Effect of Long-Chain Fatty Acids on Anaerobic Digestion

Cheng Qian

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

Master of Science

In

Environmental Engineering

John T. Novak, Chair

Doug C. Goldsmith

Gregory D. Boardman

August 9, 2013

Blacksburg, VA

Keywords: anaerobic digestion, long-chain fatty acid, solids removal, lipid concentration

Effect of Long-chain Fatty Acids on Anaerobic Digestion

Cheng Qian

ABSTRACT

Anaerobic digestion is commonly used for waste sludge treatment to reduce the volume of sludge production and to generate methane. Recently, there has been interest in adding fats, oils and greases to digesters to increase methane production.

Long-chain fatty acids are one of the major components of fats so their fate in anaerobic digestion is of interest. An investigation was carried out to study whether long-chain fatty acids (LCFAs) have an effect on digestion of waste sludge under anaerobic conditions. Four different kinds of LCFAs were used in this study. The 18 carbon series with 0, 1, 2 and 3 double bonds were studied to evaluate the degree of saturation on fatty acid degradation. Due to their molecular structure, unsaturated LCFAs are more soluble than saturated LCFAs. Oleic, linoleic, linolenic acid with an ascending number of double bonds were tested as representatives for three different degrees of saturation. In addition, stearic acid, a saturated fatty acid was also tested.

LCFAs were added to sewage sludge at concentrations ranging from 5% to 20% on a weight basis and the pH, solids reduction and COD reduction were determined. The results suggested that in addition to degrading in the digesters, all unsaturated acids contributed additional solids removal, compared to the control group. In contrast, stearic acid did not affect the solids removal. The COD reduction was similar to solids reduction in that additional COD was destroyed when

unsaturated LCFAs were added to the sludge. The mechanism for additional solids reduction is not known.

When a mixture of stearic and oleic acid (1:2 by mass ratio) was fed to the digester to investigate if increasing stearic solubility by dissolving it in oleic acid could enhance solids and COD reduction, increased solids reduction occurred. Compared to the results of only feeding stearic acid, the degradation was improved by the mixture. This suggests that solubility of LCFA plays a role in the process and could be utilized through altering parameters of operation.

ACKNOWLEDGEMENT

I would like to express my most sincere gratitude to my advisor, Dr. John T. Novak, for receiving his guidance, understanding, patience, and kindness throughout my graduate study at Virginia Tech. This thesis would not have been possible without his invaluable advices and generous help. He encouraged me to develop more independent thinking and gain a deeper insight on the study. His suggestions have a great influence on the long-term career path of my future. I'm very grateful for the opportunity of being one part of the research group. I would also like to thank to all the members of the research group for their generous help, especially Anna Maria, Renzun Zhao, Ritika Kacker, and Kartik Radhakrishnan. Working with them inspired both my study and life.

I would also like to thank to my committee members, Dr. Gregory Boardman and Dr. John Little, for their guidance and advice on my study.

Finally, I owe my deepest gratitude to my parents and husband, Jianliang Zhang. Without their continuous support and encouragement, I cannot imagine how I could have been through the entire graduate study in the United States as an international student. I also wish to thank to my friends I met at Virginia Tech for their warm support in my most difficult times.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

1 Introduction

The conventional activated sludge (CAS) process is the most widely applied process for biological wastewater treatment around the world. However, one of the largest issues for both municipal and industrial wastewater treatment plants (WWTPs) is the high production of waste sludge as a by-product of physical, chemical and biological processes during wastewater treatment (Appels, Baeyens, Degrève, & Dewil, 2008; Tyagi & Lo, 2011). Treatment and disposal of excess sewage sludge from WWTPs accounts for no less than half of the total plant operating cost (Wei, Van Houten, Borger, Eikelboom, & Fan, 2003). Incineration, landfill and land application are main methods for sludge disposal, but incineration and land application of sewage sludge can raise health risks due to contamination from the presence of heavy metals, pathogens or toxic organic chemicals in sludge. Meanwhile, the regulations based on sludge disposal are becoming more and more stringent. Different strategies are explored for not only minimizing sludge quantities but also for improving its characteristics. For instance, anaerobically treated wastewater sludge is more stable and capable of being used as an alternative soil-conditioner and fertilizer.

Anaerobic digestion (AD) is considered as one of the most effective technologies in waste sludge treatment and is applied widely at WWTPs for many advantages including reduced sludge volume and mass of sludge, sludge stabilization and the potential to generate biogas. The main component of biogas, methane, represents one of the renewable energy resources, which can be converted to heat and electricity (Nasir, Mohd Ghazi, & Omar, 2012). A variety of strategies are being developed to optimize anaerobic digestion performance and increase biogas production (Silvestre, Rodríguez-Abalde, Fernández, Flotats, & Bonmatí, 2011). These include pretreatment

to improve digestability, multistage digestion and co-digestion with other wastes. One of the wastes used for co-digestion is grease. Greases can increase gas production due to their high caloric content, so addition of greases to anaerobic digesters has recently received attention.

Lipids are one of the major organic components in municipal wastewater (Chipasa & Medrzycka, 2006). They are grouped together as fats, oils, and greases (FOG), some of which are essential for human health. Biological treatment processes are commonly used to remove lipids from wastewater (Chipasa & Medrzycka, 2006). Thus, the accumulation of considerable amounts of LCFA into sludge raises a concern as to the effect LCFAs might have on subsequent sludge treatment. Since anaerobic treatment is mainly used for stabilizing waste sludge, the performances of LCFA in oxygen-free digesters with sludge is important for industrial and municipal infrastructures to ensure effective operation.

In addition, co-digestion of sewage sludge with other organic wastes has been shown to exhibit some benefits for improving gas production. Lipids as substrate for anaerobic digestion have higher theoretical methane yield compared to other substrates such as protein and carbohydrates (Neves, Oliveira, & Alves, 2009). Some researchers report that fats co-digested with sludge increase methane yield (Davidsson, Lövstedt, la Cour Jansen, Gruvberger, & Aspegren, 2008).

This study was initiated as a result of a previous study of oleic acid addition to anaerobic digesters. It was found that addition of oleic acid to a lab-scale digester resulted in approximately 5% additional solids removal compared to a control digester (Fraser, 2010). This additional solids removal was from the initial sludge so it appeared that the addition of oleic acid would not only generate more gas from degradation of the oleic acid but would generate additional gas from degradation of more sewage sludge. Therefore, an enhancement of solids removal by

adding fatty acids to sewage sludge under anaerobic digestion was of interest. The same sludge resource, anaerobic digesters and feeding methods used by Fraser (2010) were applied in this study in order to investigate the phenomenon that occurred in Fraser's study.

2 Objective

The objective of this study was to investigate the effect of the addition of LCFAs to waste sludge during anaerobic digestion. An understanding of this process is useful for optimal design and effective operation for waste sludge treatment. Furthermore, it allows the industries and municipal WWTPs to improve parameters and performances of digestion process. Therefore, four LCFAs with different degrees of saturation, all straight chain with 18 carbons, were used in this study: stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), where the 0, 1, 2 and 3 refer to the number of double bonds into LCFA structure. The additional concentration of each LCFA inoculated to the daily loading of sludge was targeted in the range from 5% to 20% by weight.

In addition, a mixture of oleic and stearic acid was tested under the same conditions as the other single LCFAs. This was done to determine if an unsaturated fatty acid could solubilize a saturated fatty acid and thereby improve its degradability.

3 Literature Review

3.1 Sewage Sludge

Lipids, carbohydrates and proteins compose the bulk of the organic in sewage sludge (Jeris & McCarty, 1965). Municipal sewage sludge is typically classified into two categories, primary and secondary or waste activated sludge (WAS). Properties of sludge are commonly described by total solids (TS) and volatile solids (VS), which indicates biodegradability of the sludge content in order to decrease in sludge volume and biologically stabilize bio-solids. The dry organic content in sludge varies from 60% to 80%. These constituents are separated by their degradability, as follows: a fraction degradable only under anaerobic condition, a fraction degradable only under aerobic conditions, a fraction degradable both under anaerobic and aerobic conditions, and a fraction that is not degradable (Tomei, Braguglia, Cento, & Mininni, 2009).

3.2 Anaerobic Digestion

AD is a well-known and successfully developed technology for sewage sludge stabilization. It has been applied at WWTPs for a century. Its performance can accomplish the goal of waste sludge treatment, which is to alter the water content for volume and mass reduction, remove biodegradable organic fraction of sludge for further odor prevention, control pathogens and generate a stabilized product for safe final disposal (Carrère et al., 2010). Moreover, the production of biogas (mainly CH_4 and CO_2) during this process is also an advantage that could allow energy recovery at treatment plants in an environmentally-friendly way. For example, 23.5

million m^3 of biogas at 15 WWTPs in Finland was produced and 20.3 million m^3 was utilized as electricity, heat, and other forms of energy in 2006 (Luostarinen, Luste, & Sillanpaa, 2008). The biogas provided by AD can contribute to the energy requirement for WWTPs in the range from 20% to 40% (Long, Aziz, Reyes Iii, & Ducoste, 2012).

The anaerobic degradation of organic matter contained in sludge is separated into four sequential steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the first step, the particulates are hydrolyzed: proteins are converted to amino acids, carbohydrates to simple sugar, and lipids to long chain fatty acids. These more soluble compounds are further degraded by subsequent acidogenesis and acetogenesis phases. The conversion produces acetic acid, carbon dioxide and hydrogen. Through the final step of methanogenesis, methane is produced as biogas by two main groups of methanogens which utilize different electron donors. The biodegradability of particulate organics in raw sludge is dependent on its various characteristics during the hydrolysis process. The rates of later phases are also affected by whether anaerobic microorganisms accept hydrolyzed organic substances as substrates. Thus hydrolysis is generally considered as the rate-limiting step (Appels, et al., 2008; Tomei, et al., 2009). An overview of the degradation pathway in the anaerobic digestion process is given in Figure 1.

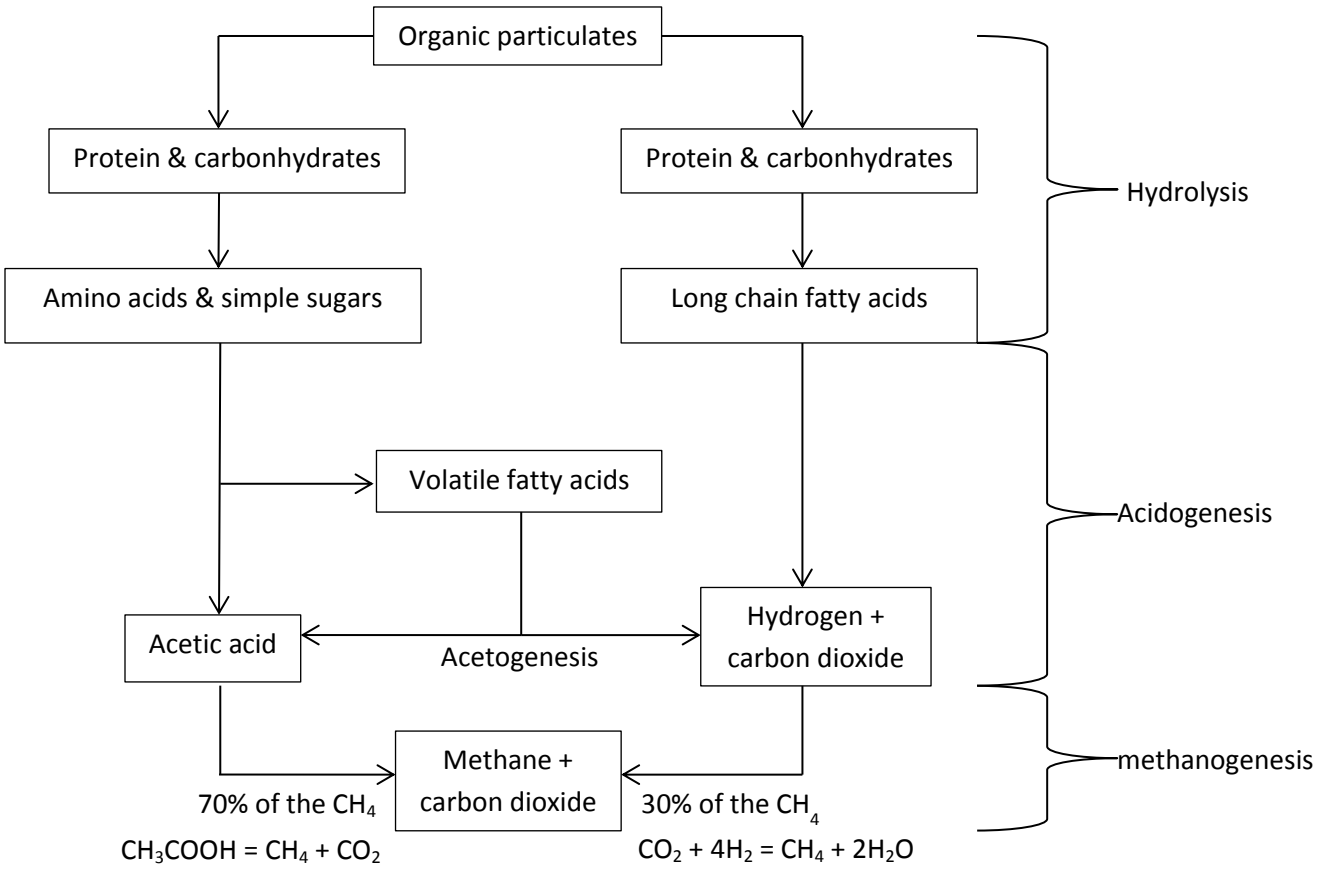


Figure 1: The multi-steps of anaerobic digestion process (Filipe & Grady, 1998), used under fair use, 2013.

3.2.1 Affecting Parameters

Methanogenic organisms are sensitive to pH because pH influences enzyme activity. Thus each group of microbes has its own optimum pH range. That specific optimal range for methanogenic organisms is between 6.5 and 7.4. More acidic and basic environments may decrease the production rate of methane (Appels, et al., 2008; Gomec, Kim, Ahn, & Speece, 2002).

Temperature has a significant effect on the operation of anaerobic digestion. The control of a stable operating temperature is important, since the adaptation of microbes to temperature fluctuation is slow. Daily variations in temperature should not be allowed, with the change limited to ± 1 °C (Garber, 1982). Both mesophilic (30-38 °C) and thermophilic (50-60 °C) processes are applied. Higher temperature (thermophilic) has several advantages including: enhanced breakdown of organics attributed to the increased biological and chemical reaction rate, improved solids dewaterability, and pathogen inactivation. On the other hand, anaerobic operations under thermophilic conditions encounter some negative effects on process stability and high energy cost for heating (Appels, et al., 2008; Buhr & Andrews, 1977; Zoetemeyer, Arnoldy, Cohen, & Boelhouwer, 1982). Thermophilic sludge has lower microbial diversity compared to the sludge digested at mesophilic temperatures, while thermophilic bacteria are more active and have higher maximum specific growth rate (Öztürk, 1991; Sekiguchi et al., 1998). The relationship between ammonia and temperature was examined in anaerobic digestion process by Angelidaki and Ahring (1994) since ammonia is beneficial to the growth of anaerobic bacteria as long as it does not exceed a certain concentration that can be toxic to methangenic activity. More free ammonia is produced with increased temperature and aggravates the ammonia inhibition, thus this problem can be resolved by decreasing the temperature or limiting the amount of ammonia by decreasing the loading.

Volatile fatty acids (VFAs) as one of the intermediates are important parameters for the control of the AD process. VFAs accumulate in the anaerobic digester due to some operating factors such as organic overloading (Switzenbaum, Giraldo-Gomez, & Hickey, 1990). A drop in pH resulting from the accumulation of VFAs could be inhibitory to the system. In order to avoid AD process failure, the specific VFA concentration has been monitored and used as a process

indicator for a long time (Pind, Angelidaki, Ahring, Stamatelatou, & Lyberatos, 2003). The ratio of propionate to acetate is suggested to show a stable process if its value is below 1.4 (Hill, Cobb, & Bolte, 1987). Hill & Holmberg (1988) reported that the iso-forms of butyric and valeric acids were also effective indicators of an AD process without stress when either of their concentrations were no more than 5 mg/L. However, Ahring et al. (1995) did not believe that the use of absolute concentrations of individual VFA to point out a system imbalance was a meaningful indicator. The substrates in the waste affect the metabolism considerably during the AD process and hence the levels of different intermediates. Therefore, each AD system has its own specific levels of VFAs to determine the process status and a change in VFA concentration could be a process balance shift that is not necessarily a sign of process failure (Pind, et al., 2003).

Solids retention time (SRT) is also a key factor for the design and operation of AD process. The populations of bacteria in the digesters require an adequate retention time to sustain themselves and metabolize substrates thoroughly. SRT, by definition, is associated with the sizing of the digester since the digesters are non-recycle systems. The concept of SRT establishes a relationship between the microbial world and operating condition. A sufficient SRT can prevent bacteria washout, minimize the effect of temperature fluctuation, and reduce the risk of the occurrence of inefficient mixing (Parkin & Owen, 1986). A proper design of SRT should be based on the rate-limiting step that is usually considered as the hydrolysis phase and leads to optimum system performances (Mahmoud, Zeeman, Gijzen, & Lettinga, 2004). Typically detention times between 15 and 20 days are used for mesophilic digestion.

Due to the variation and complexity of sludge, the optimal operating parameters depend on a case-by-case assessment. In addition, different process configurations can alter metabolic

pathways for more favorable intermediate productions that provide more capability for further methanogenesis (Azbar, Ursillo, & Speece, 2001). The characteristic of the waste hence plays a crucial role in the reactor configuration. The most common low-rate and high-rate anaerobic reactors are the continuously stirred-tank reactor (CSTR) and up-flow anaerobic sludge blanket reactor (UASB), respectively. Generally, UASB reactors are more readily applicable to soluble and easily degradable wastes rather than insoluble lipids and LCFAs discussed in this study. Less operational difficulties have been addressed in CSTR systems for lipid treatment by researchers, in contrast to frequent lipid treatment failures happened in UASBs (Long, et al., 2012).

3.3 Lipids

Lipids, characterized either as fats or oils and greases (FOG), are one of the most common organic constituents found in municipal wastewater and wastewater coming from food processing industries, slaughterhouses, dairy industries, and oil refineries (Cavaleiro, Pereira, & Alves, 2008; Fernández, Sánchez, & Font, 2005).

Lipids are mainly composed of neutral fats and long-chain fatty acids (LCFAs). Fatty acids are carboxylic acids with a long hydrophobic aliphatic tail, which is distinguished by the degree of saturation. A LCFA is defined as a fatty acid with an aliphatic tail longer than 12 carbons. Fatty acids containing one or more double bonds are known as unsaturated. The position of carbon atom bonded to the double bond determines two distinct configurations of unsaturated fatty acids: cis or trans. In nature, cis configurations more commonly occur in unsaturated LCFA. The cis configuration means that the adjacent carbons atoms are on the same side of the double bond. By

contrast, trans configuration is shaped as a straight chain similar to the saturated fatty acid. As a result of the chemical structure, the cis configuration lacks flexibility compared to that of the saturated fatty acids as well as the trans isomer (Sousa, Smidt, Alves, & Stams, 2009). However, Kabara et al. (1977) confirmed that the cis isomer is more active than the trans isomer.

The activated sludge process is efficient at removing of lipids from wastewater. Hrudey (1982) observed that greater than 80% of the lipids were adsorbed to activated sludge from the aqueous phase in less than 20 minutes' contact time. Nevertheless, the occurrence of sludge flotation and washout during the operation due to the adsorption of fatty acids onto biomass was also widely reported (Cirne, Paloumet, Bjornsson, Alves, & Mattiasson, 2007a). This reflects on a fact that most of the lipids may remain in sewage sludge, which is of concern for sludge treatment.

Anaerobic co-digestion of sewage sludge with lipid-rich wastes or digestion with sludge containing lipids has gained increasing attention in the wastewater community. It has been shown that co-digestion has several benefits including dilution of inhibitory compounds and improvement of biogas yields due to the nutrient supply and synergistic phenomenon by the co-substrate (Cavaleiro, et al., 2008; J. C. Kabouris et al., 2009; Mata-Alvarez, Macé, & Llabrés, 2000). Lipid-rich waste is one of the most attractive substrates being studied because of its high methane potential and low nitrogen content which reduces the potential for ammonia inhibition.

Under anaerobic conditions, neutral fats can be readily hydrolyzed (lipolyzed) into free LCFAs and glycerol. The hydrolysis is catalyzed by extracellular lipase released by acidogenic bacteria (Masse, Masse, Kennedy, & Chou, 2002; Wan, Zhou, Fu, & Li, 2011). Through syntrophic acetogenesis, LCFAs are successively oxidized to short chain fatty acids via β-oxidation. Before the fatty acids enter the β-oxidation circle, they are activated with coenzyme A (Long, et al.,

2012). β-oxidation repeatedly shortens the carbon chains of the LCFAs by two carbons and subsequently converts them to acetate and hydrogen at the end of β-oxidation circle. Eventually, acetate and hydrogen are utilized by methanogens to form methane (DiRusso, Black, & Weimar, 1999; Novak & Carlson, 1970; Sousa, et al., 2009; Weng & Jeris, 1976). Meanwhile, glycerol is fermented to acetate and hydrogen and the fermentation forms 1,3-propanediol as one of the intermediate products (Biebl, Zeng, Menzel, & Deckwer, 1998). The mechanism of β- oxidation is illustrated in Figure 2.

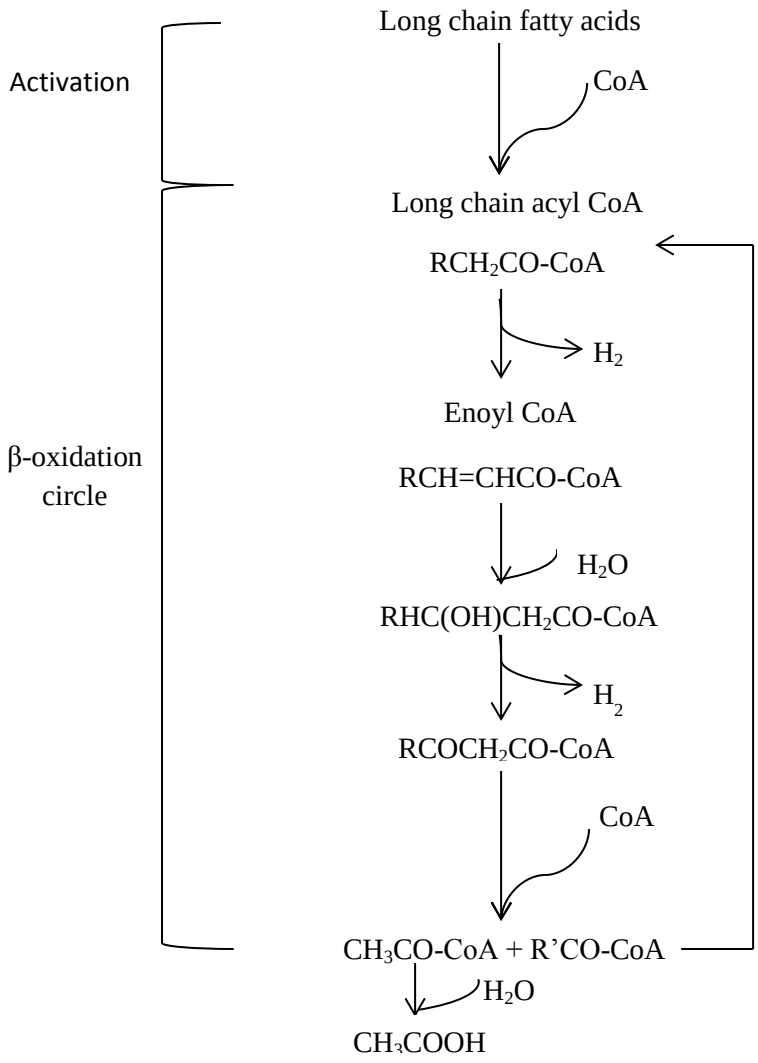


Figure 2: β-oxidation circle of LCFAs.

It has been suggested that unsaturated LCFAs are unlike saturated fatty acids, which just follow the β-oxidation pathway. For unsaturated LCFAs, some investigators believe that complete chain saturation occurs before entering the β-oxidation circle (DiRusso, Black, & Weimar, 1999;

Novak & Carlson, 1970; Sousa, et al., 2009; Weng & Jeris, 1976). Hence, the pathway of unsaturated LCFA degradation is a sequential step: hydrogenation and β-oxidation.

Using oleic acid (C18:1) as an example:

Hydrogenation:

$C_{18}H_{34}O_2$ (C18:1) + H_2 = $C_{18}H_{36}O_2$ (C18:0)

β-oxidation:

$CH_3(CH_2)_{16}COOH$ (C18:0) + $2H_2O$ = $CH_3(CH_2)_{14}COOH$ (C16:0) + CH_3COOH + $2H_2$

Some researchers reported that full hydrogenation may not be achieved prior to β-oxidation (Sousa, et al., 2009). Lalman and Begley (2000, 2001) found that palmitic (C16:0) and myristic acids (C14:0) were observed as intermediates during the anaerobic degradation process of linoleic (C18:2) or oleic acid (C18:1) instead of stearic acid (C18:0). This suggests that unsaturated LCFAs may go through β-oxidation directly without full saturation in the process of anaerobic degradation.

3.3.1 Inhibition

LCFAs have been reported to show an acute toxicity to anaerobic microbes, such as syntrophic acetogens and methanogens (Cirne, et al., 2007a). The inhibition of LCFAs causes a lag phase at the start-up of some batch experiments so that the methane production from acetate and the degradation processes are retarded. The methane production from hydrogen is slowed but no lag

period is observed (Hanaki, Matsuo, & Nagase, 1981). Approximately, 70% of the methane produced during the anaerobic degradation is formed from acetate (Jeris & McCarty, 1965). Compared to hydrogenotrophic methanogens, acetogens and acetotrophic methanogens are pointed out to be more sensitive to inhibition (Hanaki, et al., 1981).

The mechanism behind the LCFA toxicity on anaerobic bacteria is considered to be an interaction between the cell wells of microbes and LCFAs due to the adsorption, which is primarily responsible for the toxicity. The accumulation of LCFAs onto the sludge surface by adsorption obstructs normal biomass transfer, limits essential nutrient transportation, and subsequently affects bacterial activity in anaerobic environments (Pereira, Pires, Mota, & Alves, 2005). Moreover, the adsorption phenomenon is also responsible for operational problems like sludge floatation and washout that may result in treatment failure of wastewater or biomass containing high levels of LCFAs (Long, et al., 2012).

Rinzema et al. (1994) found that the toxicity was primarily related to LCFA concentration rather than the LCFA:biomass ratio described in early research. They also evidenced the existence of lethal threshold concentrations of LCFAs. The studies of Rinzema et al. (1994) and Angelidaki et al. (1992) showed that the toxicity of LCFAs was nonreversible if the concentration of LCFA exceeded a critical threshold level. Nevertheless, anaerobic bacteria exhibit adaptation to the intermediates accumulated during LCFA hydrolysis.

However, recent studies conducted by Pereira et al. (2004) and Hwu et al. (1998) found that the inhibitory effect is not permanent and can be reversible after the degradation and mineralization of LCFAs occurs. As a result of their studies, the LCFAs associated with loose structure of sludge has higher adsorption and accumulation rate that means suspended sludges are more

favorable for efficient degradation of LCFAs than granular sludges. The mass transfer limitation is taken into account and further investigation suggests the initial lag phase and temporarily decreased methanogen activity caused by LCFAs is a physical limitation rather than metabolic inhibition (Pereira, et al., 2005). This is in agreement with the finding of Hwu et al. (1998) that methane production increased with the physical process of LCFA accumulation onto the sludge gradually and complete biodegradation to methane could be attained even at very high LCFA concentration. The results of the batch tests analyzed by Palatsi et al. (2010) demonstrated the recovery of methanogenic activity and confirmed the transport limitation theory. In addition, Pereira et al. (2005; 2004) pointed out the significant enhancement of sludge performance and methane production related to LCFA mineralization.

From a microbial viewpoint, gram-positive bacteria are generally more susceptible to the inhibition caused by intermediate and LCFAs than gram-negative bacteria (Kabara, et al., 1977; Sheu & Freese, 1973). With advanced molecular techniques, Sousa, et al. (2008) brought a new insight on microbial community structure by finding out populations related to the *Clostridiaceae* and *Syntrophomonadaceae* families, both of which are important in LCFA degradation. Palatsi et al. (2010) observed that there is no new microbial population developed over time, and believed that microbial acclimation to LCFA is ascribed to the physiological nature of existing hydrogenotrophic methanogens and LCFA degrading bacteria.

3.3.2 Affecting Factors

The biodegradation of LCFA in reactors as other organic compounds is influenced by factors such as molecular structure of the compound (degree of saturation for LCFA), solubility of the compound in the media containing microorganisms, environmental factors (pH, temperature, nutrients, etc.), and organic loading level (Chipasa & Medrzycka, 2006).

3.3.2.1 Temperature

Hwu et al. (1997) explored the temperature effect on acetate-utilizing methanogenesis, which was exposed to the toxicity of oleic acid in anaerobic digestion. Mesophilic conditions were recommended as a better option for process temperature since thermophilic bacteria were more vulnerable to LCFA inhibition. In addition, they correlated the conclusion with the structure of the sludge that more densely packing of sludge layers allows mesophilic sludge have more strength and stability than thermophilic sludge (Quarmby & Forster, 1995). Bayr et al. (2012) evaluated anaerobic co-digestion of lipid-rich wastes at 35 and 55 °C and the result of the thermophilic treatment failure is consistent with the finding of Hwu et al. (1997).

3.3.2.2 Concentration

(Angelidaki & Ahring, 1992; Koster & Cramer, 1987; Arjen Rinzema, et al., 1994) concluded that the inhibition of LCFAs is highly concentration dependent. The LCFA loading rate also affects various treatment systems, especially for high-rate reactors.

Oleic acid is the most abundant component in LCFA-containing wastewater and described as one of the strongest inhibitors among LCFA. Thus it has been the most frequently targeted for research (C.-S. Hwu, et al., 1998; C. S. Hwu & Lettinga, 1997). It has been reported by Alves et al. (2001) that the 50% inhibition concentration of oleic acid for acetoclastic and hydrogenophilic bacteria was 50 mg/L and 200 mg/L, respectively. This result was based on adding oleic acid in the range from 100 to 900 mg/L. Angelidaki and Ahring (1992) found the initial inhibitory concentration of oleic acid started from 100 mg/L to higher than 200 mg/L but addition of a higher concentration did not promote the further inhibition. A larger scale of oleic concentration was tested by Cirne et al. (2007b), ranging from 5% to 47% (w/w, COD). They found that the limitation of LCFA degradation occurred in the tests with higher than 40% lipids.

3.3.2.3 Degree of Saturation

The study of Sousa, et al. (2008) implied that the saturation degree of LCFA might be attributed to the different bacterial composition involved in LCFA degradation. Unsaturated fatty acids require additional enzymes that breakdown double bonds in the process of metabolism (Shoukry & Schulz, 1998). With respect to different levels of saturation, LCFA might differ with regard to their degradation rates in anaerobic systems. Koster and Cramer (1987) believed that the toxicity of unsaturated LCFAs increased with increasing double bonds. In the case of aerobic treatment, the position of the double bonds did not affect appreciably the metabolism of corresponding unsaturated LCFA, reported by Loehr and Roth (1968). They also found trans compounds

presented a much slower rate of biological oxidation than cis compound and even slower than the corresponding saturated compound.

3.3.2.4 Solubility

Fatty acids are also characterized by solubility that is dependent on molecular structure (Bober & Garus, 2006). LCFAs are generally not soluble in water because their long hydrocarbon chain is hydrophobic. With increasing length of carbon chains, the solubility of LCFAs decreases in water (Bober & Garus, 2006). The solubility of stearic acid (C18:0) is about 0.29 mg per 100g water at 20°C, while that of palmitic acid (C16:0) is raised to 0.72 mg per water at the same temperature (Ralston & Hoerr, 1942). As well as the number of carbon, the degree of saturation also affects the solubility. Unsaturated LCFAs have higher solubility than the saturated ones with identical chain length.

The low solubility of LCFA is thought to be partially responsible for the slow rate of LCFA degradation (Kim, Han, & Shin, 2004).

3.3.2.5 Reactor Configuration

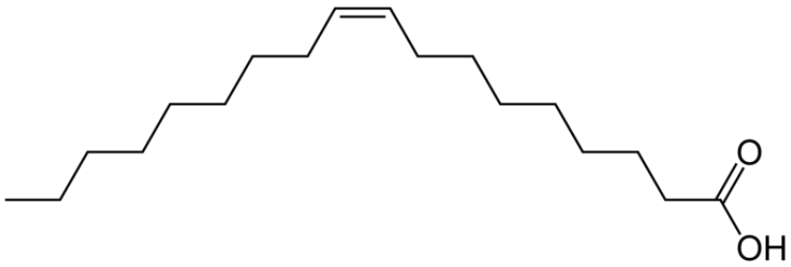
The issue of sludge floatation and washout has been largely concerned with treating wastes containing lipids or LCFAs in UASB reactor. In spite of high LCFA loading rate applied in the system, locally excess adsorption of LCFAs onto sludge takes place even at a low loading rate and that is the main reason for floatation and washout of biomass (C.-S. Hwu, et al., 1998; Arjen

Rinzema, Alphenaar, & Lettinga, 1993). Rinzema et al. (1993) concluded that conventional UASB could easily encounter local overloading and accumulation of LCFAs, unlike a CSTR which allows sufficient mixing and contact between substrates and biomass. Moreover, good mixing improves degradation of LCFAs and the subsequent methane production rate (Pereira, Pires, Mota, & Alves, 2002). Therefore, the CSTR system is preferable for LCFA treatment due to its relatively high mixing condition (Long, et al., 2012). Hanaki, et al. (1981) also suggested feeding at constant rate could avoid overloading and shock loading of LCFAs and keep the system in healthy condition.

Oleic (designated as C18:1), linoleic (C18:2), linolenic (C18:3), and stearic (C18:0) acids are common LCFA found in vegetable and animal fats and oils. Accordingly, they are also easily detected in wastewater and wastes from related industries. These LCFA were used in this study. The chemical and skeletal formulas of the LCFA are shown as follows:

Oleic acid

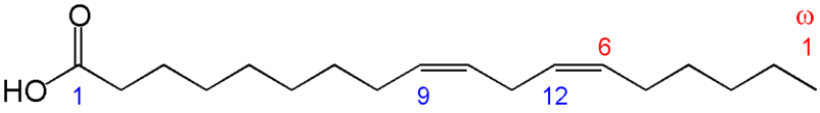
Formula: $C_{18}H_{34}O_2$



Lipid number: C18:1

Linoleic acid

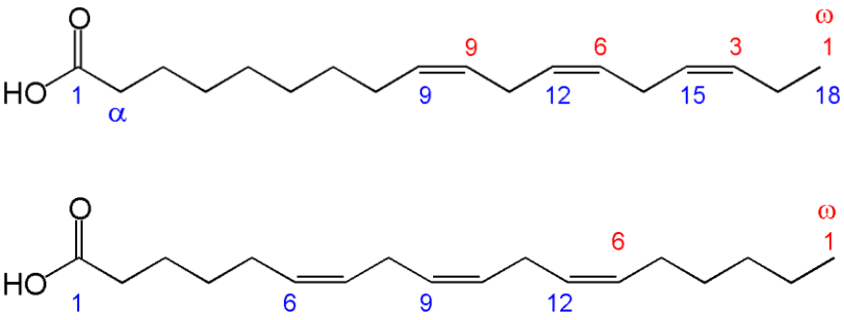
Formula: $C_{18}H_{32}O_2$



Lipid number: C18:2

Linolenic acid

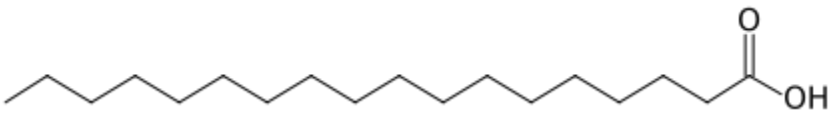
Formula: $C_{18}H_{30}O_2$



Lipid number: C18:3

Stearic acid

Formula: $C_{18}H_{36}O_2$



Lipid number: C18:0

4 Materials and Methods

Sludge Samples

The sludge used in this study was collected from the District of Columbia Water and Sewage Authority (DCWASA) Blue Plains wastewater treatment plant. The raw sludge consisted of thickened primary and secondary sludge, both of which were diluted to approximately 3% total solids before being mixed together at a ratio of one to one by weight to prepare for the influent sludge. During the entire period of experimentation, all the raw and prepared sludge was kept refrigerated at 4 °C.

Seed Sludge

The influent sludge was spiked with different LCFAs investigated in this study one at a time. The LCFAs were all purchased from MP Biomedicals and stored at 4 °C. The mixture of the influent sludge and a specific type of LCFA was prepared and fed into the anaerobic digester every day. In order to evaluate the effect of different LCFA concentrations, the proportion of LCFA to the influent sludge (mass basis) was selected as 5%, 10% and 20%. Based on a sludge loading and withdrawal rate of 500 ml per day, the calculation of daily LCFA dose for 10% is as follows:

The influent sludge: 3% total solids = 30,000 mg/L

The quantity of LCFA added per day: (30,000 mg/L) × (0.5 L) × 10 % = 1,500 mg = 1.5 g

Hence, 0.7g and 3 g of LCFA was applied to obtain 5% and 20% LCFA contained in the feed, respectively. It could be assumed that the overall solids concentration of 3% influent sludge increased based on the additional LCFA.

The following LCFAs were in the feeding sludge and studied at different concentrations, which are tabulated in table 1.

Table 1: Study matrix for LCFAs.

LCFA	Concentration of LCFA		
	5%	10%	20%
oleic acid	√	√	√
linoleic acid	√	√	√
linolenic acid	√	√	√
stearic acid		√	√
stearic + oleic acid		√	

Anaerobic Digester Set-up

The anaerobic digesters are polyethylene plastic tanks supplied by Hobby Beverage Equipment Company. They have a 5 gallon capacity with a cylindrical shape and a conical bottom. The lid and bottom ball valve provide an anaerobic environment. Gas produced in the tank was circulated via a peristaltic pump that provided continuous mixing. The lab-scale digesters were

set up in the constant temperature room at 37 °C (mesophilic condition) to keep the temperature stable. The configuration of one of the three digesters is shown in Figure 3.

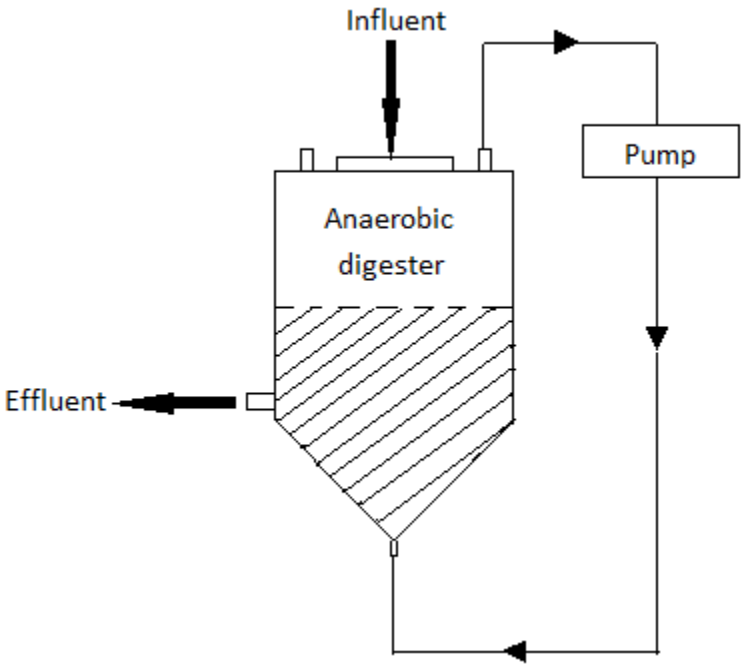


Figure 3: Configuration of the anaerobic digester applied in the study.

All of the digesters were operated with a total volume of 10 L sludge. On a daily basis, 500 ml sludge was withdrawn first for further analysis and then the same amount of sludge was fed into each digester. The effluent and influent was equally quantified in order to maintain a 20-day SRT. One of the digesters was operated as the control along with the other digesters receiving different levels of LCFAs. The feeding sludge containing LCFA was not transferred into any of the reactors until a sufficient time of operation with only the influent sludge had been carried out. The microbes in the anaerobic digesters require adequate time for acclimation to the new environment. It took one month for the sludge to be stabilized. After the stabilization, the digesters except the control started to receive the 500 ml seed sludge (the mixture of sludge and

LCFAs) instead of the influent sludge every day. All the feedings went into the anaerobic tanks from the tops.

Sampling and Analysis

Total solids (TS), Volatile solids (VS), pH and chemical oxygen demand (COD) were tested and determined according to Standard Methods (APHA, AWWA, & WEF, 1989). All the experiments were performed in triplicate. In terms of statistical analysis, one sided t-test and one way analysis of variance (ANOVA) were applied for hypothesis tests to determine if there were significant differences from varied data groups. The significance level was constantly equal to 0.05.

5 Results

5.1 pH

Proper pH is essential for methanogens to successfully survive in the anaerobic digester. From Figure 4, the data of pH for 10% oleic acid is shown to be in the range of 7.4 to 7.7 and has a tendency to be stable over the operating period. The pH data presented was typical of all the digesters. Both of the pH values obtained from the control effluent and effluents associated with LCFAs indicate the environment for anaerobic bacteria is sound and stable.

In the study of Kabara et el. (1977), fatty acids could be described as surfactants and exhibited their strongest actions against microbes in an acidic environment. For example, the pH from 6.0 to 6.5 is an inhibitory environment for microbes with the occurrence of fatty acids. Based on the range of the pH values, the anaerobic environment in this study should not stimulate the toxicity of LCFAs to bacteria activity.

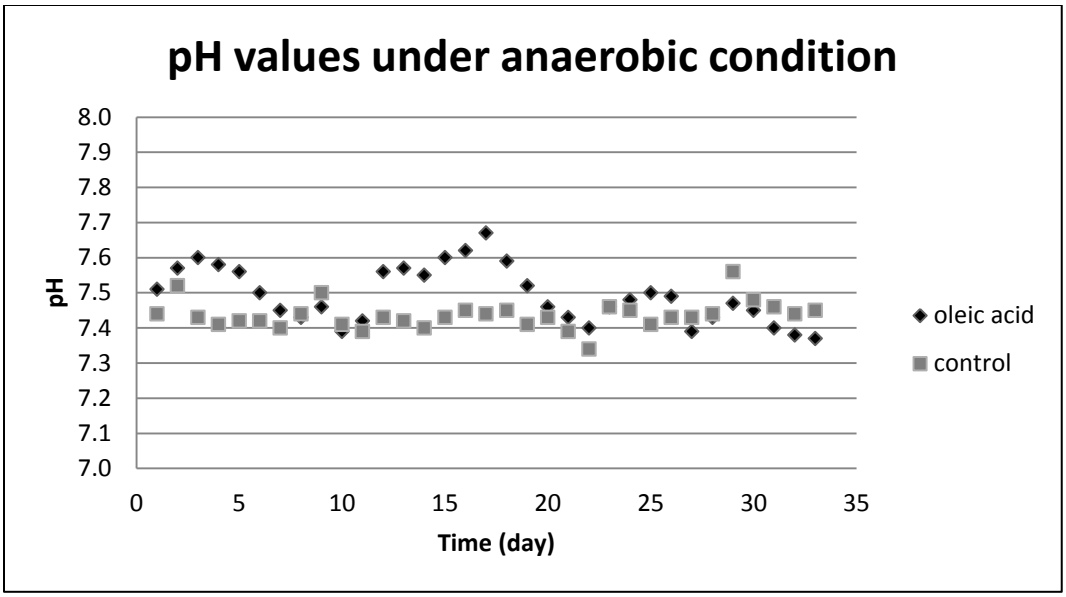


Figure 4: pH measurements**.**

5.2 Solids

5.2.1 Oleic Acid (C18:1)

Oleic acid is one of the most common LCFAs found in various natural fats and oils. The oily form of the oleic acid utilized in the experiment is odorless and yellowish. Fraser (2010) investigated the addition of 10% oleic acid and a combination of oleic, linoleic and linolenic acid at equal weights into the anaerobic sludge. The sum of three LCFAs' weights is also up to 10% of total solids concentration. The circumstances of his experimentation such as materials and environmental factors were very similar to that of this study.

For the study of oleic acid, a level of 10% was initially added to the digester and the concentration of the fatty acid was then changed to 5% and then to 20%.

The effluent sludge and feed sludge prior to digestion were both characterized by TS, VS and COD. It was assumed that complete degradation of all the fatty acids occurred during the anaerobic digestion. That could lead to complete conversion to biogas. Based on this assumption, the effluent solids in the digester receiving additional oleic acid should share similar characteristics with the effluent of the control sludge, which would be reflected in the solids data.

5.2.1.1 Additional Reduction of Solids

At the beginning of the study, 10% of oleic acid was fed into the digester and evaluated via solids reduction. For the control and the oleic acid-amended digester, the TS and VS data shows consistency over the operating period as shown in Figure 5 and 6. Based on the consistency of the data, the steady-state period was selected and is also indicated in Figure 5 and 6. Compared to the control, the digester with 10% additional oleic acid was observed to average a lower solids concentration from the effluent results for both TS and VS, which is in accordance with the data of Frasier (2010). This indicated that the addition of oleic acid improved the degradation of the original sludge.

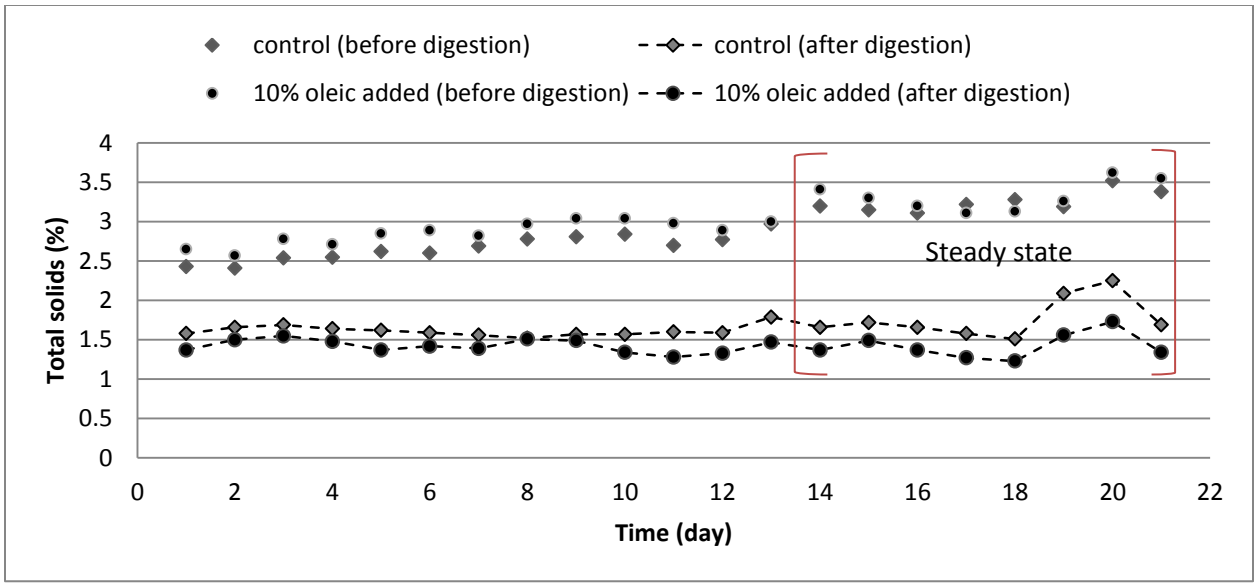


Figure 5: Comparison of TS (%) between the control and sludge added with 10% oleic acid.

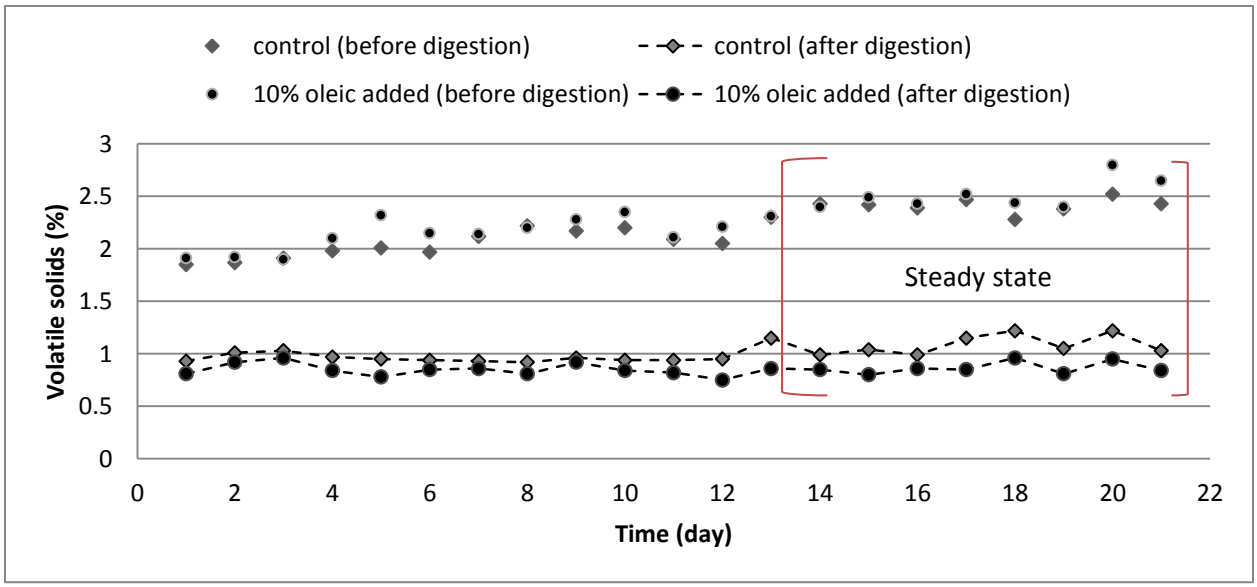


Figure 6: Comparison of VS (%) between the control and sludge added with 10% oleic acid.

In order to assess the statistical significance, the following tests were performed: two-sample, one sided t-tests for means were run by the R project to provide a statistical analysis of TS and VS reductions.

The alternative hypotheses for both TS and VS data are listed as follows:

1. Before the digestion, the population mean of solids data from the control is less than that of the sludge seeded with 10% oleic acid.

2. After the digestion, the average solids values of the control is greater than that of the 10% oleic acid-amended sludge.

Both of the p values for the effluent data provided in Table 2 were lower than the significance level $\alpha = 0.05$, therefore the null hypothesis was rejected and there was a statistical difference from each other in means with 95% confidence. However, the first alternative hypothesis could not be accepted due to the p values slightly higher than 0.05. By way of rerunning two-sided t-tests for the solids data from the control and 10% oleic acid-added sludge before the digestion, significant differences between two groups of data were not obtained ($p = 0.1364$ for TS; $p = 0.1839$ for VS), although the TS and VS means of the oleic acid-added group did have larger number than that of the control group.

Table 2: Statistical analysis of solids data.

Control / 10% additional oleic acid	p value	
	Before digestion	After digestion
TS	0.06821	2.774e-06
VS	0.09196	7.151e-08

The outcomes of the statistical tests demonstrate that additional solids removal was accomplished by seeding with oleic acid, based on the assumption that the solids from the 10% oleic acid was completely consumed in the anaerobic digester. This assumption was not confirmed but could be determined by an analysis of the long chain fatty acid content of the digesters. That analysis was considered to be beyond the scope of this study. Whether or not the oleic acid was fully biodegraded, the addition of oleic acid resulted in a lower effluent solids concentration compared to the control sludge without fatty acids. Figure 7 illustrates the proportion of additional solids reduction based on the mass balance method.

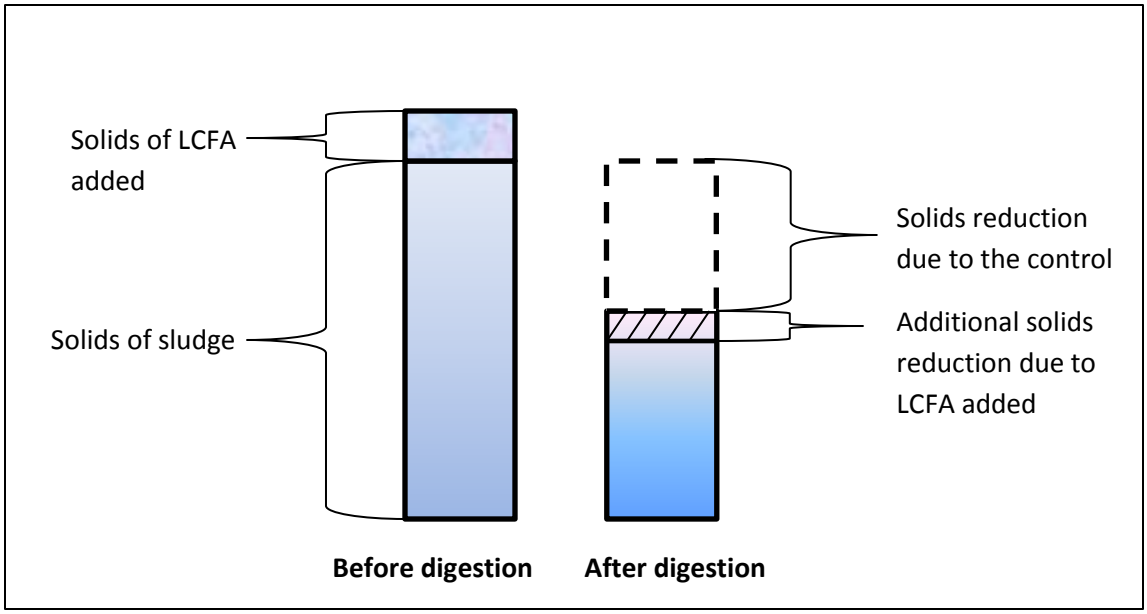


Figure 7: Illustration of additional reduction contributed by added oleic acid at the level of 10%.

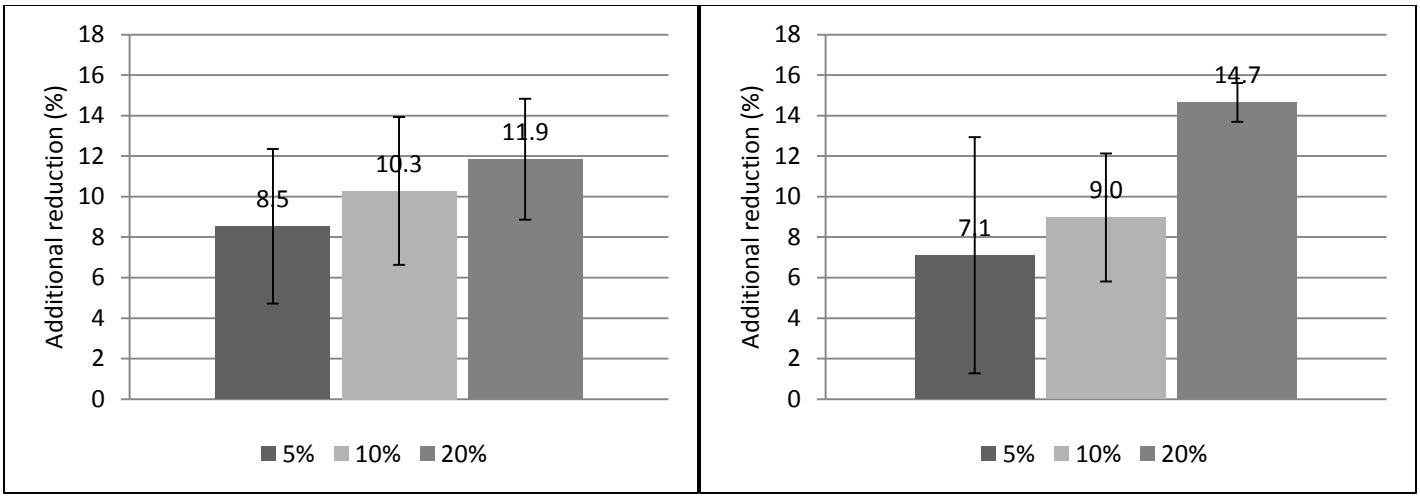
5.2.1.2 Concentration

The effect of oleic acid addition on the sludge biodegradation efficiency was evaluated in terms of oleic acid concentrations ranging from 5% to 20%. The percentages of average solids reduction are presented in Table 3, corresponding to different levels of oleic acid addition. An enhancement of solids removal could be observed from both TS and VS with increasing concentrations of additional oleic acid.

Table 3: Average total solids reduction according to three different concentrations (5%, 10% and 20%) of oleic acid.

Concentration of oleic acid	0%	5%	10%	20%
Total TS reduction (%)	41.8	51.1	56.6	57.1
Total VS reduction (%)	53.5	61.1	63.3	71.5

Additional solids reduction contributed by oleic acid is plotted in Figure 8 with regard to concentration of oleic acid. As seen in Figure 8, the additional degradation of solids increased with increasing addition of oleic acid. An average of 10% additional TS was destroyed with the occurrence of additional oleic acid, while at least 7% additional reduction was found in terms of VS. Compared to Figure 8 (a), the increased VS reduction percentage was greater and implicated more efficiency in degradation.



(a) TS (b) VS

Figure 8: Average additional (a) TS and (b) VS reduction by adding different concentrations of oleic acid only from 5% to 20%.

One-way ANOVA was applied to analyze if any significant difference occurred among different data groups of additional solids removal. The dependent variable was set as TS first and the single factor was the concentration of oleic acid added to the digester, which was symbolized as four letters in the data frame for 0% (the control), 5%, 10% and 20%. As a result, the p value was equal to 2e-04 that means there was a significant effect of LCFA concentration on extra TS reduction. Since each of the four data groups mentioned above was significantly different from the control, a post hoc comparison was conducted with Tukey HSD test to compare differences between the digesters receiving LCFAs. No significant difference was seen for 5%, 10% and 20% from each other, though all of them were statistically different from the control. By running identical tests for additional VS reduction, p values from the ANOVA summary was recorded as 4.03e-05 that confirmed the concentration factor associated with increased VS destruction as

well as TS. The statistical results of the Tukey HSD are summarized in Table 4. The influence of oleic concentration on TS and VS reduction were alike in the statistical analysis except the VS comparison between 5% and 20% of additional oleic acid was different. That implied that increasing the amount of oleic acid increased the VS removal.

Table 4: Tukey HSD analysis on additional VS removal data.

Results	Source of variation (concentration of oleic addition)
Significant difference	0% and 5% ($p = 0.019$) 0% and 10% ($p = 0.0032$) 0% and 20% ($p = 1.9\times10^{-5}$) 5% and 20% ($p = 0.012$)
No significant difference	5% and 10% ($p = 0.82$) 10% and 20% ($p = 0.070$)

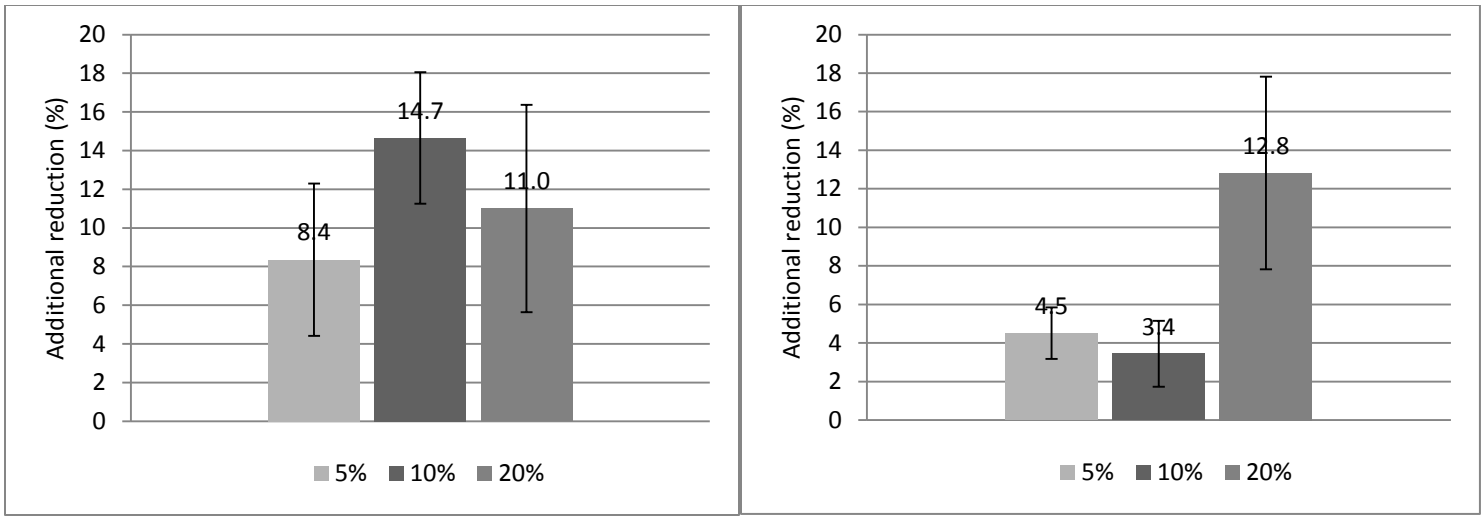
5.2.2 Linoleic Acid (C18:2)

Linoleic acid is an unsaturated LCFA with an 18-carbon chain and two cis double bonds, described as a colorless liquid oil. In order to investigate the effect of different degrees of unsaturation, linoleic acid was used in this study by varying its concentration from 5% to 20%. In Table 5, the results of the solids removal for linoleic acid were highly variable, but still showed enhanced degradation compared to the control.

Table 5: Average total solids reduction according to three different concentrations (5%, 10% and 20%) of linoleic acid.

Concentration of linoleic acid	0%	5%	10%	20%
Total TS reduction (%)	31.8	40.8	51.7	45.8
Total VS reduction (%)	43.1	54.9	51.6	58.6

As plotted in Figure 9, additional TS and VS reduction was observed, but the results showed no consistent trend. Statistical tests were performed to compare 0% (the control), 5%, 10% and 20% of linoleic acid addition. The p value obtained from the ANOVA test for extra TS and VS reduction was 8.40×10^{-4} and 4.65×10^{-4}, respectively. Although the Tukey HSD test conducted later did not show any tendency of solids reduction to increase with increased adding of linoleic acid, the statistical outcomes can be interpreted that the concentration of linoleic acid contained in the sludge also affects the solids data and the addition of linoleic acid is of assistance to the solids degradation to some extent.



(a) TS (b) VS

Figure 9: Average additional (a) TS and (b) VS reduction by adding different concentrations of linoleic acid only from 5% to 20%.

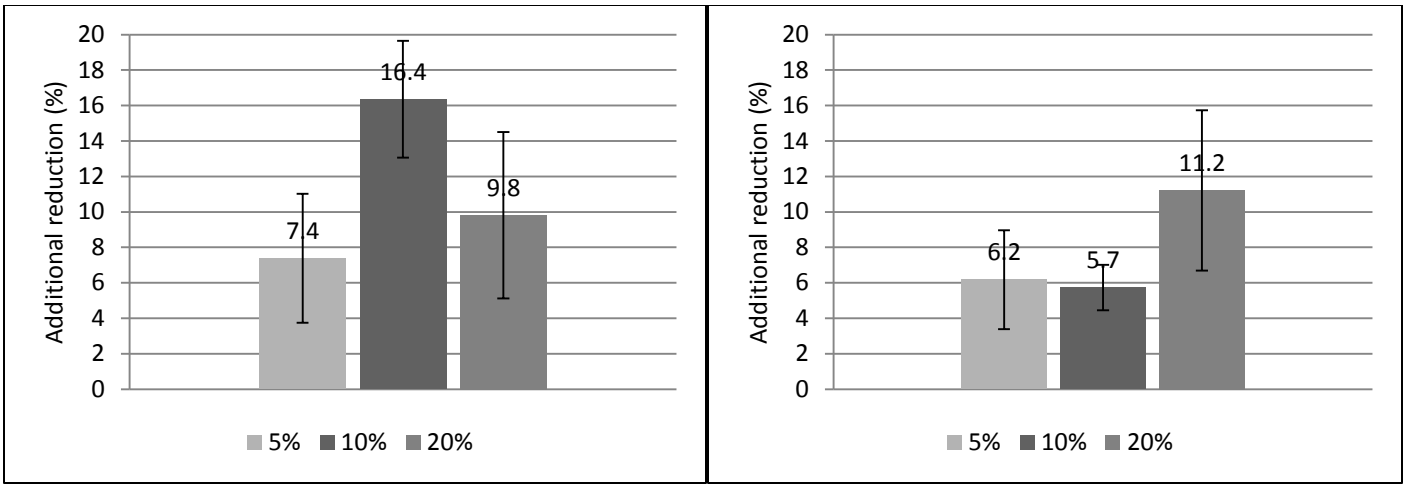
5.2.3 Linolenic Acid (C18:3)

Linolenic acid, an 18-carbon unsaturated LCFA with three double bonds, was also studied. Different levels of linolenic acid addition from 0% to 20% related to solids reduction data is summarized in Table 6.

Table 6: Average total solids reduction according to three different concentrations (5%, 10% and 20%) of linolenic acid.

Concentration of linoleic acid	0%	5%	10%	20%
Total TS reduction (%)	33.2	48.9	53.4	44.6
Total VS reduction (%)	45.3	56.6	53.9	57.0

Figure 10 illustrates the additional solids removal contributed by linolenic acid. Compared to linoleic acid, it exhibited a similar pattern for extra solids reduction. It can be seen that a certain improvement on solids removal occurred due to the feeding of linolenic acid to the digester. An ANOVA test provided statistical information on the relationship of different solids data affected by varying levels of linolenic acid addition from 0% (the control) to 20%. The results concluded that a statistical difference did occur within four concentration groups ($p = 9.40e\text{-}05$ for TS; $p = 0.021$ for VS). The solids data with 5%, 10% and 20% of linolenic acid addition did not differ significantly from each other by running Tukey HSD test. However, all of them were significantly different from the control.



(a) TS (b) VS

Figure 10: Average additional (a) TS and (b) VS reduction by adding different concentrations of linolenic acid from 5% to 20%.

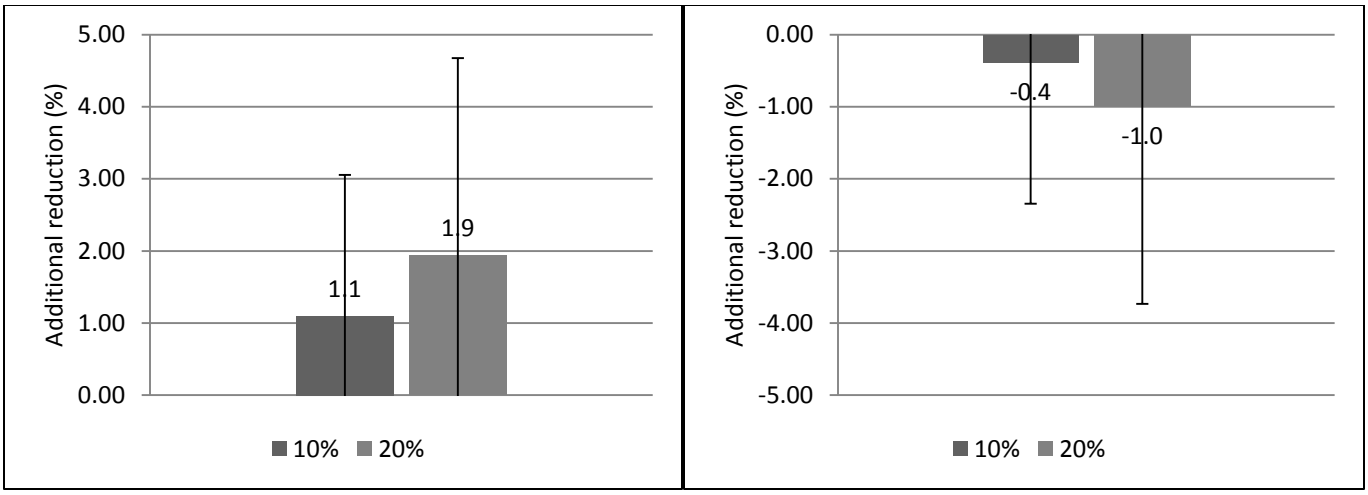
5.2.4 Stearic Acid (C18:0)

Stearic acid is a very common 18-carbon saturated fatty acid. Its physical appearance is white powder and the solubility in water is as low as 3mg/L at 20°C (Ralston & Hoerr, 1942). A concentration of 10% and 20% of stearic acid was moderately stirred with the influent sludge and then fed to the digesters. For the different concentrations of stearic acid added, Table 7 lists the performances of total solids reduction and the additional solids reduction is calculated and plotted in Figure 11.

Table 7: Average total solids reduction according to two different concentrations (10% and 20%) of stearic acid.

Concentration of stearic acid	0%	10%	20%
Total TS reduction (%)	43.2	44.4	45.9
Total VS reduction (%)	49.4	52.0	53.1

The charts in Figure 11 suggests that most of the stearic acid was biodegraded in the anaerobic digester but the sludge did not receive any extra benefit of solids destruction from the additional stearic acid at either 10% or 20%, in contrast to the unsaturated LCFAs. The statistical tests were conducted on the solids data of the effluents from the digesters with 0% (the control), 10% and 20% of stearic acid. The results of ANOVA test show the concentration did not play a role in solids degradation for either TS ($p = 0.35$) or VS ($p = 0.87$). Moreover, no significant difference from every two groups of the data including the control was concluded from the Tukey HSD test.



(a) TS (b) VS

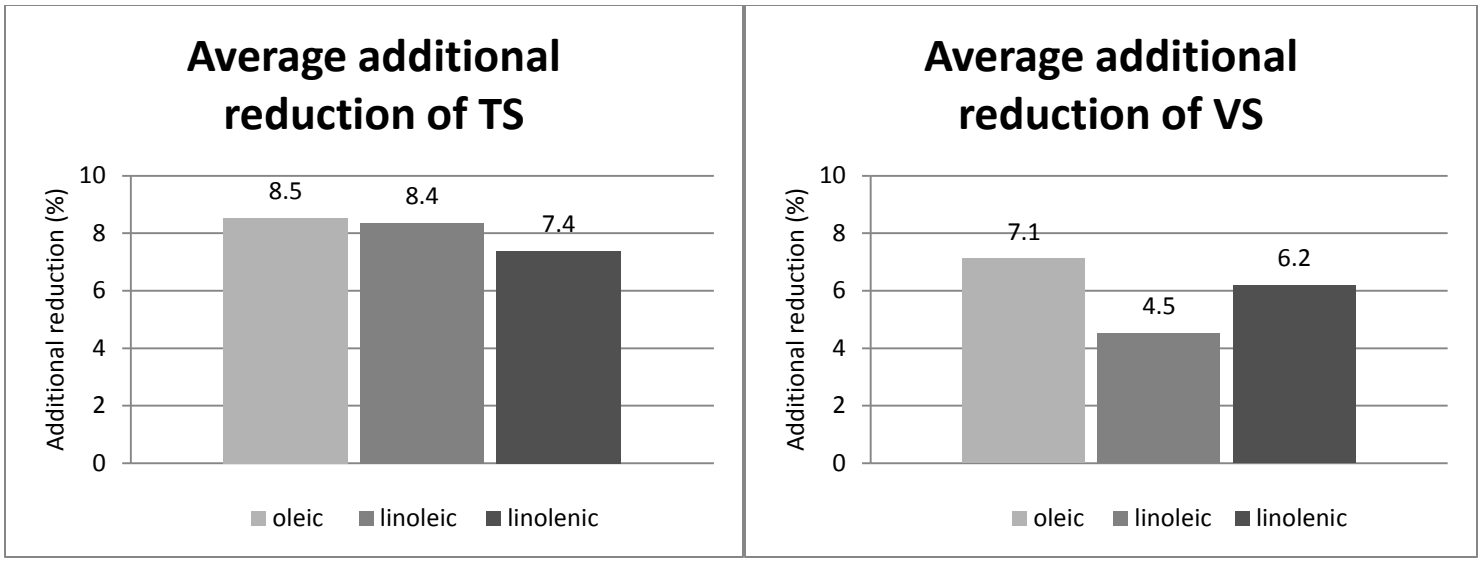
Figure 11: Average additional (a) TS and (b) VS reduction by adding stearic acid from 10% to 20%.

The anaerobic degradation of stearic acid is regarded as a slower process than that of the unsaturated LCFAs with equal chain length (Lalman & Bagley, 2001; Novak & Carlson, 1970). Compared to oleic, linoleic and linolenic acid, stearic acid is more insoluble in water due to its physical structure. Therefore, whether the stearic acid dispersed well and had enough contact with bacteria in the anaerobic digester was a concern since the majority of content in sludge is water (Yin, Han, Lu, & Wang, 2004).

5.2.5 Comparison

The concentration of unsaturated LCFAs added to the sludge proved to be a key factor in the performance of solids reduction by statistical methods. To evaluate the effect of unsaturation, the additional solids reduction of oleic, linoleic, linolenic and stearic acid was analyzed in one plot, which is shown in Figures 12, 13 and 14 referring to three different concentrations of LCFA addition. Three comparisons were used to determine if a pattern was followed due to different degrees of saturation.

5.2.5.1 Concentration: 5%



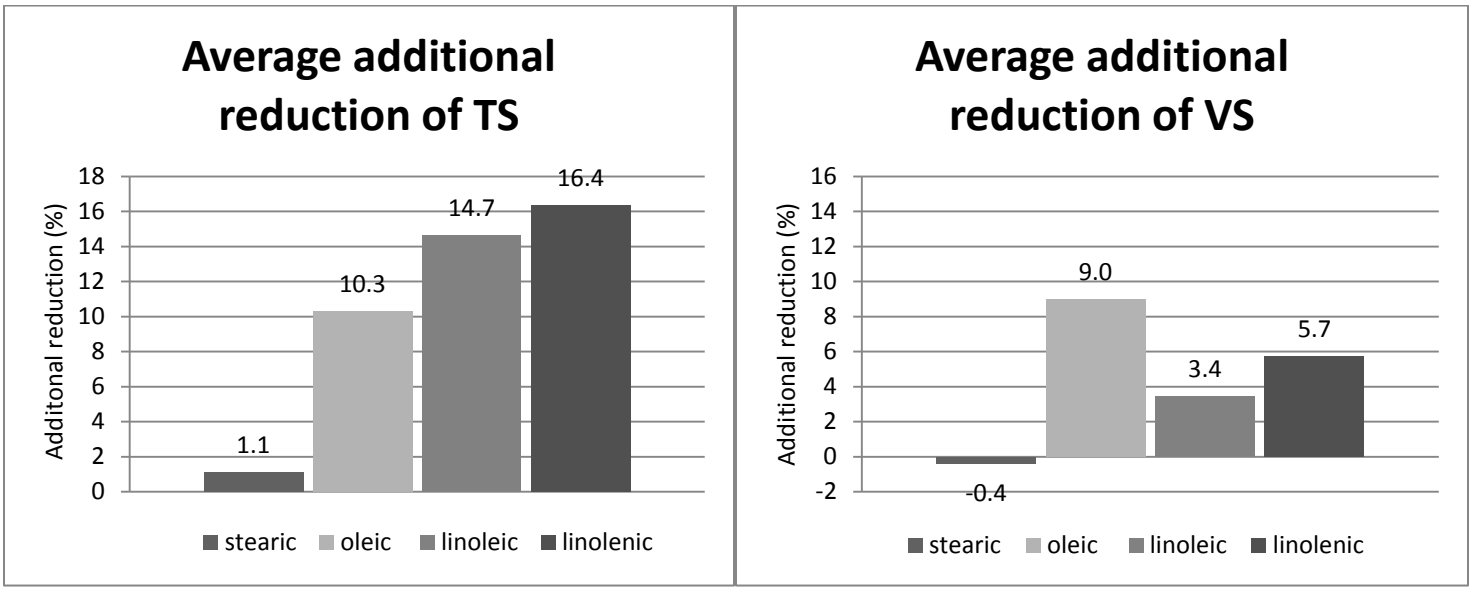
(a) TS

(b) VS

Figure 12: Average additional reduction of (a) TS and (b) VS by feeding 5% of oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids

The data in Figure 12 shows that oleic acid had a slightly better capability for both of the solids removal when only 5% of three LCFAs added to the sludge under absolute the same condition. The performances of linoleic and linolenic acids varied according to the TS and VS data. ANOVA was run in order to determine if the means of three independent samples significantly differed from each other. As a result, a p-value of 0.9174 and 0.6555 was recorded for TS and VS, respectively. Since they were smaller than α (= 0.05) that meant the null hypotheses of equal mean were accepted. In turn, the three kinds of unsaturated LCFAs presented no statistical difference in solids reduction. However, the previous statistical tests carried out for individual fatty acids all showed a significant difference from the control.

5.2.5.2 Concentration: 10%



(a) TS

(b) VS

Figure 13: Average additional reduction of (a) TS and (b) VS by feeding 10% of stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids.

As shown in Figure 13, the stearic data presented were much lower than other fatty acids and there was no contribution to extra solids removal. With respect to TS reduction, the amounts of additional removal for different fatty acids increased with increasing degrees of unsaturation. For VS reduction, the results showed no trend with saturation level. It is probable that the variability in feed and variation in the analysis accounted for this variability.

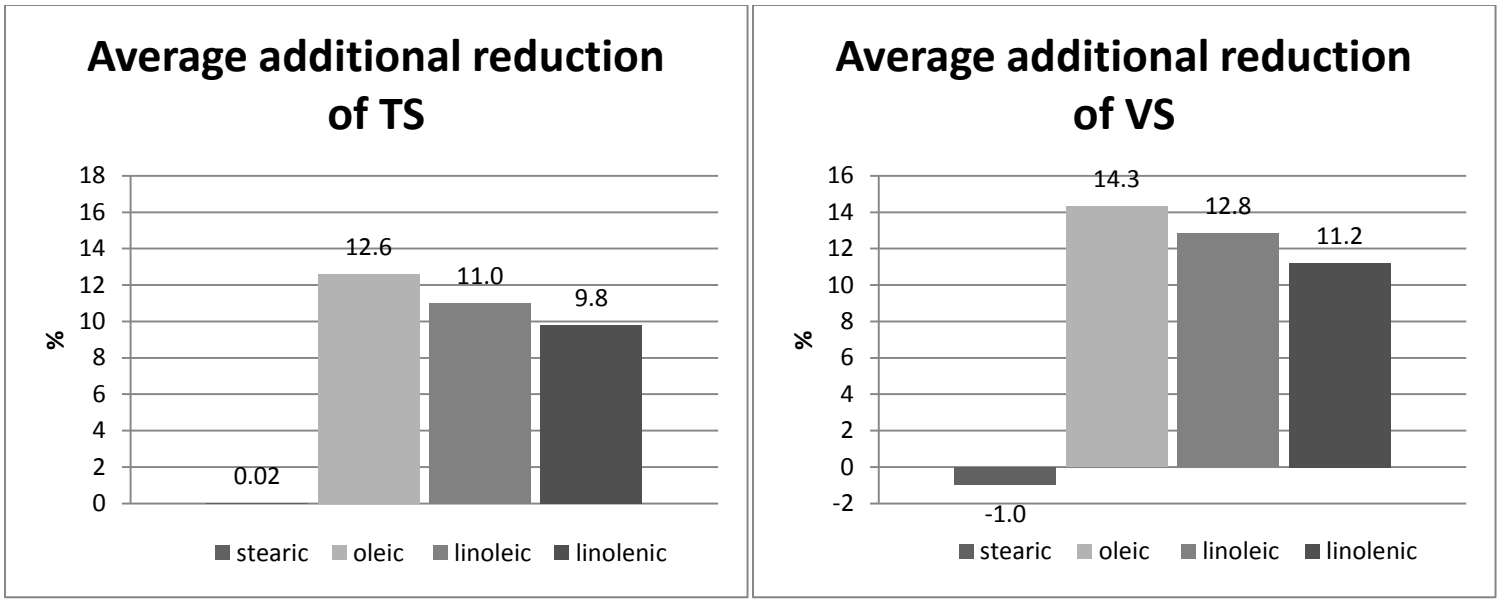
A summary of ANOVA and Tukey HSD test for TS data is listed in Table 8. The results indicated that the unsaturation level was correlated to the additional TS destruction with the occurrence of 10% LCFAs in the sludge. In spite of stearic acid being significantly different

from the other LCFAs, the variation in the three unsaturated fatty acids was not statistically significant, although one of the p values slightly less than 0.05 and might suggest oleic and linolenic acid were marginally different from each other. For the VS data, the similar results could also be found by running the statistical tests.

Table 8: Statistical results for TS according to 10% dosage of different LCFAs.

Results	Source of variation (degree of unsaturation)
Significant difference	C18:0, C18:1, C18:2 and C18:3 ($p = 7.057e-06$) C18:0 and C18:1 ($p = 2.0\times10^{-3}$) C18:0 and C18:2 ($p = 3.7\times10^{-5}$) C18:0 and C18:3 ($p = 9.0\times10^{-6}$) C18:1 and C18:3 ($p = 0.047$)
No significant difference	C18:1 and C18:2 ($p = 0.20$) C18:2 and C18:3 ($p = 0.85$)

5.2.5.3 Concentration: 20%



(a) TS

(b) VS

Figure 14: Average additional reduction of (a) TS and (b) VS by feeding 20% of stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids.

From Figure 14, the opposite tendency occurred in the TS data for three unsaturated fatty acids compared to the 10% addition when the concentration of acids was 20%. A decrease took place in TS and VS removal when the degree of unsaturation increased. The 20% stearic acid did not contribute to additional solids removal which was consistent with the pattern that occurred in the test of 10% LCFAs. It is possible that 20% LCFAs showed inhibition to the microorganism in the digester and inhibition intensified as the solubility of LCFAs increased.

The results of statistical tests conducted for the 20% additional LCFAs were similar to that of the analysis on the LCFAs at the level of 10% addition, since both of the p values resulted from ANOVA tests were lower than 0.05 in Table 9. The following post hoc test showed that the three

unsaturated LCFAs were not significantly different from each other, while they all differed from the stearic acid.

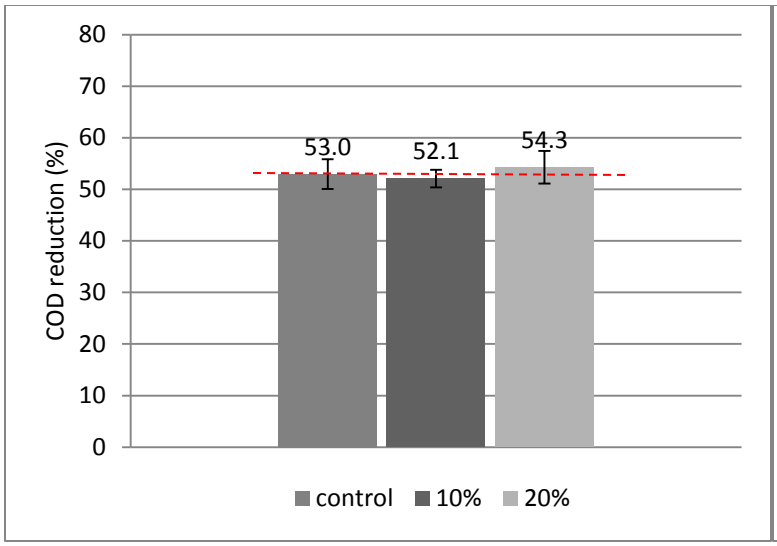
Table 9: ANOVA analysis according to 20% dosage of different LCFAs.

Source of variation: C18:0, C18:1, C18:2 and C18:3	Results: significant difference
TS	$p = 0.015$
VS	$p = 0.0018$

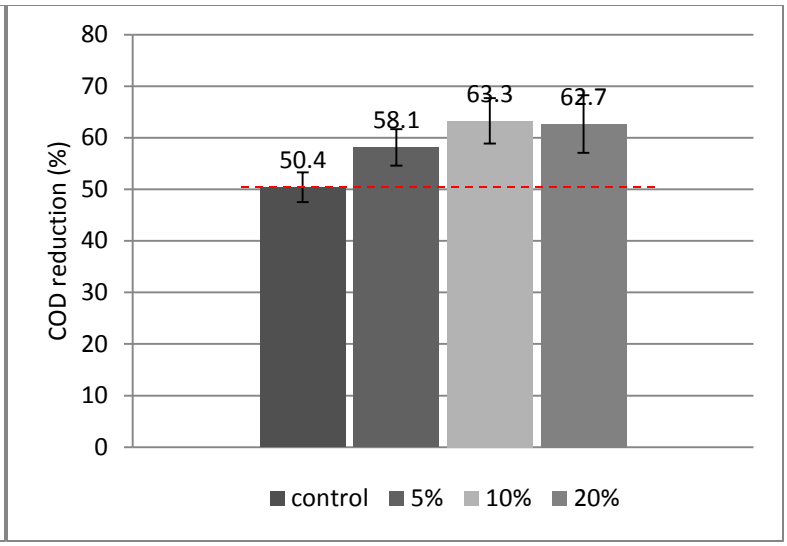
5.3 COD Reduction

Both VS and COD measurement reflect the organic matter in the sample (Pind, et al., 2003). As the solids data illustrated above, the COD values from the LCFA-amended feeding were increased due to the additional LCFAs. An additional COD reduction could also be obtained if the effluent COD of the LCFA-amended sludge was lower than the effluent COD from the control, based on the assumption that the LCFAs were fully degraded.

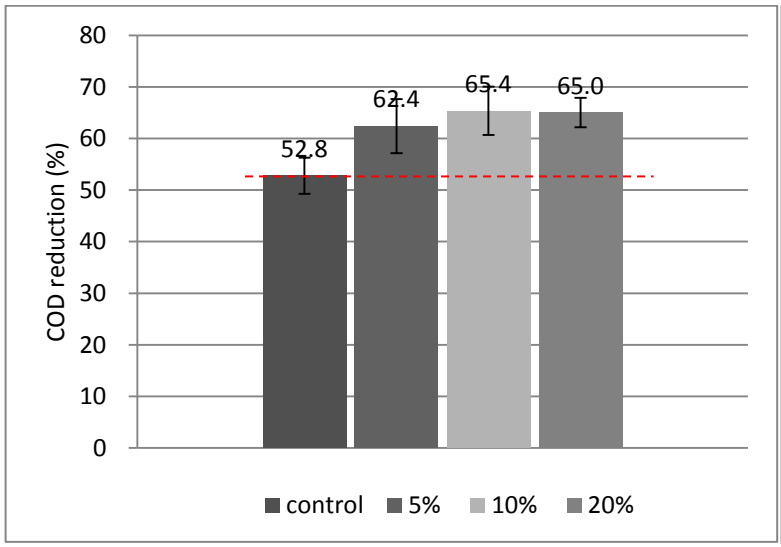
The average COD reduction is plotted for four LCFAs with various concentrations in Figure 15. Stearic acid differed the most from the three unsaturated LCFAs and did not show any more COD reduction compared to the control.



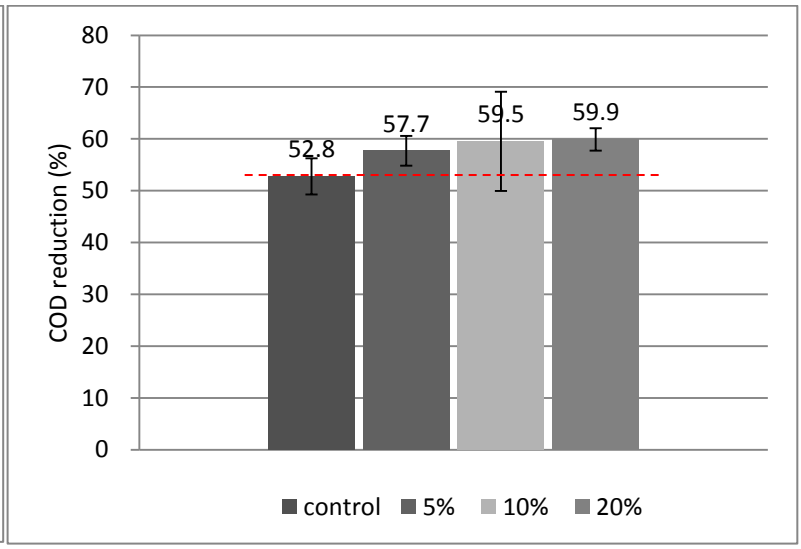
(a) Stearic acid



(b) Oleic acid



(c) Linoleic acid



(d) Linolenic acid

Figure 15: Average COD reduction by adding different concentrations of (a) stearic acid, (b) oleic acid, (c) linoleic acid, and (d) linolenic acid.

The average additional COD reduction from all the LCFA levels added to the digesters is provided in Table 10. Average additional COD reduction was not given individually for each adding level, since the COD removal among all the concentrations applied to every LCFA was close to each other and the data was consistent with low variation, as shown by the error bars in Figure 15. ANOVA and Tukey HSD were performed to examine the concentration impact on each LCFA addition. The consistency in COD data was proved by statistical tests that no significant difference occurred for all the LCFAs from varying concentration addition, but they were all significantly different from the control except for stearic acid. The statistical analysis on the COD data could be correlated to that on the VS data. The average additional COD reduction for stearic acid suggested that complete degradation of stearic acid was not accomplished in the digester.

Table 10: Average additional COD reduction.

LCFA	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Average additional COD reduction (%)	-2.8	8.0	10.5	5.3

5.4 Mixture of stearic and oleic acid

The stearic acid was investigated individually in the previous study and its relatively low solids and COD removal compared to the other LCFAs had raised an attention. Since stearic acid was the only saturated LCFA in the study and had the lowest solubility in water, one hypothesis was addressed that the low solubility impeded the anaerobic microbe access. That might result in difficulties either in its own degradation or utilization as energy source by bacteria. Therefore, the following study was added to see if the improved solubility of stearic acid that resulted from combining it with oleic acid could promote the degradation of stearic acid in the digester.

5.4.1 Experimental Design

A batch study was conducted to investigate the effect of oleic acid on the solubility of stearic acid. The procedure was generally described in the following steps. A beaker was filled with 20 ml oleic acid, which is clear colorless liquid. In attempt to obtain a 1:1 ratio (w/w) of stearic and oleic acid, 18 g of white powder of stearic acid was added to the oil liquid since the density of stearic and oleic acid is 0.84 and 0.89 g/ml, respectively. The solution was magnetically stirred under room temperature (summer).

After an at least two-hour mixing, abundant solids were observed in suspension in the solvent, indicating super-saturation. Nevertheless, a clear solution occurred and stearic acid was completely dissolved by leaving it overnight.

The purpose of this study was to determine if one of the benefits of the addition of unsaturated LCFA was to dissolve saturated fatty acids, making them more bioavailable. Since stearic acid could dissolve in oleic acid, a mixture of the two fatty acids was fed to the digester.

The preparation of daily feeding with sludge is shown in Table 11. The total amount of the LCFA mixture was 1.5 g to add 10% of the total daily feeding (w/w). In order to accelerate the dissolution rate, the proportion of stearic acid in the solution was dropped from 1:1 to 1:2. In addition, the room with all the digesters kept the constant temperature higher than room temperature, which provided extra assistance to the dissolution process.

Table 11: The list of daily feeding.

Name	Quantity
Sludge (1:1 of primary and secondary sludge)	500 ml
Oleic acid	1.0 g
Stearic acid	0.5 g

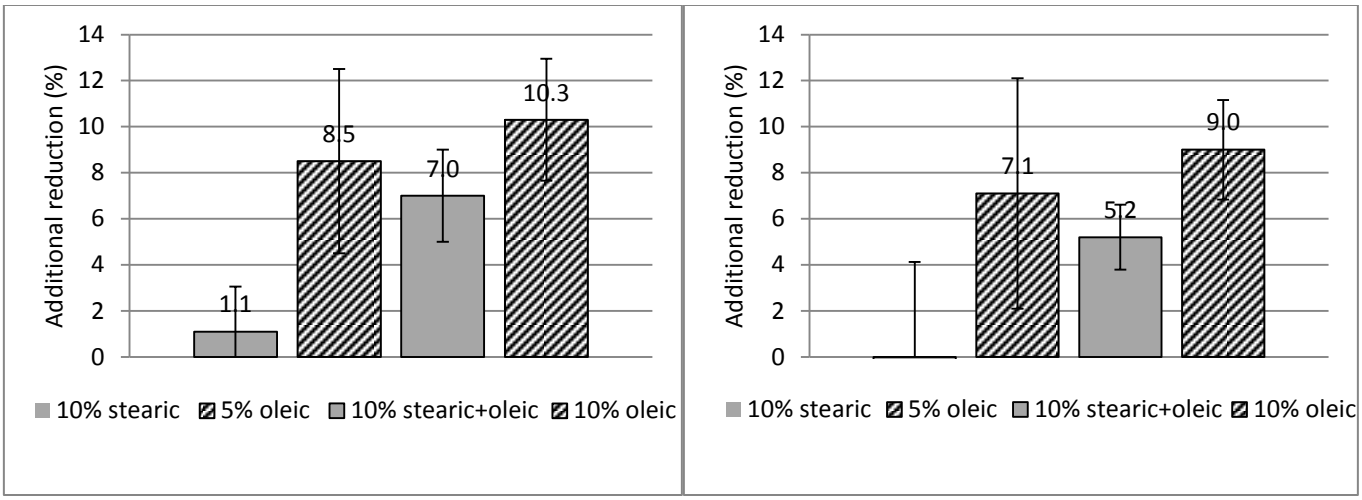
5.4.2 Solids Removal

The total solids reduction is obtained from the combination of stearic and oleic acid in Table 12. Meanwhile, the additional solids reduction related to the mixture was compared with oleic acid as well as oleic acid at the same level of addition to the digester. The comparison is provided in Figure 16.

Table 12: Average solids reduction referring to 10% mixture of stearic and oleic acid.

Digester	Control	Stearic + oleic acid (10% in total)
Total TS reduction (%)	45.2	56.4
Total VS reduction (%)	53.7	60.4

As seen in Figure 16, the performance of stearic acid in solids removal was improved by being mixed with oleic acid. Numerically, the mixture of fatty acids still presented lower additional solids reduction than oleic acid only, so statistical tests were run later for its significance. ANOVA and a post hoc test was established on the comparison of four data groups including the control, 10% stearic acid only, 10% oleic acid only and 10% of the LCFA mixture. In spite of the stearic acid, all the other data groups were significantly different from the control. The p value for the mixture and stearic acid only was 0.0067 (TS) and 0.046 (VS), indicating that dissolving stearic acid in oleic acid did increase the solids removal compared to the addition of stearic acid only. However, the results from the comparison between the mixture and oleic acid only turned out that they did not differ statistically (the p value was equal to 0.29 for TS and 0.35 for VS). Since over 60% of the mixture was composed of oleic acid, the stearic acid in the dissolution did not contribute much to the sludge degradation by comparing the result from 5% additional oleic acid with that from10% additional mixture. Therefore, the dissolution of stearic acid into oleic acid did not benefit the anaerobic degradation in this study.



(a) TS (b) VS

Figure 16: Average additional (a) TS and (b) VS reduction by adding 10% of stearic acid only, 5% and 10% of oleic acid only and 10% mixture of oleic and stearic acid.

The standard deviation of the solids data from the stearic acid only indicated that the variability of the data points was relatively high, which could be a possibility of stearic acid's poor dispersion in the digester due to its low solubility.

5.4.3 COD Reduction

As shown in Figure 17, the COD reduction from the mixture was higher than that from the control. As a result of ANOVA run on the effluent of the control, the mixture and oleic acid only, the p value was 7.135e-06 indicating a significant difference from the three data groups. The post hoc test also showed a statistical difference between the mixture and the control ($p = 8.50 \times 10^{-5}$),

but no such difference was observed between the mixture and oleic acid only ($p = 0.24$). The statistical results were in accordance with that performed on the VS reduction for the LCFA mixture. Stearic acid was partially degraded in the digester based on the calculation of additional COD reduction provided above.

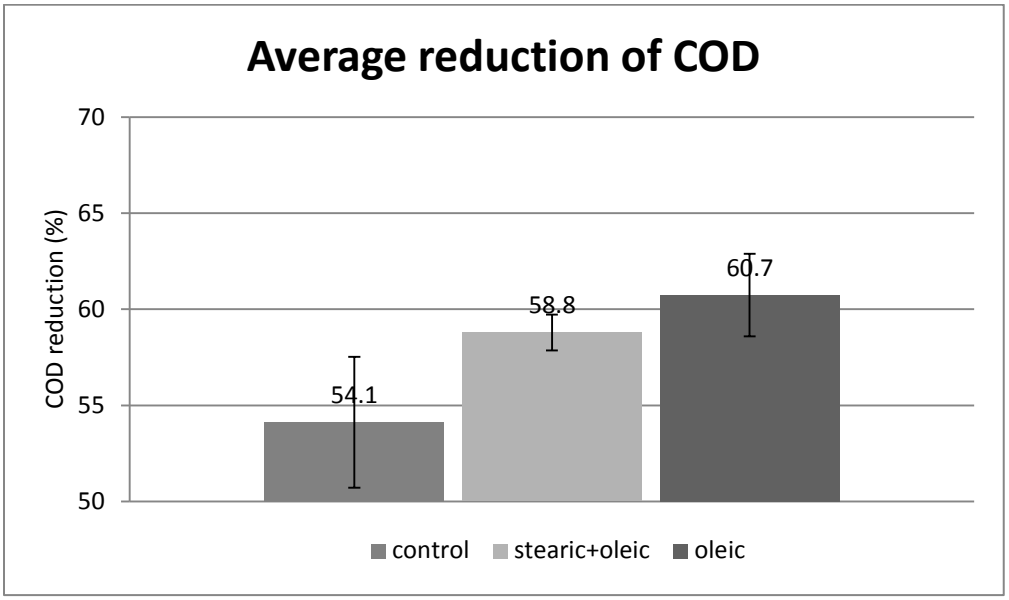


Figure 17: Average COD reduction during the operation of 10% mixture (stearic and oleic acid) compared with adding 10% oleic acid only.

6 Discussion

Unsaturated fatty acids have two configurations: cis or trans. The shape of trans configuration is similar to that of the saturated fatty acid. All the unsaturated LCFAs used in this study are in cis configuration. The molecules of LCFAs with a cis double bond cannot pack as tightly as their counterpart saturated fatty acids with a straight chain. In addition, the intermolecular distance between the molecules of unsaturated LCFAs becomes enlarged with increased number of cis double bonds contained in the carbon chain. The closer the molecular packing is, the more difficult the hydrophilic part of LCFAs will be ionized in water (Kanicky & Shah, 2002). In accordance to this structure-function relationship, the solubility in water of the LCFAs studied follows an order of: linolenic acid > linoleic acid > oleic acid > stearic acid. Since the sludge is mainly composed of water, the solubility of LCFAs in sludge should be a similar situation as that in water.

The pH values were around 7 and stayed stable during the operation. In this neutral range of pHs, the environment in the digester was beneficial not only to the normal metabolism of anaerobic microbes but also to the maintenance of fatty acids' ionized form (Sousa, et al., 2009).

The solids data analyzed by the comparison of additional solids reduction and statistical tests proved that the concentration of the LCFA addition and saturation of the LCFAs' carbon chain were key factors that have impacts on solids degradation in anaerobic digestion. All the solids reduction associated with the unsaturated LCFAs was significantly different from the control. The unsaturated fatty acids in the digester improved the performance of solids destruction in addition to complete self-biodegradation. However, the unsaturated LCFAs such as linoleic and linolenic acid did not show a distinct pattern with increased concentration from 5% to 20%,

while within this concentration range the additional solids reduction became greater with more oleic acid added.

Statistically, the addition of 5%, 10% and 20% linoleic and linolenic acid varied highly with regard to solids destruction data so an optimum concentration for solids reduction could not be determined. In the case of the saturated fatty acid, stearic acid, it was significantly different from the other three unsaturated LCFAs at every addition level and no statistical difference was observed when compared to the control and was different from the same concentration of three unsaturated LCFAs.

The trend of COD reduction was similar to the solids reduction. Most of the COD reduction for varied LCFAs at different concentrations was consistent with the corresponding VS destruction, which supported the conclusion from the solids data.

It was suggested by Loehr and Roth (1968) that one of the reasons causing the slow and poor degradation of saturated LCFAs might be difficult breaking them down to shorter chain compounds compared to fatty acids with double bonds. Another explanation was that the bacteria could not readily metabolize the saturated LCFAs due to their low solubility. Therefore, a mixture of stearic and oleic acid (1:2, w/w) was fed into the digester in order to investigate the impact of solubility on solids reduction. The solubility of stearic acid was enhanced by dissolving it in oleic acid as well as the solids and COD reduction. Confirmed by statistical analysis, the results were significantly different from the addition of stearic acid only. Kabara, et al. (1977) believed that the mechanism for gram-positive bacteria and fatty acids was because of hydrophilic and hydrophobic parts of LCFAs' molecules. It might be concluded that the stearic acid with increased solubility could be more easily accessible and utilized by bacteria as an

energy source, and subsequently improve the anaerobic degradation of sludge. However, in this study, after the assessment, the stearic acid in the mixture did not play an important role in the increased sludge degradation.

The inhibition phenomenon cited from the literature did not retard the anaerobic digestion in this study. It was probably due to the SRT and mixing condition. The SRT of the system might be long enough for microorganisms to acclimate to LCFAs. During the adaptation period, the anaerobic microbes could recover from the initial inhibition of LCFAs. From the results of solids and COD data, the contact between LCFAs and bacteria seemed adequate that indicated a well mixing condition.

The successful operation on anaerobic co-digestion of FOG with sewage sludge has been reported by many studies (Kabouris et al., 2008; J. C. Kabouris, et al., 2009). Several advantages are achieved from co-digestion including increased methane yield. The extra solids reduction obtained from the co-digestion with LCFAs in this study would be beneficial to sludge treatment. However, a lot of uncertainties would be associated with full-scale operation. The unclear mechanism related to solubility and a high variation in different concentrations of LCFA addition needs further study and investigation.

7 Conclusions

The conclusions from this study on the effect of LCFAs to anaerobic sludge are summarized as follows:

- Oleic acid improves additional solids reduction as its concentration in the feed increases. Linoleic and linolenic acid show no statistical difference from different concentrations added to the digesters.
- For unsaturated LCFAs, the degree of unsaturation does not affect sludge degradation.
- For the saturated LCFA in this study, stearic acid does not improve sludge digestion.

References

Ahring, B., Sandberg, M., & Angelidaki, I. (1995). Volatile fatty acids as indicators of process imbalance in anaerobic digestors. *Applied Microbiology and Biotechnology, 43*(3), 559-565. doi: 10.1007/bf00218466

Alves, M. M., Vieira, J. A. M., Pereira, R. M. A., Pereira, M. A., & Mota, M. (2001). Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part II: Oleic acid toxicity and biodegradability. *Water Research, 35*(1), 264-270. doi: 10.1016/s0043-1354(00)00242-6

Angelidaki, I., & Ahring, B. K. (1992). EFFECTS OF FREE LONG-CHAIN FATTY-ACIDS ON THERMOPHILIC ANAEROBIC-DIGESTION. *Applied Microbiology and Biotechnology, 37*(6), 808-812.

Angelidaki, I., & Ahring, B. K. (1994). Anaerobic thermophilic digestion of manure at different ammonia loads: Effect of temperature. *Water Research, 28*(3), 727-731. doi: 10.1016/0043-1354(94)90153-8

APHA, AWWA, & WEF. (1989). Standard Methods for the Examination of Water and Wastewater.

Appels, L., Baeyens, J., Degrève, J., & Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science, 34*(6), 755-781. doi: 10.1016/j.pecs.2008.06.002

Azbar, N., Ursillo, P., & Speece, R. E. (2001). Effect of process configuration and substrate complexity on the performance of anaerobic processes. *Water Research, 35*(Compendex), 817-829.

Bayr, S., Rantanen, M., Kaparaju, P., & Rintala, J. (2012). Mesophilic and thermophilic anaerobic co-digestion of rendering plant and slaughterhouse wastes. *Bioresource Technology, 104*(0), 28-36. doi: 10.1016/j.biortech.2011.09.104

Biebl, H., Zeng, A. P., Menzel, K., & Deckwer, W. D. (1998). Fermentation of glycerol to 1,3-propanediol and 2,3-butanediol by <i>Klebsiella pneumoniae</i&gt. *Applied Microbiology and Biotechnology, 50*(1), 24-29. doi: 10.1007/s002530051251

Bober, K., & Garus, M. (2006). RP‐HPTLC Application in the Investigation of Solubility in Water of Long‐Chain Fatty Acids. [Article]. *Journal of Liquid Chromatography & Related Technologies, 29*(19), 2787-2794. doi: 10.1080/10826070601001476

Buhr, H. O., & Andrews, J. F. (1977). The thermophilic anaerobic digestion process. *Water Research, 11*(2), 129-143. doi: 10.1016/0043-1354(77)90118-x

Carrère, H., Dumas, C., Battimelli, A., Batstone, D. J., Delgenès, J. P., Steyer, J. P., & Ferrer, I. (2010). Pretreatment methods to improve sludge anaerobic degradability: A review. *Journal of Hazardous Materials, 183*(1–3), 1-15. doi: 10.1016/j.jhazmat.2010.06.129

Cavaleiro, A. J., Pereira, M. A., & Alves, M. (2008). Enhancement of methane production from long chain fatty acid based effluents. *Bioresource Technology, 99*(10), 4086-4095. doi: 10.1016/j.biortech.2007.09.005

Chipasa, K. B., & Medrzycka, K. (2006). Behavior of lipids in biological wastewater treatment processes. *Journal of Industrial Microbiology & Biotechnology, 33*(8), 635-645. doi: 10.1007/s10295-006-0099-y

Cirne, D. G., Paloumet, X., Bjornsson, L., Alves, M. M., & Mattiasson, B. (2007a). Anaerobic digestion of lipid-rich waste-Effects of lipid concentration. *Renewable Energy, 32*(Compendex), 965-975.

Cirne, D. G., Paloumet, X., Bjornsson, L., Alves, M. M., & Mattiasson, B. (2007b). Anaerobic digestion of lipid-rich waste - Effects of lipid concentration. *Renewable Energy, 32*(6), 965-975. doi: 10.1016/j.renene.2006.04.003

Davidsson, Å., Lövstedt, C., la Cour Jansen, J., Gruvberger, C., & Aspegren, H. (2008). Co-digestion of grease trap sludge and sewage sludge. *Waste Management, 28*(6), 986-992. doi: 10.1016/j.wasman.2007.03.024

DiRusso, C. C., Black, P. N., & Weimar, J. D. (1999). Molecular inroads into the regulation and metabolism of fatty acids, lessons from bacteria. *Progress in Lipid Research, 38*(2), 129-197. doi: 10.1016/s0163-7827(98)00022-8

Fernández, A., Sánchez, A., & Font, X. (2005). Anaerobic co-digestion of a simulated organic fraction of municipal solid wastes and fats of animal and vegetable origin. *Biochemical Engineering Journal, 26*(1), 22-28. doi: 10.1016/j.bej.2005.02.018

Filipe, C. D. M., & Grady, J. (1998). *Biological Wastewater Treatment, Second Edition, Revised and Expanded*: Taylor & Francis.

Fraser, K. D. (2010). Increased anaerobic digestion efficiency via the use of thermal hydrolysis, from http://scholar.lib.vt.edu/theses/available/etd-07122010-193345/

Garber, W. F. (1982). Operating Experience with Thermophilic Anaerobic Digestion. *Journal (Water Pollution Control Federation), 54*(8), 1170-1175.

Gomec, C. Y., Kim, M., Ahn, Y., & Speece, R. E. (2002). The role of pH in mesophilic anaerobic sludge solubilization. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 37*(10), 1871-1878. doi: 10.1081/ese-120015467

Hanaki, K., Matsuo, T., & Nagase, M. (1981). Mechanism of inhibition caused by long-chain fatty acids in anaerobic digestion process. *Biotechnology and Bioengineering, 23*(7), 1591-1610. doi: 10.1002/bit.260230717

Hill, D. T., Cobb, S. A., & Bolte, J. P. (1987). USING VOLATILE FATTY-ACID RELATIONSHIPS TO PREDICT ANAEROBIC DIGESTER FAILURE. *Transactions of the Asae, 30*(2), 496-501.

Hill, D. T., & Holmberg, R. D. (1988). Long chain volatile fatty acid relationships in anaerobic digestion of swine waste. *Biological Wastes, 23*(3), 195-214. doi: 10.1016/0269-7483(88)90034-1

Hrudey, S. E. (1982). Factors Limiting Emulsified Lipid Treatment Capacity of Activated Sludge. *Journal (Water Pollution Control Federation), 54*(8), 1207-1214.

Hwu, C.-S., Tseng, S.-K., Yuan, C.-Y., Kulik, Z., & Lettinga, G. (1998). Biosorption of long-chain fatty acids in UASB treatment process. *Water Research, 32*(5), 1571-1579. doi: 10.1016/s0043-1354(97)00352-7

Hwu, C. S., & Lettinga, G. (1997). Acute toxicity of oleate to acetate-utilizing methanogens in mesophilic and thermophilic anaerobic sludges. *Enzyme and Microbial Technology, 21*(4), 297-301. doi: 10.1016/s0141-0229(97)00050-1

Jeris, J. S., & McCarty, P. L. (1965). The Biochemistry of Methane Fermentation Using ${\rm C}^{14}$ Tracers. *Journal (Water Pollution Control Federation), 37*(2), 178-192.

Kabara, J., Vrable, R., & Lie Ken Jie, M. (1977). Antimicrobial lipids: Natural and synthetic fatty acids and monoglycerides. *Lipids, 12*(9), 753-759. doi: 10.1007/bf02570908

Kabouris, C., J., Tezel, U., Pavlostathis, S. G., Engelmann, M., Todd, A. C., & Gillette, R. A. (2008). The Anaerobic Biodegradability of Municipal Sludge and Fat, Oil, and Grease at Mesophilic Conditions. *Water Environment Research, 80*(3), 212-221. doi: 10.2175/106143007x220699

Kabouris, J. C., Tezel, U., Pavlostathis, S. G., Engelmann, M., Dulaney, J., Gillette, R. A., & Todd, A. C. (2009). Methane recovery from the anaerobic codigestion of municipal sludge and FOG. *Bioresource Technology, 100*(15), 3701-3705. doi: 10.1016/j.biortech.2009.02.024

Kanicky, J. R., & Shah, D. O. (2002). Effect of Degree, Type, and Position of Unsaturation on the pKa of Long-Chain Fatty Acids. *Journal of Colloid and Interface Science, 256*(1), 201-207. doi: 10.1006/jcis.2001.8009

Kim, S. H., Han, S. K., & Shin, H. S. (2004). Two-phase anaerobic treatment system for fat-containing wastewater. *Journal of Chemical Technology and Biotechnology, 79*(1), 63-71. doi: 10.1002/jctb.939

Koster, I. W., & Cramer, A. (1987). Inhibition of Methanogenesis from Acetate in Granular Sludge by Long-Chain Fatty Acids. *Applied and Environmental Microbiology, 53*(2), 403-409.

Lalman, J. A., & Bagley, D. M. (2000). Anaerobic degradation and inhibitory effects of linoleic acid. *Water Research, 34*(17), 4220-4228. doi: 10.1016/s0043-1354(00)00180-9

Lalman, J. A., & Bagley, D. M. (2001). Anaerobic degradation and methanogenic inhibitory effects of oleic and stearic acids. *Water Research, 35*(12), 2975-2983. doi: 10.1016/s0043-1354(00)00593-5

Loehr, R. C., & Roth, J. C. (1968). Aerobic Degradation of Long-Chain Fatty Acid Salts. *Journal (Water Pollution Control Federation), 40*(11), R385-R403.

Long, J. H., Aziz, T. N., Reyes Iii, F. L. d. l., & Ducoste, J. J. (2012). Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. *Process Safety and Environmental Protection, 90*(3), 231-245. doi: 10.1016/j.psep.2011.10.001

Luostarinen, S., Luste, S., & Sillanpaa, M. (2008). Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. *Bioresource Technology, 100*(Compendex), 79-85.

Mahmoud, N., Zeeman, G., Gijzen, H., & Lettinga, G. (2004). Anaerobic stabilisation and conversion of biopolymers in primary sludge—effect of temperature and sludge retention time. *Water Research, 38*(4), 983-991. doi: 10.1016/j.watres.2003.10.016

Masse, L., Masse, D. I., Kennedy, K. J., & Chou, S. P. (2002). Neutral fat hydrolysis and long-chain fatty acid oxidation during anaerobic digestion of slaughterhouse wastewater. *Biotechnology and Bioengineering, 79*(1), 43-52. doi: 10.1002/bit.10284

Mata-Alvarez, J., Macé, S., & Llabrés, P. (2000). Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology, 74*(1), 3-16. doi: 10.1016/s0960-8524(00)00023-7

Nasir, I. M., Mohd Ghazi, T. I., & Omar, R. (2012). Anaerobic digestion technology in livestock manure treatment for biogas production: A review. *Engineering in Life Sciences, 12*(3), 258-269. doi: 10.1002/elsc.201100150

Neves, L., Oliveira, R., & Alves, M. M. (2009). Fate of LCFA in the co-digestion of cow manure, food waste and discontinuous addition of oil. *Water Research, 43*(20), 5142-5150. doi: 10.1016/j.watres.2009.08.013

Novak, J. T., & Carlson, D. A. (1970). KINETICS OF ANAEROBIC LONG CHAIN FATTY ACID DEGRADATION. *Journal of the Water Pollution Control Federation, 42*(Compendex), 1932-1943.

Öztürk, M. (1991). Conversion of acetate, propionate and butyrate to methane under thermophilic conditions in batch reactors. *Water Research, 25*(12), 1509-1513. doi: 10.1016/0043-1354(91)90181-o

Palatsi, J., Illa, J., Prenafeta-Boldú, F. X., Laureni, M., Fernandez, B., Angelidaki, I., & Flotats, X. (2010). Long-chain fatty acids inhibition and adaptation process in anaerobic thermophilic digestion: Batch tests, microbial community structure and mathematical modelling. *Bioresource Technology, 101*(7), 2243-2251. doi: 10.1016/j.biortech.2009.11.069

Parkin, G., & Owen, W. F. (1986). FUNDAMENTALS OF ANAEROBIC DIGESTION OF WASTEWATER SLUDGES. *Journal of Environmental Engineering, 112*(Compendex), 867-920.

Pereira, M. A., Pires, O. C., Mota, M., & Alves, M. M. (2002). Anaerobic degradation of oleic acid by suspended and granular sludge: identification of palmitic acid as a key intermediate. [Article; Proceedings Paper]. *Water Science and Technology, 45*(10), 139-144.

Pereira, M. A., Pires, O. C., Mota, M., & Alves, M. M. (2005). Anaerobic biodegradation of oleic and palmitic acids: Evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. *Biotechnology and Bioengineering, 92*(Compendex), 15-23.

Pereira, M. A., Sousa, D. Z., Mota, M., & Alves, M. M. (2004). Mineralization of LCFA associated with anaerobic sludge: Kinetics, enhancement of methanogenic activity, and effect of VFA. *Biotechnology and Bioengineering, 88*(Compendex), 502-511.

Pind, P., Angelidaki, I., Ahring, B., Stamatelatou, K., & Lyberatos, G. (2003). Monitoring and Control of Anaerobic Reactors

Biomethanation II. In B. Ahring, B. Ahring, I. Angelidaki, J. Dolfing, L. Euegaard, H. Gavala, F. Haagensen, A. Mogensen, G. Lyberatos, P. Pind, J. Schmidt, I. Skiadas & K. Stamatelatou (Eds.), (Vol. 82, pp. 135-182): Springer Berlin / Heidelberg.

Quarmby, J., & Forster, C. F. (1995). A comparative study of the structure of thermophilic and mesophilic anaerobic granules. *Enzyme and Microbial Technology, 17*(6), 493-498. doi: 10.1016/0141-0229(94)00051-r

Ralston, A. W., & Hoerr, C. W. (1942). THE SOLUBILITIES OF THE NORMAL SATURATED FATTY ACIDS. *The Journal of Organic Chemistry, 07*(6), 546-555. doi: 10.1021/jo01200a013

Rinzema, A., Alphenaar, A., & Lettinga, G. (1993). Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactors. *Process Biochemistry, 28*(8), 527-537. doi: 10.1016/0032-9592(93)85014-7

Rinzema, A., Boone, M., Knippenberg, K. v., & Lettinga, G. (1994). Bactericidal Effect of Long Chain Fatty Acids in Anaerobic Digestion. *Water Environment Research, 66*(1), 40-49.

Rinzema, A., Boone, M., Van Knippenberg, K., & Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environment Research, 66*(Compendex), 40-49.

Sekiguchi, Y., Kamagata, Y., Syutsubo, K., Ohashi, A., Harada, H., & Nakamura, K. (1998). Phylogenetic diversity of mesophilic and thermophilic granular sludges determined by 16S rRNA gene analysis. *Microbiology, 144*(9), 2655-2665. doi: 10.1099/00221287-144-9-2655

Sheu, C. W., & Freese, E. (1973). Lipopolysaccharide Layer Protection of Gram-Negative Bacteria Against inhibition by Long-Chain Fatty Acids. *Journal of Bacteriology, 115*(3), 869-875.

Shoukry, K., & Schulz, H. (1998). Significance of the reductase-dependent pathway for the beta-oxidation of unsaturated fatty acids with odd-numbered double bonds - Mitochondrial metabolism of 2-trans-5-cis-octadienoyl-CoA. *Journal of Biological Chemistry, 273*(12), 6892-6899. doi: 10.1074/jbc.273.12.6892

Silvestre, G., Rodríguez-Abalde, A., Fernández, B., Flotats, X., & Bonmatí, A. (2011). Biomass adaptation over anaerobic co-digestion of sewage sludge and trapped grease waste. *Bioresource Technology, 102*(13), 6830-6836. doi: 10.1016/j.biortech.2011.04.019

Sousa, D. Z., Pereira, M. A., Alves, J. I., Smidt, H., Stams, A. J. M., & Alves, M. M. (2008). Anaerobic microbial LCFA degradation in bioreactors. [Article]. *Water Science & Technology, 57*(3), 439-444.

Sousa, D. Z., Smidt, H., Alves, M. M., & Stams, A. J. M. (2009). Ecophysiology of syntrophic communities that degrade saturated and unsaturated long-chain fatty acids. [Review]. *Fems Microbiology Ecology, 68*(3), 257-272. doi: 10.1111/j.1574-6941.2009.00680.x

Switzenbaum, M. S., Giraldo-Gomez, E., & Hickey, R. F. (1990). Monitoring of the anaerobic methane fermentation process. *Enzyme and Microbial Technology, 12*(10), 722-730. doi: 10.1016/0141-0229(90)90142-d

Tomei, M. C., Braguglia, C. M., Cento, G., & Mininni, G. (2009). Modeling of Anaerobic digestion of sludge. *Critical Reviews in Environmental Science and Technology, 39*(Compendex), 1003-1051.

Tyagi, V., & Lo, S.-L. (2011). Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: an up to date review. *Reviews in Environmental Science and Biotechnology, 10*(3), 215-242. doi: 10.1007/s11157-011-9244-9

Wan, C., Zhou, Q., Fu, G., & Li, Y. (2011). Semi-continuous anaerobic co-digestion of thickened waste activated sludge and fat, oil and grease. *Waste Management, 31*(Compendex), 1752-1758.

Wei, Y. S., Van Houten, R. T., Borger, A. R., Eikelboom, D. H., & Fan, Y. B. (2003). Minimization of excess sludge production for biological wastewater treatment. *Water Research, 37*(18), 4453-4467. doi: 10.1016/s0043-1354(03)00441-x

Weng, C., & Jeris, J. S. (1976). BIOCHEMICAL MECHANISMS IN METHANE FERMENTATION OF GLUTAMIC AND OLEIC ACIDS. *Water Research, 10*(1), 9-18. doi: 10.1016/0043-1354(76)90151-2

Yin, X., Han, P., Lu, X., & Wang, Y. (2004). A review on the dewaterability of bio-sludge and ultrasound pretreatment. *Ultrasonics Sonochemistry, 11*(6), 337-348. doi: 10.1016/j.ultsonch.2004.02.005

Zoetemeyer, R. J., Arnoldy, P., Cohen, A., & Boelhouwer, C. (1982). INFLUENCE OF TEMPERATURE ON THE ANAEROBIC ACIDIFICATION OF GLUCOSE IN A MIXED CULTURE FORMING PART OF A 2-STAGE DIGESTION PROCESS. *Water Research, 16*(3), 313-321. doi: 10.1016/0043-1354(82)90191-9