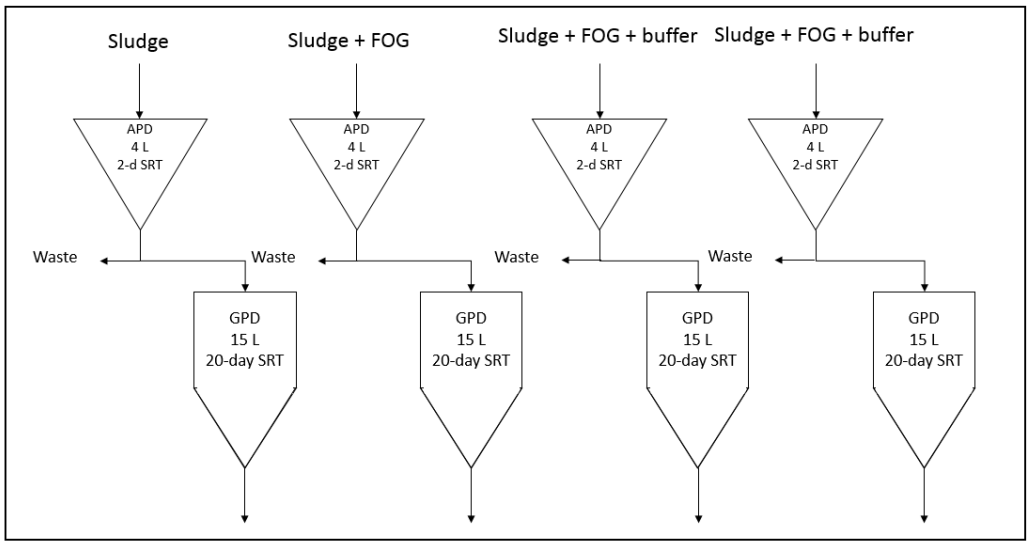

Figure 7 Schematic representation of two-phase co-digestion systems during the first period

Analytical Methods

Total solids (TS), total volatile solids (TVS), chemical oxygen demand (COD) were measured according to Standard Methods 2540B, 2540B, and 5220C, respectively (Clesceri et al., 1998). The pH was measured using an Oakton pH 11 series pH meter with temperature compensation. The meter was calibrated daily with standard buffer solutions (Cole-Parmer Instrument Co., Chicago, IL).

Volatile Fatty Acid Analysis

Samples were centrifuged for 5 minutes at 35,000 rpm (IEC NH-SII centrifuge). Then, the supernatants were filtered through a 0.45 µm membrane filter prior to being analyzed for volatile fatty acids (VFA). The analysis was performed using a Shimadzu gas chromatograph (Model HP 5890) with flame ionization detector (FID). A Nukol^{TM} column (15m x 53mm capillary column with 0.5 µm film thickness) was used. Helium gas was used as the carrier gas at a flow rate of 16 ml/min along with hydrogen (45 ml/min), air (450 ml/min), and nitrogen (30 ml/min).

Fatty Acid Methyl Esters Analysis

LCFAs were converted to Fatty Acid Methyl Esters (FAMEs) prior to analysis with a gas chromatograph (GC; Model HP 6890) with FID). LCFAs were extracted by adding 2-mL of a hexane:MTBE (1:1) solution and 10 µL of sulfuric acid to 10 mL samples in a glass tube, which was vigorously shaken for 45 seconds. The process was repeated three times to maximize the amount of LCFAs extracted. Solvents were then evaporated from the samples under a nitrogen gas stream. A 3-mL transesterification fluid (methanol:chloroform:hydrochloric acid, 10:1:1) was added to the extracted LCFA samples and heated to 90 °C for 30 minutes. Then, a 2-mL extract solution containing hexane and chloroform at a 4:1 ratio was used to extract methyl esters for the chromatography analysis. Fatty acid methyl esters (FAME) were analyzed on a GC (Hewlett Packard Model 6890) with FID using a capillary column (100m x 0.25mm ID x 0.2 µm film thickness) with hydrogen as the carrier gas (35 ml/min). The temperature was held at 170 °C for 2.5 minutes, increased to 250 °C at a rate of 4 °C/min, and then held at 260 °C for 2 minutes.

Headspace Analyses

Headspace analyses for methane (CH_4) and carbon dioxide (CO_2) were performed using a Shimadzu GC (Model GC-14A) with a thermal conductivity detector (TCD). A Haysept D column with 4-m length and a 6.35 mm inner diameter was used. Helium was used as the carrier

gas at a flow rate of 17 mL/min. The TCD temperatures were 40, 70, and 110 °C with a detector current of 150 mA.

Results

Effect of pH on acid-phase co-digestion of FOG and the overall performance of TPD

The effect of pH on hydrolysis and acidogenesis in acid-phase co-digestion systems was investigated in this study. The pH of all APDs decreased from 7.2 to 4.5 within the first 12 days (Figure 8). Adjustments in pH were initiated on day 19.

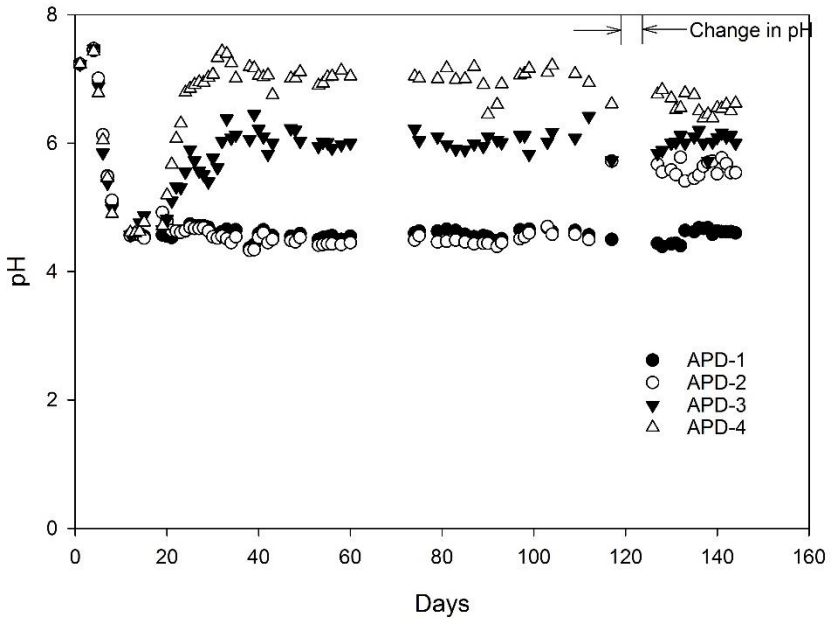

Figure 8 pH conditions in the acid-phase digesters during both operating periods

The pH adjustment obviously affected solubilization of sludge (Figure 9). The increase in soluble chemical oxygen demand (SCOD) was noticeable at a pH of 6.0. The SCOD of the APDs, at pH 4.5, 5.5, 6.0, 6.5, and 7.0, increased by up to 23%, 27%, 38%, 26%, and 29% of the total chemical oxygen demand (TCOD), respectively. Generally, the SCOD was not significantly different among APDs with a controlled pH. However, the pH-6 APD had significantly higher SCOD and VFAs than the one without pH adjustment (Figure 10). VFA production was found to

correspond to increase in SCOD. The maximum VFA concentration was detected at pH 6.0 (10.7 g/L COD). A decrease in VFA production was found with the APDs at pH 6.5 and 7.0, as compared to VFAs at pH 6.0. However, all VFA production from APDs with pH adjustment was higher than the control (6.5 g/L COD). It is important to note that the percent VFA production accounted for approximately 81% to 128% of SCOD. This indicates that SCOD was mainly from VFAs. At pH 6, maximum carbohydrate hydrolysis and VFA production could come from the suitable environments for acid-forming bacteria (Demirel and Yenigün, 2002; Zoetemeyer et al., 1982). However, at low pH conditions (pH 5.0), undissociated acids can be produced that will inhibit acidogenesis(Babel et al., 2004).

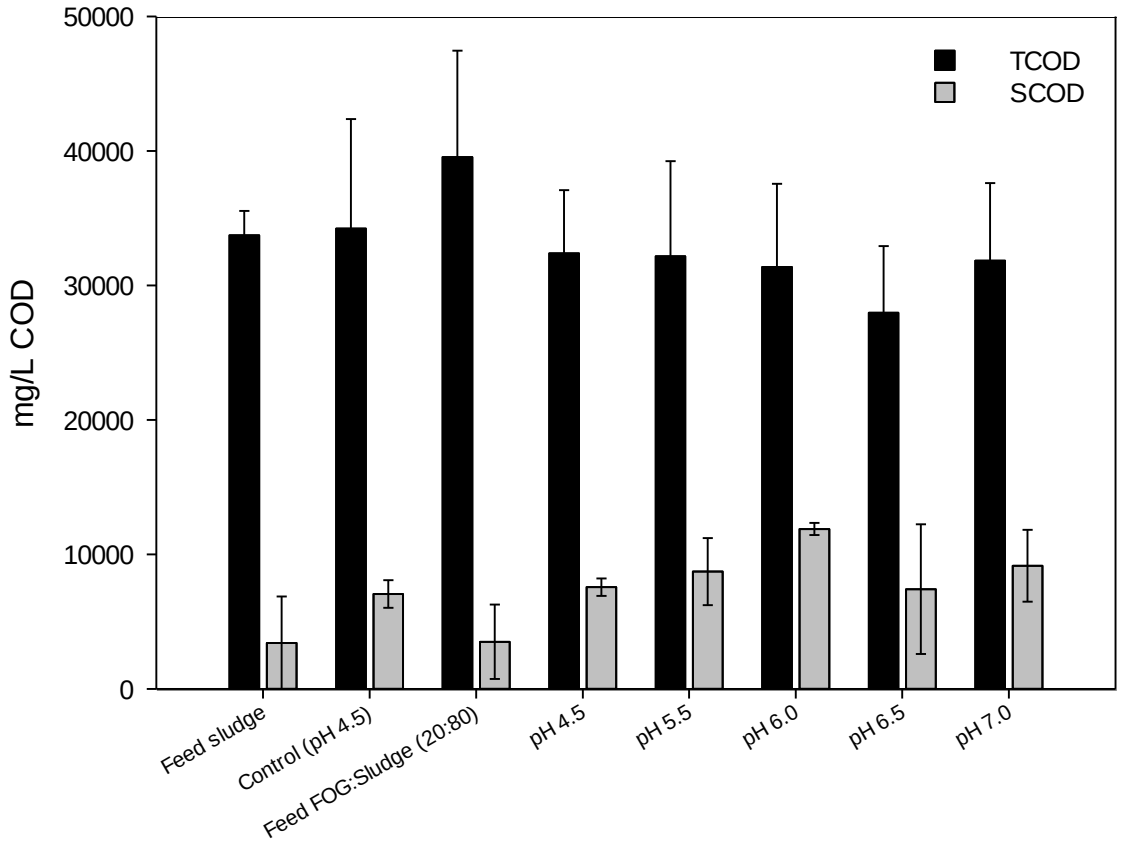

Figure 9 Chemical oxygen demand of the feed sludge and APD effluents at different pH conditions

During anaerobic digestion, carbohydrates, proteins, and lipids can be converted to VFAs. The distribution of VFA depends on the pH. The main VFA product from the APDs with

a controlled pH (pH of 5.5, 6.0, 6.5, and 7.0) was acetic acid. The acetic acid, at pH 6 and 7, accounted for 36% and 33% of the total VFAs, respectively. Propionic acid was found to be the main VFA constituent at pH 4.5, accounting for 39% of the total VFA (Figure 11). Propionic acid as a main product is not desirable in anaerobic digestion since methanogens cannot directly utilize the product (Harper and Pohland, 1986). Horiuchi et al. (2002) suggested that selective production can be controlled by adjusting the pH. However, no significant difference in VFA distribution was observed at the different pH conditions of this study.

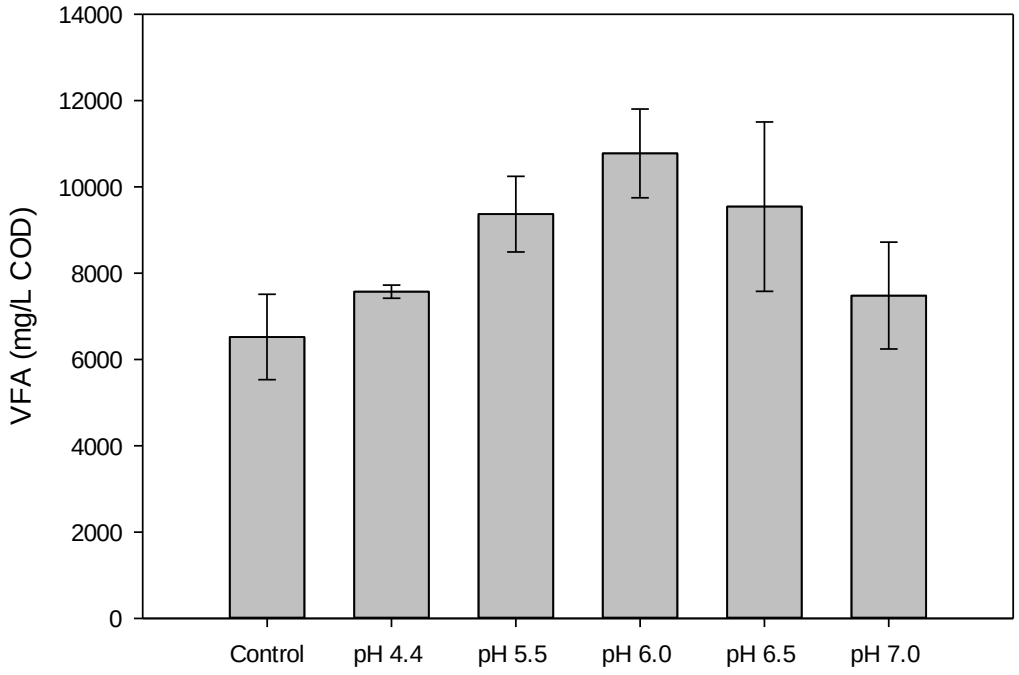

Figure 10 VFA production in acid-phase digesters

The increase in hydrolysis and acidogenesis rates directly correlated to biogas production. At pH 6, methane production was significantly different than at the lower pH conditions (Figure 12). However, there was no significant difference in methane production among the higher pH conditions (pH 6.0, 6.5, and 7.0), with production rates of 5.9, 5.1, and 5.3 L of CH_4/day/15-L digester, respectively. Nevertheless, methane production from GPDs with FOG addition was significantly different than the GPD without FOG addition. At pH 4.5, 5.5, 6.0, 6.5, and 7.0, methane production increased, as compared to the GPD without FOG addition, by 55%, 66%,

107%, 81%, and 86%, respectively. Correlations between VFA production from APDs and methane production from GPDs were observed (Figure 13). The VFAs that were readily converted to methane gas corresponded to higher methane production. LCFAs might also play an important role in producing methane. This can be observed, at pH 7, where relatively high methane in the GPD was produced with low VFAs, but high LCFAs, from the APD. The biogas primarily consisted of methane and carbon dioxide (approximately 67% methane) in all gas-phase reactors with FOG addition. Methane in the gas-phase reactor without FOG addition was approximately 64% of total biogas. No significant difference among methane production was observed in the GPDs with FOG addition. The results generally illustrate the benefits of co-digesting municipal sludge with FOG.

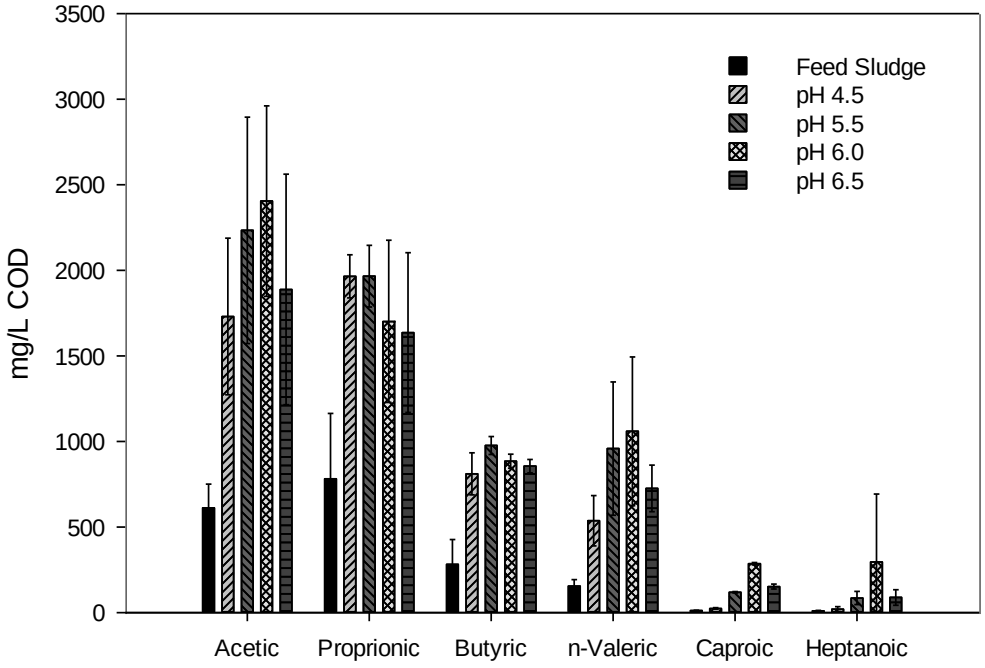

Figure 11 VFA composition in each acid-phase digester

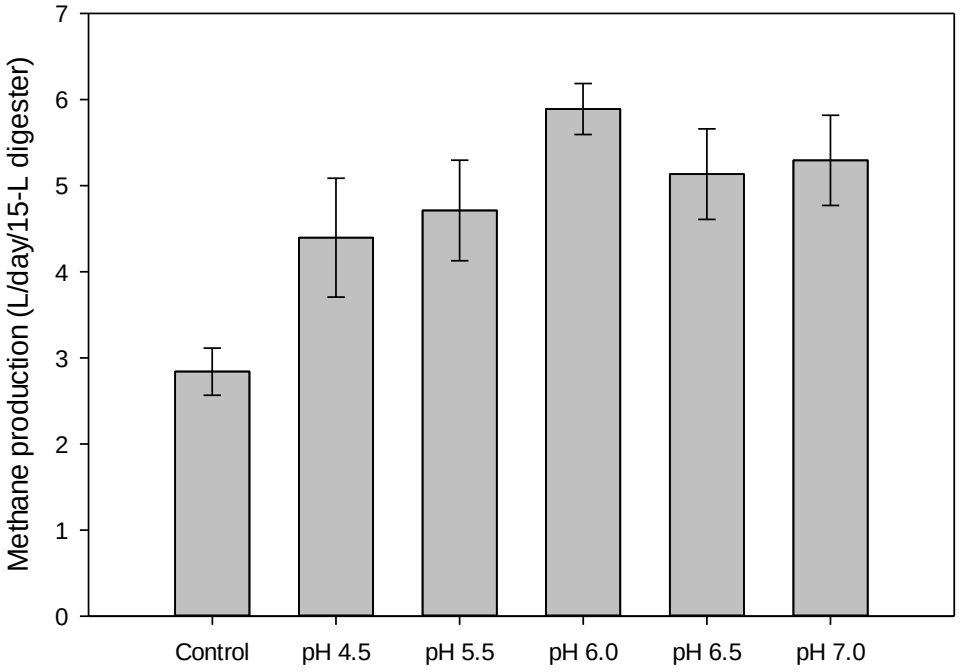

Figure 12 Methane productions from gas-phase digesters at different pH conditions

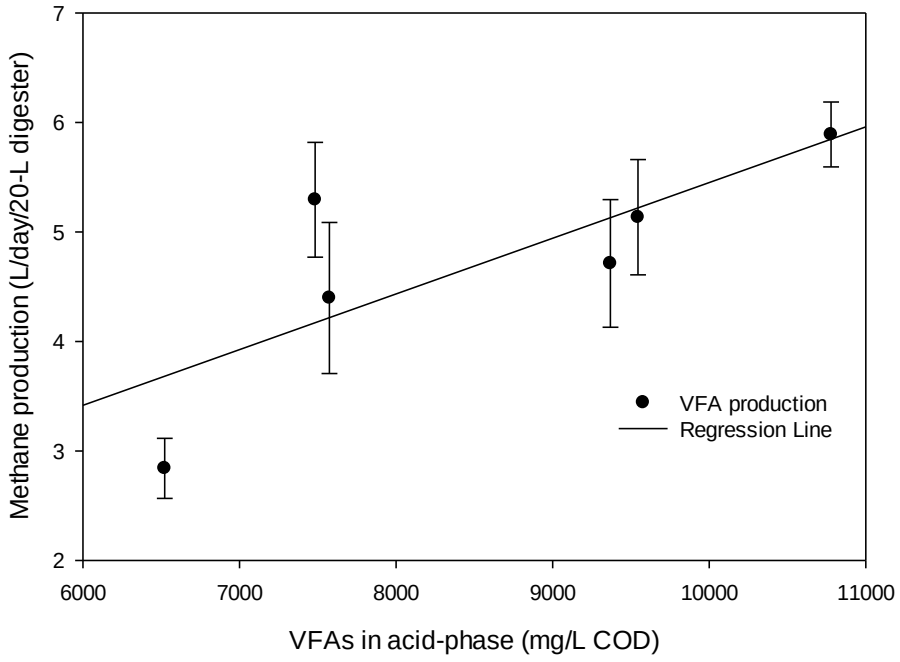

Figure 13 Correlations between methane production in GPDs and VFAs in APDs

FOG degradation mechanisms in APDs

The main LCFA concentrations in feed sludge, GPD effluents, and APD effluents are shown in Table 7. Oleic acid (C18:1) accounted for approximate 34% of total LCFAs in feed sludge with FOG addition, while palmitic acid (C16:0) accounted for 36%. Unsaturated fatty acids were almost completely degraded, while saturated fatty acids were produced or not degraded in the acid-phase systems (Figure 14). According to the mass balance analysis, the percentages of LCFA recovery from APD effluents at pH 4.5, 6.0, and 7.0 were approximately 43%, 67%, and 62% of the total LCFAs from the feed sludge, respectively. Assuming no saturated LCFA degradation in the APDs, the low percent recovery of the LCFAs might have resulted from LCFA adsorption onto biomass that passed in the effluent or accumulation at the surface of the APDs. In other literature, palmitic acid (C16:0) was found to be adsorbed onto biomass surfaces (Pereira et al., 2005; Palatsi et al., 2012). Palmitic acid (C16:0) was present in less amounts in the APDs' effluents, especially at pH 4.5. This could be the result of LCFA accumulation that was later seen in the APDs.

Table 7 LCFA concentrations (mg/L COD) of feed sludge and digesters' effluents from both APDs and GPDs

	12:0	**14:0**	**15:0**	**16:0**	**16:1**	**18:0**	**18:1**	**18:2**	**18:3**
Feed Sludge	26	157	32	1238	23	638	249	36	9
Feed Sludge (20%FOG)	62	289	48	1910	65	917	1831	192	49
APD (Control)	23	126	30	1103	26	811	166	13	5
GPD Effluent	**0**	1	0	46	0	19	5	2	0
APD (pH 4.5)	26	146	28	1172	10	660	263	17	7
GPD Effluent	0	4	0	74	0	30	13	5	0
APD (pH 5.5)	29	148	31	1182	22	1055	177	40	4
GPD Effluent	0	0	0	43	0	19	10	3	0
APD (pH 6.0)	32	179	41	1541	20	1640	148	10	8
GPD Effluent	0	3	0	60	0	32	10	2	0
APD (pH 6.5)	43	172	37	1348	26	1286	173	23	6
GPD Effluent	0	0	0	45	0	31	9	3	0
APD (pH 7.0)	46	209	43	1510	16	1327	203	8	6
GPD Effluent	0	4	0	62	0	35	11	2	0

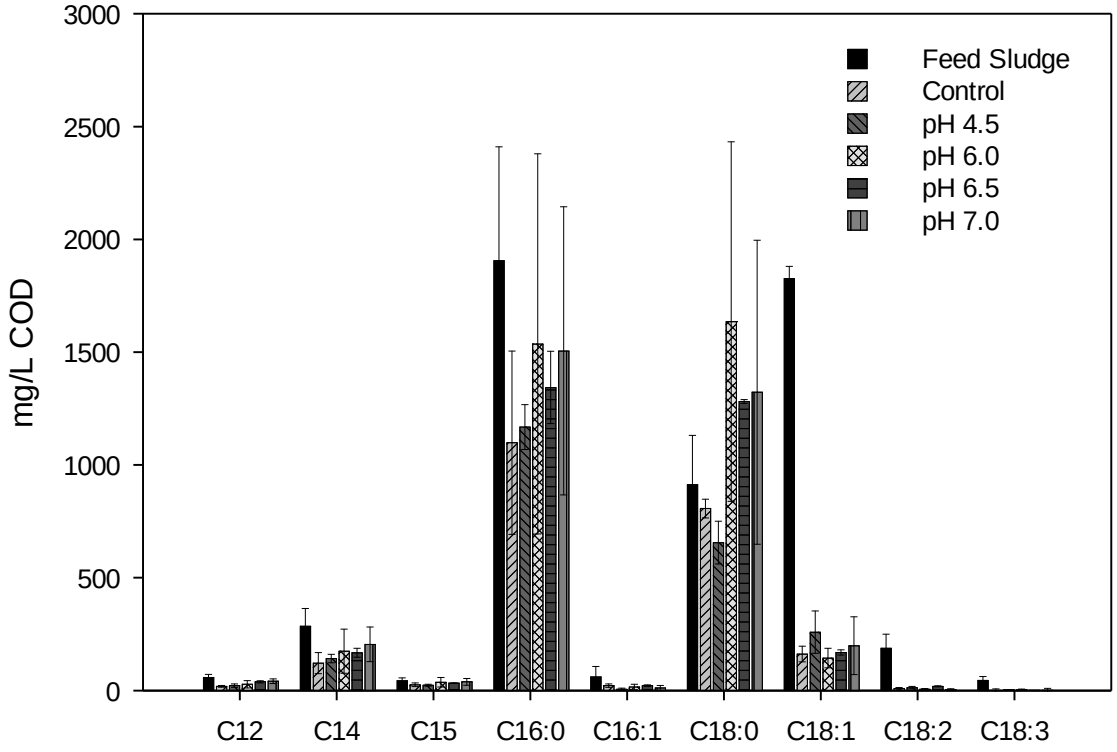

Figure 14 LCFA concentrations (mg/L COD) of feed sludge and APD effluent at different pH

The patterns of degradation and production of LCFAs were similar in all APDs, where unsaturated fatty acids were degraded and saturated fatty acids were produced or not degraded (Figure 15). APDs effectively removed unsaturated LCFAs, especially C18:1, which was one of the most abundant fatty acids seen in this study. Cis/trans isomerism of unsaturated 18-carbon chains were found in this study to have different degradation properties. Only 60% of C18:1cis was degraded, while almost 100% of C18:1trans was degraded in all of the APDs. Stearic acid production was observed in all of the APDs with pH adjustment, while no C18:0 production was found in the APD without pH adjustment. The absence of C18:0 production can explain the accumulation of 14.3 g LCFA material in the APD without pH adjustment at the end of the trial. C16:0 and C18:0 were noted to be the main components of grease balls that accumulated on top of the liquid layers. The production of C18:0 is not recognized as an intermediate product of the

degradation of unsaturated 18-carbon chains. One possible explanation for the C18:0 production is that unsaturated C18-carbon chains might be saturated by the high H^+ ion concentration or high partial pressure of hydrogen gas in the APDs (Miron et al., 2000).

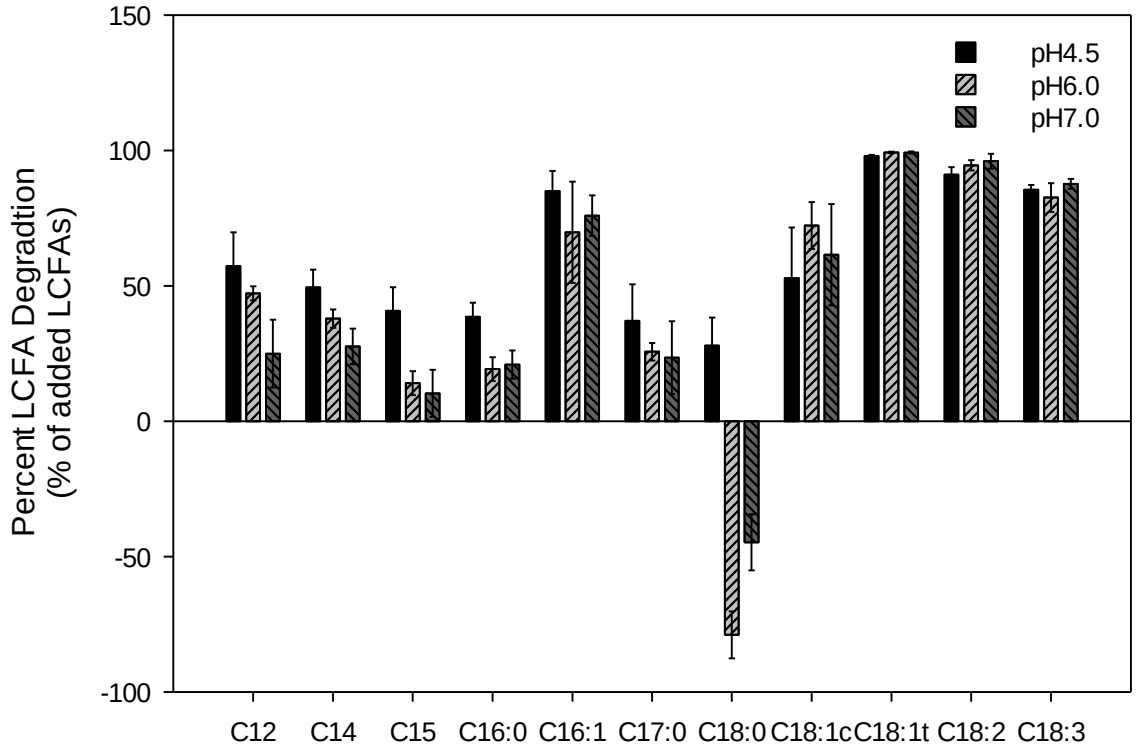

Figure 15 Percent LCFA degradation in the APD effluents where negative values indicate the production

Effect of pH on LCFA accumulation on top layer of sludge fluids

Accumulated LCFA material was found floating on top of the liquid layers after each sampling period. The accumulated LCFA material (or "grease balls") was present in all digesters, including the one without FOG addition. However, very little to no LCFA accumulation was found in the APD without FOG addition, as shown in Table 8.

Table 8 Total VFA, LCFA, and accumulated LCFA materials over 112-day period (first operating period)

	pH	Total VFA (mg/L)	Total LCFAs (mg/L)	Accumulated LCFAs (g)	Accumulated LCFA (g/L)
Feed	5.25 (±1.30)	1703 (±526)	2033 (±547)	0	0.0
Control	4.54 (±0.08)	4113 (±1509)	749 (±163)	1.0	0.3
pH 4.5	4.46 (±0.11)	4508 (±1509)	838 (±720)	14.3	3.6
pH 6.0	5.95 (±0.38)	6034 (±1751)	1292 (±1072)	6.0	1.5
pH 7.0	7.00 (±0.20)	4901 (±1320)	1209 (±577)	3.5	0.9

At pH 4.5, the maximum amount of LCFA material measured was 14.3 grams. It is important to note that the accumulated LCFA material with a diameter less than about 5.0 mm was not quantified due to the difficulty in removing it from biomass. Digesters with higher pH levels had higher VFA and LCFA concentrations in effluents and less accumulated LCFA materials. This indicates that high pH can cause LCFAs to become more soluble and less likely to precipitate. The composition of accumulated LCFA material is shown in Figure 16.

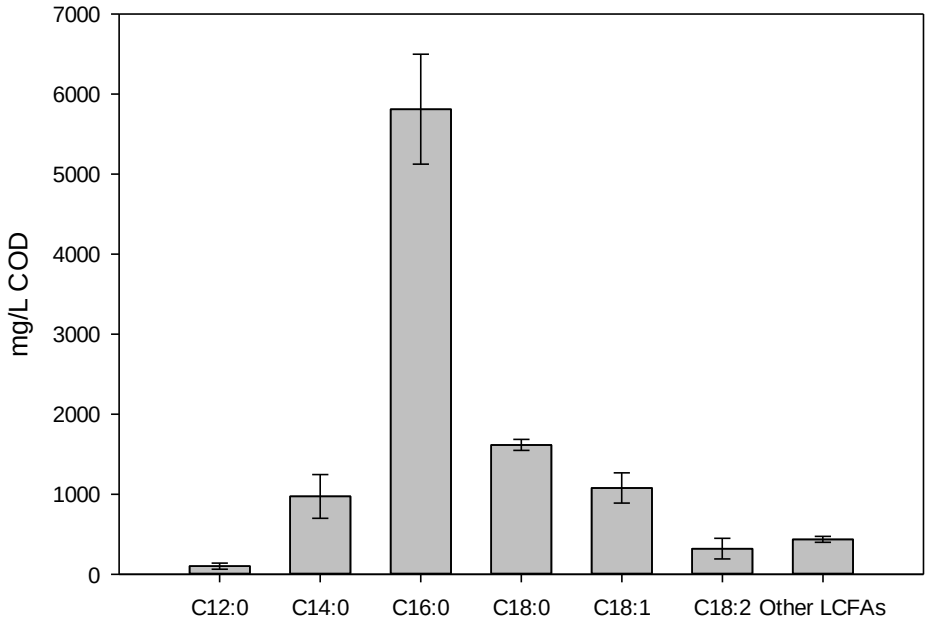

Figure 16 LCFA composition in a grease ball

Palmitic acid (C16:0), the most abundant LCFA in the grease balls, accounted for approximately 56% of the total LCFAs. Stearic acid (C18:0) was the second most abundant (16% of the total LCFAs). A mass balance analysis was done in order to track the fate of LCFAs in the APDs (Figure 17). Unsaturated fatty acids, such as C16:1, C18:1, C18:2, and C18:3 were degraded to approximately 80%. C18:1trans, the most abundant unsaturated LCFAs in the FOG, was degraded up to 99%. The unsaturated fatty acids could possibly be converted to saturated fatty acids such as C16:0 and C18:0. The production of palmitic acid (C16:0) was not found because C16:0 accumulated and floated on top of the liquid layer. However, the production of stearic acid (C18:0) was significant (Figure 17). At higher pH conditions, C18:0 appeared to be more soluble in the APDs. This could explain the high amounts of C18:0 in the effluents and less accumulation in the APDs.

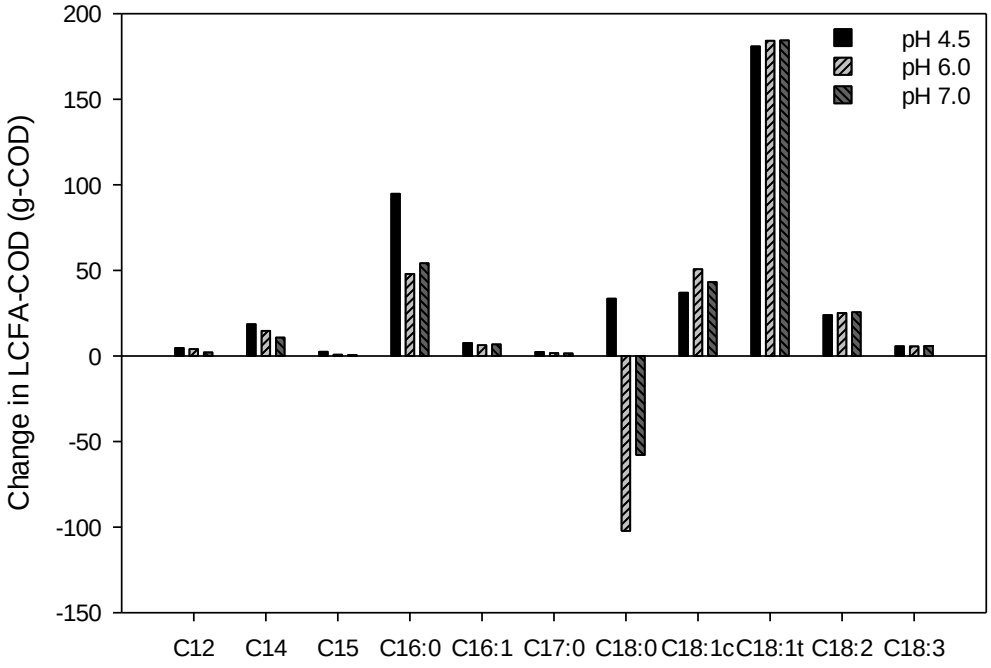

Figure 17 Degradation of LCFAs in g COD with negative numbers indicating production during the first operating period

LCFA accumulation in the APDs was observed during both operating periods. Higher pH corresponded to less LCFA accumulation. The accumulation rates showed a similar trend in the two periods (Figure 18). The rates were expected to be similar, but the total LCFA accumulation would be different due to different operating durations. The APDs were operated for 112 and 48 consecutive days during the first and second operating periods, respectively. In the first operating period, the accumulation rate was expected to be higher than in the second, shorter period because as the grease balls grew, more surface area was available to adsorb more LCFAs.

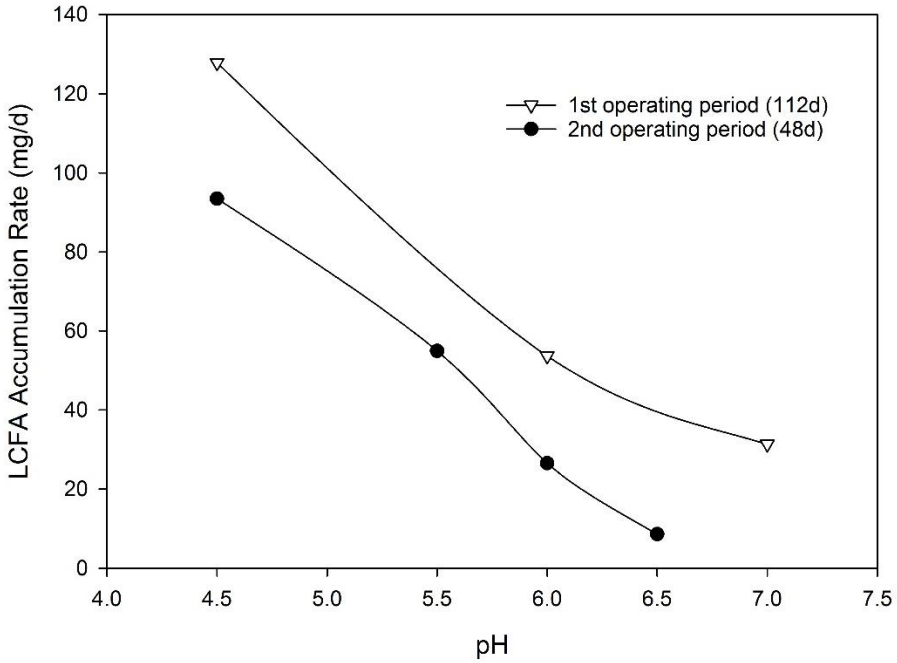

Figure 18 LCFA accumulation rates at different pH conditions in the acid-phase digesters during the 1st operating period (112 days) and 2nd operating period (48 days)

Discussion

Addition of FOG into two-phase systems was shown to increase methane production. The influence of pH on two-phase, anaerobic co-digestion was illustrated in this study. The overall performance of the TPDs with a controlled pH proved to be more effective in terms of sludge solubilization and methane productions. The increase in VFA production at pH 6 occurred because it was a more suitable environment for acid-forming bacteria (Zoetemeyer et al., 1982).

Moreover, undissociated acids produced at a lower pH could promote inhibition of acidogenesis (Babel et al., 2004). However, no significant difference in VFA production was observed among the APDs with pH adjustments (5.5-7.0) in this study.

In many studies, authors have found that the acid-phase digesters of two-phase systems help saturate the unsaturated fatty acids and promote the degradation of lipids (Kim et al., 2004; Kim and Shin, 2010). The LCFA loading can also be increased because saturated LCFAs are less inhibitory than unsaturated LCFAs. Komatsu et al. (1991) found that saturation of oleic acid (C18:1) occurred at a short hydraulic retention time (8-hr HRT) and palmitic acid (C16:0) accumulated in the reactor. However, the authors found that at pH 6, acidogenesis was hindered (Komatsu et al., 1991). It is important to note that these authors investigated the effects of pH on two-phase co-digestion at pH 6.0 or more and no accumulation of LCFA materials was mentioned. In most studies, the pH was not reported to hinder acid-phase reactor performance, but the optimum pH range remains controversial. The differences in characteristics of sludge, types of reactors, SRT, temperature, and other operational parameters is likely responsible for the controversy. However, the results of this study support that idea that the alkalinity in acid-phase digesters is an important parameter, where higher pH or pH close to neutral (6.0-7.0) showed the optimum conditions.

This study confirmed that the addition of FOG to two-phase digestion systems can affect accumulations of LCFAs in acid-phase reactors. The LCFA grease balls which accumulated and floated on top of the liquid were found in all operating digesters. The higher pH (6.0-7.0) correlated to less accumulation of LCFAs. One mechanism of LCFA accumulation is precipitation between LCFAs and divalent cations (Pereira et al., 2005). In this study, potassium phosphate (K_2HPO_4) was used to adjust pH conditions. High amounts of monovalents could decrease the divalent to monovalent ratio, which will decrease the chance of LCFA precipitation by divalent cations at a higher pH (6.0-7.0). In this study, there was significant production of stearic acid (C18:0), which is not known as an intermediate product in the degradation of unsaturated 18-carbon fatty acids. Recently, researchers have reported that unsaturated 18-carbon chains, such as oleic acid (C18:1) and linoleic acid (C18:2), are energetically favorable to degrade to palmitic acid (C16:0) without undergoing saturation to form stearic acid (C18:0) (Lalman and Bagley, 2001; Pereira et al., 2002). Lower hydrogen partial pressures in a typical,

single-phase anaerobic digester make oleic acid conversion to palmitic acid (C16:0) more energetically favorable. The same principle applies to the conversion of linoleic to myristic acid (C14:0). However, in an acid-phase digester, the hydrogen partial pressure is expected to be present due to the production of hydrogen by acid-forming bacteria. Moreover, the concentration of H^+ at a lower pH, such as pH 4.5 and 6.0, was much higher compared to the pH 7. The high H^+ concentration could result in conversion of C18:2 to C18:1, because the conversion of C18:2 to C16:0 is energetically unfavorable. Similarly, C18:1 could be converted to 18:0 instead of C16:0, as described by Lalman et al. (2001). This would explain the smaller amount of C18:0 seen in the APD at pH 7. According to the mass balance study, the amount of C18:0 in the pH-4.5 APD was less than the other APDs with pH adjustment. The absence of C18:0 occurred because it could be precipitated or adsorbed onto sludge and wasted with the effluents.

Conclusion

The influence of pH in the acid-phase, co-digestion of fats, oils, and grease (FOG) was investigated. The mechanisms of LCFA degradation and accumulation in a two-phase system were studied on a laboratory scale. The conclusions from this study are:

1. Addition fats, oils, and grease at 20% COD of the total COD concentration greatly increased methane production. The optimum methane production in the GPDs correlated to the soluble compounds and LCFAs from the APDs.
2. Unsaturated fatty acids were degraded in the APDs. Percent degradation of C18:1trans, one of the most abundant LCFAs, was as high as 99%. Saturated fatty acids were not degraded in the APDs.
3. A pH greatly affected solubilization and precipitation of LCFA materials. Higher pH corresponded to less LCFA accumulation and more saturated LCFA solubilization, especially stearic acid (C 18:0). A pH close to neutral was found to reduce LCFA accumulation in the APDs.

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Chapter 5: Concluding Remarks and Engineering Significance

Co-digestion of municipal sludge with high energy products, especially fats, oils, and grease (FOG) in order to increase methane production and recover energy has been popularly practiced in recent years. LCFAs, the main components in FOG, can cause inhibition of methanogens in an anaerobic digester. This thesis was aimed at improving our understanding of the LCFA degradation mechanisms under thermal hydrolysis and acid-phase pretreatments. A good understanding of LCFA degradation mechanisms is essential for engineering process design and operation of anaerobic co-digestion systems. This research explored two different anaerobic digestion pretreatment methods: thermal hydrolysis and acid-phase co-digestion systems. In Chapter 3, Thermal Degradation of Long Chain Fatty Acids, the effects of thermal hydrolysis pretreatment on LCFA degradation was studied. The thermal hydrolysis experiments revealed possible problems that LCFAs can cause when added to thermal hydrolysis reactors. Specific implications of this work for wastewater treatment are as following:

- **Thermal hydrolysis of LCFAs at 90-160 °C is not effective.** Both saturated and unsaturated LCFAs were stable under thermal treatments at up to 8 hours.

- **Addition of FOG to thermal hydrolysis reactors needs to be carefully considered.** Increase in the degradation of more complex molecules and less degradation of LCFAs will result in LCFA accumulation, which can later inhibit methanogens in anaerobic digesters. Moreover, LCFA accumulation can result in increases in the pressures and temperatures of thermal hydrolysis reactors.

In Chapter 4, the influence of pH and FOG degradation in acid-phase co-digestion was studied. Results confirmed that pH has an impact on the hydrolysis rate of sludge in the APDs, and that the optimum pH was 6.0. Optimum conditions in the APDs not only enhance biogas production, but also prevent possible LCFA accumulation issues. Understanding the optimum conditions and LCFA degradation in the APDs are crucial to operating anaerobic co-digestion systems. The implications of the second manuscript for wastewater treatment include the following:

- **Co-digestion of municipal sludge and fats, oils, and grease (FOG) in two-phase systems increases methane production.** FOG has great potential for increasing energy recovery in wastewater treatment facilities. However, addition of FOG into two-phase systems can a cause scum and/or grease ball formations, which can later create operating problems in the APDs.

- **pH is an important parameter in the acid-phase co-digestion of municipal wastewater.** High pH conditions (6.0-7.0) in APDs not only increases hydrolysis rate, but also reduces LCFA accumulation in the digesters. However, when the APD was at pH 6.5 or above in this study, hydrolysis rate decreased. Therefore, operators need to understand how the pH of their systems can affect desired performance.

Appendix A: Thermal Degradation of Long Chain Fatty Acids

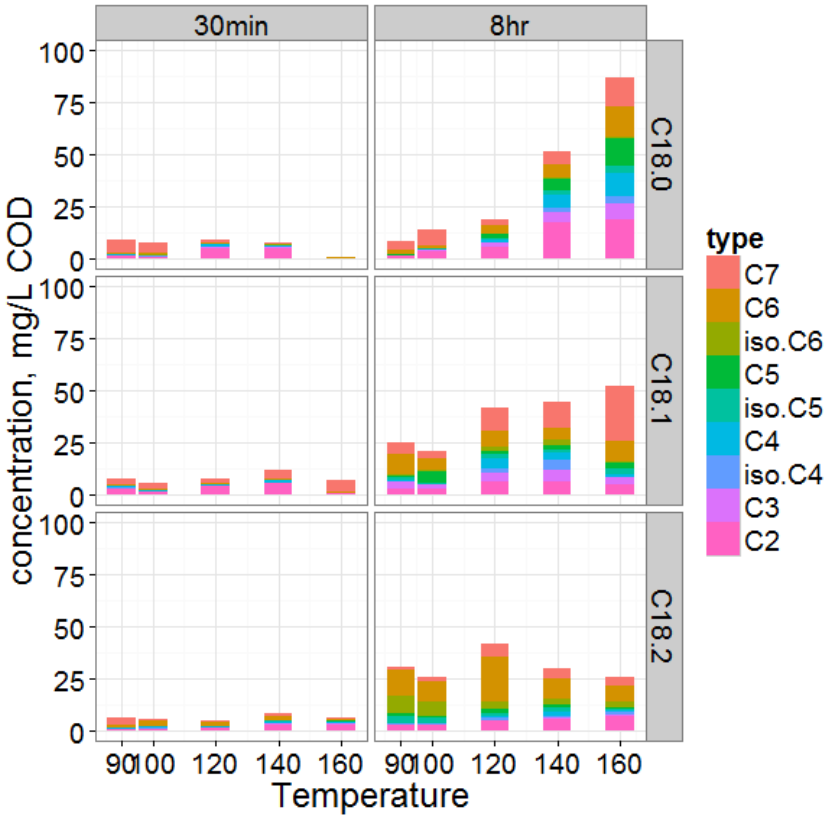

Figure 19 VFA productions at different thermal hydrolysis durations and temperatures

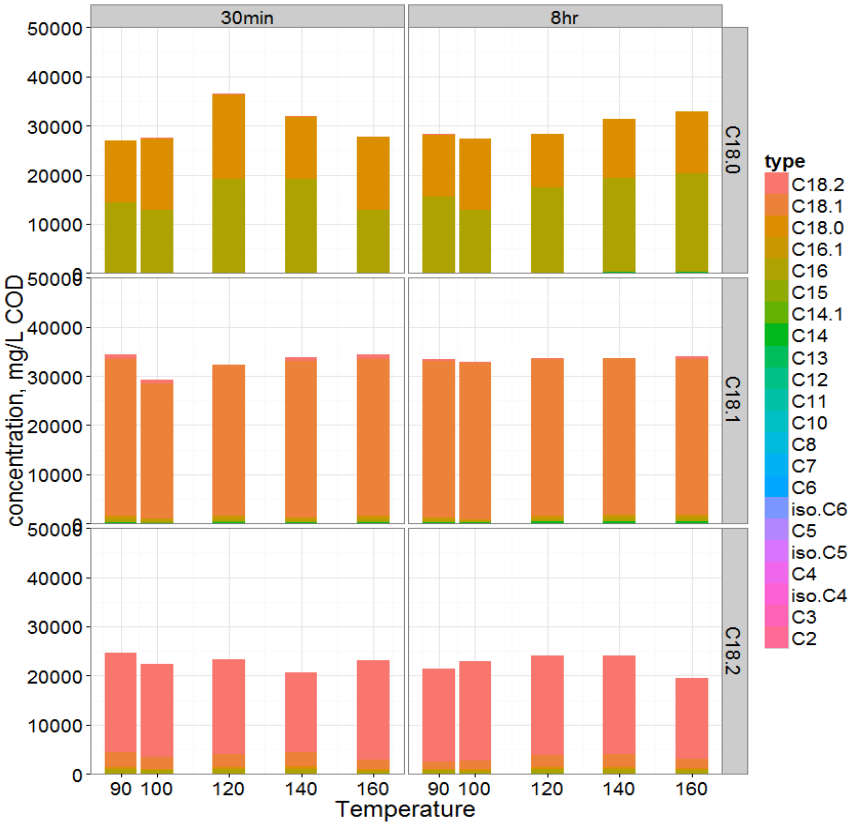

Figure 20 Total fatty acids productions at different thermal hydrolysis durations and temperatures

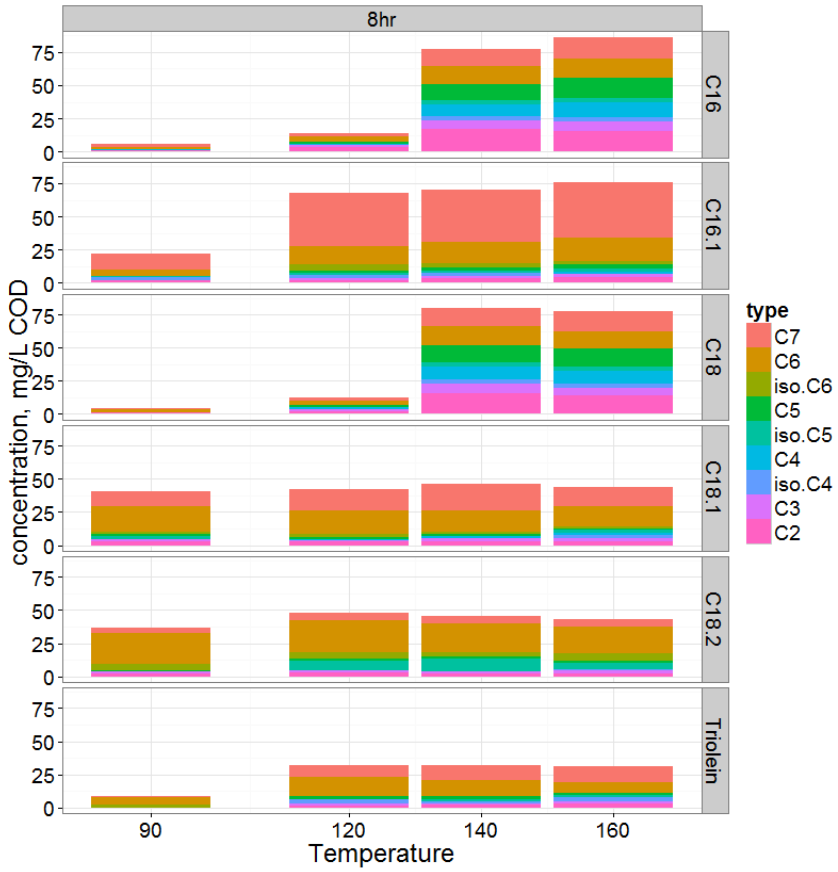

Figure 21 VFA production at 8-hr thermal hydrolysis

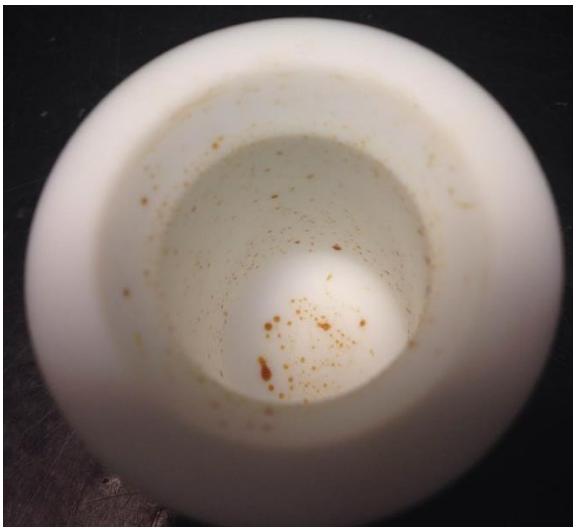

Figure 22 Unsaturated residuals after 8-hr thermal hydrolysis treatment

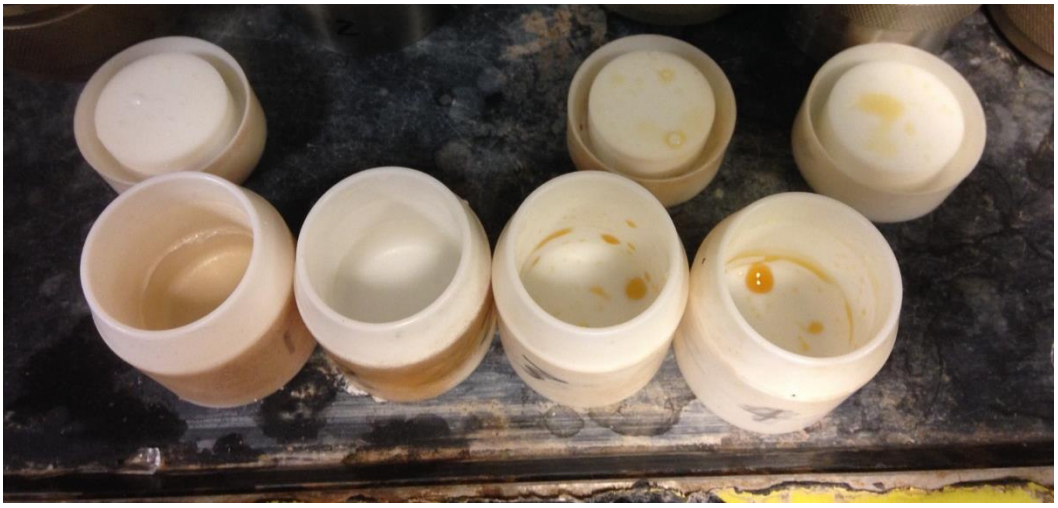

Figure 23 LCFA residuals after 8-hr thermal hydrolysis treatments: C16:0, C18:0, C18:1, and C18:2 (from left to right)

Figure 24 Thermal hydrolysis of LCFAs with (10,000 mg/L COD) of digested sludge (8-hr thermal hydrolysis)

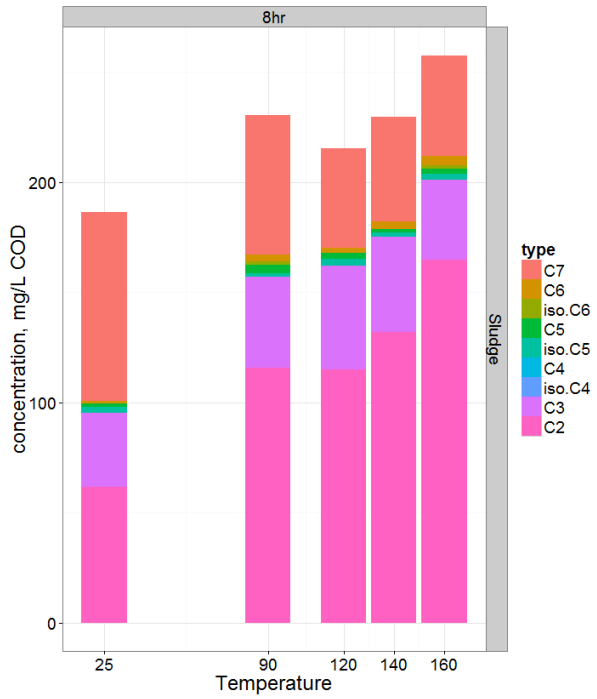

Figure 25 VFA production of municipal sludge (8-hr thermal hydrolysis)

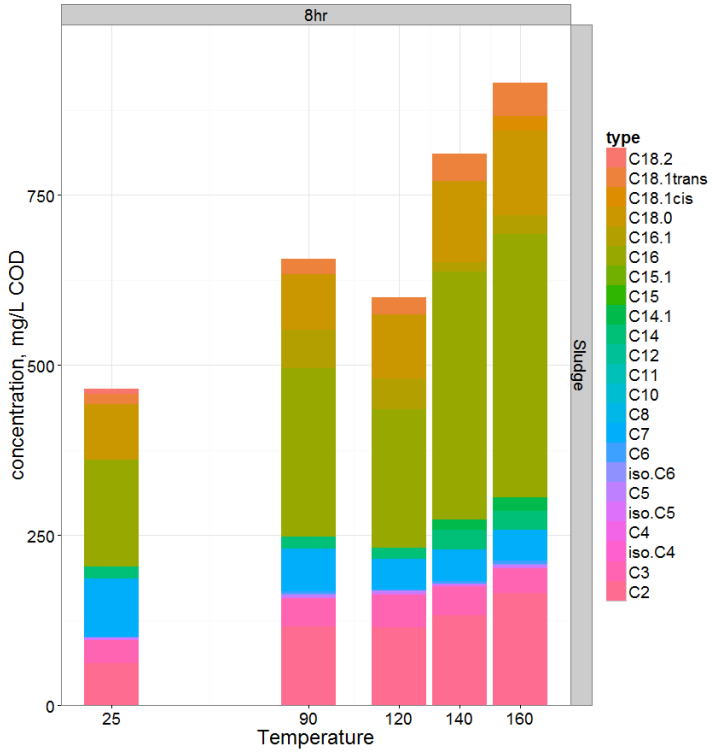

Figure 26 Total fatty acid productions of municipal sludge at different temperatures (8-hr thermal hydrolysis)

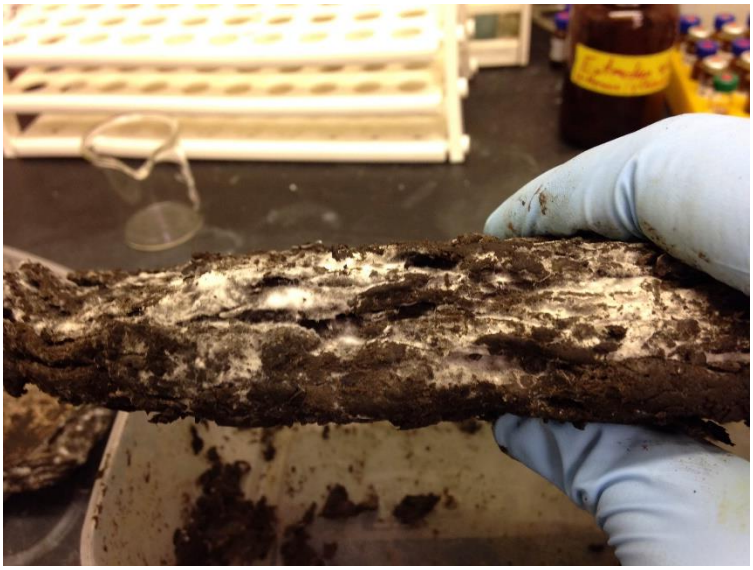

Figure 27 Scum formation from a thermal hydrolysis reactor: the scum was attached onto the wall of the reactor

Appendix B: Influence of pH and FOG Degradation in Acid-Phase Co-Digestion

Table 9 LCFA accumulation rates during 1st and 2nd operating periods

pH	1st operating period		2nd operating period	
	grams	mg/d	grams	mg/d
Control	1.0	9.2	NA	NA
4.5	14.3	127.8	4.5	93.4
5.5	NA	NA	2.6	54.9
6.0	6.0	53.6	1.3	26.5
6.5	NA	NA	0.4	8.6
7.0	3.5	31.3	NA	NA

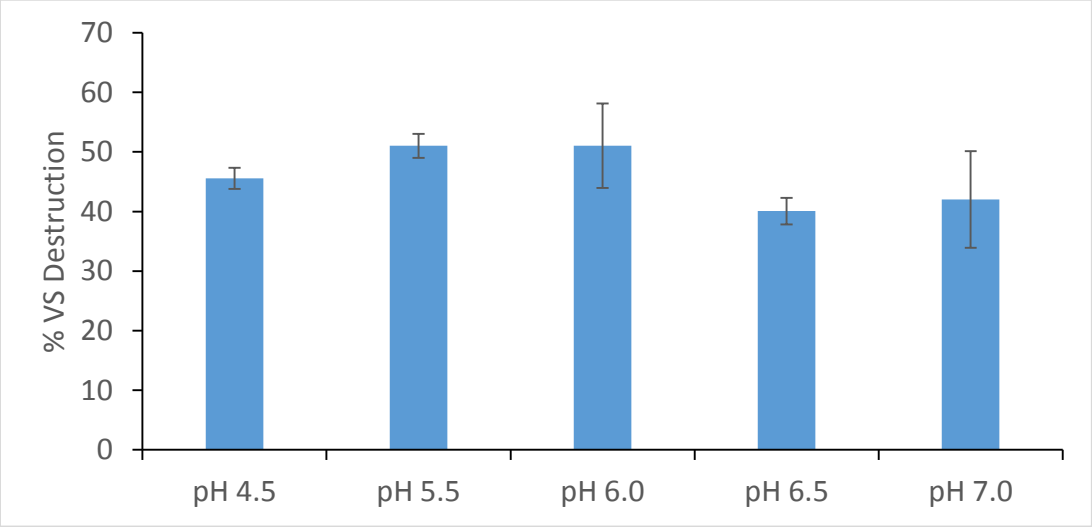

Figure 28 percent VS destruction in the GPDs at different pH conditions

Figure 29 High-density polyethylene batch fermentation reactors supplied by the Hobby Beverage Equipment Company (Temecula, California)

Figure 30 Accumulated LCFAs (grease balls) from the APDs during the 1st operating periods (left) and the grease balls (right) (ruler shown in inches)