ABSTRACT

HOSSEN, ELVIN HAFFEZ. Inducing Functional Stability of Anaerobic Co-digestion of Grease Interceptor Waste and Thickened Waste Activated Sludge. (Under the direction of Dr. Francis L. de los Reyes III and Dr. Tarek N. Aziz).

Anaerobic co-digestion of waste activated sludge with lipid-rich wastes is a promising technology in enhancing the energy production and organic degradation by a waste treatment process. However, the limitation on how much lipid-rich waste can be added as co-substrate has prevented this type of treatment process to be effectively utilized due to possible microbial inhibition towards anaerobic communities. The adsorption of LCFA from lipid-rich waste onto the cell wall of organisms eventually results in substrate and nutrient transport limitations while acute toxicity will inhibit biological activity of both aceticlastic and hydrogenotrophic methanogens, causing the instability to digesters.

In anaerobic co-digestion of lipid-rich waste and sewage sludge, it can be a challenge to achieve process stability. This study was conducted to investigate the effectiveness of a specific operating strategy that may confer greater functional stability of an anaerobic system. The strategy was to apply repeated pulse feed perturbation as a training method to induce stability thus enhancing the performance of anaerobic digesters in treating lipid-rich or high-strength wastes.

Based on the finding in this study, the training method appeared to have positive impacts towards the stability of anaerobic digesters when tested by rather high perturbation (OLR = 2.94 g VS/L-day). The trained reactor seemed to be relatively more resilient and resistant in response to stress in the form of high organic loading compared to control reactor. However, when tested with a really high organic loading rate (OLR = 4.48 g VS/L-day), the training

method did not lead to positive results as the perturbation was deemed too severe, and both reactors eventually failed due to severe inhibition and other operational problems. After the second perturbation test, the results indicate that aceticlastic methanogens in the trained reactor A showed a better performance than in B, and therefore became positively affected by the training method. As for the training strategy, determining the appropriate organic loading rate, the length of training period, and the frequency of perturbation would be an important consideration.

This study provides some insights toward creating more robust and functionally stable anaerobic co-digestion systems treating lipid-rich wastes. The result, however, would also be more comprehensive if complemented with molecular analysis to investigate the dynamics of microbial populations that determine the stability of function from anaerobic digestion system.

Inducing Functional Stability of Anaerobic Co-digestion of Grease Interceptor Waste and Thickened Waste Activated Sludge

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Environmental Engineering

Raleigh, North Carolina

2013

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DEDICATION

To Addrison Hossen and Helmiyati Amir Hamzah, who give me the greatest gift anyone could give another person: they believe in me.

BIOGRAPHY

Elvin Hossen was born in Indonesia to a working class family. As he always hated his dad's job as Civil Engineer, he instead chose to pursue a college degree in Chemical Engineering naively thinking that he would learn so much about chemistry, a consideration he would later regret. After graduated in 2009 with Bachelor of Engineering from Institut Teknologi Bandung, Elvin then worked as an assistant for Professor Tjandra Setiadi, a renowned expert in water and wastewater treatment in Indonesia, and spent some period of time working on his projects; cooperated with Ministry of Environment, oil companies, and chemical plants. However, as it had always been his desire to apply for a graduate school, he then tried to look up for some scholarships. During this process, Elvin had also finished two semesters of master's program in Petroleum Engineering at his old alma mater as a back-up plan if the scholarship did not work, but soon resigned from the program with one semester remaining after securing Fulbright scholarship to study in the US. Elvin eventually decided to pursue an MS degree in Environmental Engineering with emphasis in Environmental Process Engineering at North Carolina State University. He worked under the guidance of Dr. Francis de los Reyes and Dr. Tarek Aziz to conduct a thesis research with the topic of anaerobic co-digestion of lipid-rich wastes and thickened waste activated sludge. His long-term goal is to be a Professional Engineer within the field of Process Engineering-water and wastewater.

ACKNOWLEDGMENTS

I would like to address my gratitude to the following people who have played an important part in my two ebullient years of the graduate program at North Carolina State University:

- Late James William Fulbright, for his Fulbright scholarship that brought me to the US,
- Members of my committee, Dr. Francis de los Reyes III and Dr. Tarek Aziz as co-chairs, and Dr. Joel Ducoste for their guidance, advice, instruction and support during my study.
- David Black, our lab manager, who had been very patient in dealing with my incompetence working in Environmental Engineering Lab,
- My fellow friends and colleagues from Mann Hall 319A and Environmental Engineering graduate program, especially those who are and were in de los Reyes' research group, for their friendships. Special thanks to Ling for her insurmountable help with my research; to Jory, for his time driving me everywhere to collect samples; and Dominic, for the competition to determine who the best all-nighter of all was,
- Liya Weldegebriel, the undergraduate researcher, who assisted me in measuring all those never-ending samples,
- My Indonesian friends in Raleigh: Randy, Ayu, Yane, Mbak Uli, Debora, Mas Donny, Sekar, Mbak Rini, Sigit and Ilma, Bagus and Rasi, and Bang Darwin; my second family, for their support and fun time every weekend doing countless nights of karaoke, potluck parties, and awesome trips for the last 2 years,
- And most importantly, to my family: Mama, Papa, and my sister and her family. Thank you for always being there for me, even though we were separated by 10,000 miles of distance and 12 hours of time.

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CHAPTER 1 INTRODUCTION

1.1 Background

Anaerobic digestion of sewage sludge to stabilize and reduce solids concentration has increased the efficiency and sustainability of wastewater treatment compared to conventional treatment methods such as landfilling and incineration (Davidsson et al., 2008; Luostarinen et al., 2009). As a treatment process, anaerobic stabilization has been used on municipal sludge for over a century and is being recognized as a key technology for the future of a bio-based economy (Mata-Alvarez et al., 2000; Verstraete et al., 2005; De Vrieze et al., 2013). Besides the high degree of sludge stabilization and volume reduction, anaerobic digestion also offers environmental and economic benefits, such as nutrient recycling and energy production in the form of biogas that consists of methane, carbon dioxide, and hydrogen sulfide (Bougrier et al., 2006; Luostarinen et al., 2009). This growing acknowledgement of the advantages of this biological process, coupled with a better understanding of anaerobic chemistry, microbiology, and advance in process engineering as well as stricter environmental regulations, have led to more intense study and development to improve the performance of anaerobic digestion.

One of the strategies to improve anaerobic digestion is the addition of other organic waste as co-substrate. Anaerobic co-digestion is a process where energy-rich organic waste materials, such as Fats, Oils, and Grease (FOG), lipid-rich waste such as grease trap sludge, or food wastes can be added to digester along with sewage sludge, manure, or other easily degradable substrates to enhance biogas production and organic matter degradation (Wan et

al., 2011). Several advantages of anaerobic co-digestion are: (1) producing relatively higher methane yield, (2) increasing the availability of feedstock, (3) providing more balanced nutrients, and (4) improving the economics of the anaerobic digestion facility, and hence the wastewater treatment plant.

Among available co-substrates for anaerobic digestion, lipid-rich wastes are known to be one of the most attractive options due to their high methane production potentials. Davidson et al. (2008) reported that methane yield increased by 9-27% when grease trap sludge (10-30% of total VS added). Wang (2013) even showed that the addition of grease interceptor waste (65.5% by weight of VS) to thickened waste activated sludge (TWAS) can increase the methane yield by 317%. Luostarinen et al. (2009) observed a 66% increase in methane yield with the addition of grease trap waste (up to 46% of total VS), while Kabouris et al. (2008) also reported that the addition of dewatered FOG to sewage sludge increased the methane production by 2.95 times.

Despite the reported benefits of co-digestion of lipid-rich waste with sewage sludge, however, it is still uncommon to use it as a sole substrate in anaerobic digesters. The amount of lipid-rich waste that can be added as co-substrate is limited due to possible inhibition caused by long-chain fatty acids (LCFA) on anaerobic communities. The adsorption of LCFA onto cell wall of organisms eventually results in substrate and nutrient transport limitations while acute toxicity will inhibit biological activity of both aceticlastic and hydrogenotrophic methanogens (Noutsopoulos et al., 2013). Studies also reported that anaerobic digestion of high strength lipid wastes may develop wide operational problems

such as sludge flotation, digester foaming, clogging of gas collection and handling systems, and blockage of pumps and pipes (Long et al., 2012).

Reliable conversion of organic substrates will result in methane production and certain redundancy towards stress, and successful anaerobic treatment requires the stable function of a complex, interdependent microbial community (McMahon et al., 2004; De Vrieze et al., 2013). In anaerobic co-digestion of lipid-rich wastes and sewage sludge, the accumulation of LCFA can be seen as the stress that occurs to the anaerobic system. In many cases, growing stress, accompanied by low functional stability of microbial community will eventually lead to anaerobic digesters failure.

In anaerobic co-digestion of lipid-rich waste and sewage sludge, it can be a challenge to achieve process stability. However, several researchers have reported that the inhibition of anaerobic digestion due to accumulation of LCFA is reversible, and a specific recovery strategy can be employed (Palatsi et al., 2009). Some practices such as the addition of adsorbents, dilution with active inocula to increase the microbe/LCFA ratio, addition of micronutrients, and change in feeding patterns for acclimation have been used to overcome inhibition of methanogens thus creating a more stable and robust anaerobic community (Wan et al., 2011).

It has been observed by several researchers that the structure of the microbial community is a determining factor for functional stability of anaerobic digestion. The community shift is required during the recovery process of anaerobic microorganisms after it exposure to stress such as overloading (Delbés et al., 2000), which later suggested the ability of more stable anaerobic communities to use multiple metabolic pathways. Fernandez (1999) and McMahon

(2004) also showed that there is a close link between flexibility within the microbial community and the stability of function, supported by the claim that digester whose community structure had experienced more profound changes due to stress in the past had better functional stability than the one which did not struggle.

Given the aforementioned discovery, applying the theory to the case of anaerobic digestion with lipid-rich wastes as co-substrate would be an attractive strategy to achieve higher functional stability. Functionally stable anaerobic co-digesters will result in more reliable methane production and better recovery times following stress and perturbation. Considering stress such as overloading is a recurring event, it is interesting to evaluate whether repeated stress can induce the functional stability to even higher level and be a promising strategy.

1.2 Research Objectives and Hypotheses

The main objective of this study was to evaluate whether a higher degree of functional stability could be achieved by applying intentional stress on the anaerobic co-digestion system thus inducing the stability of the microbial community and creating a more robust co-digestion system. To achieve the goal, transient perturbations in the form of pulse-feed organic overloading were used to create periodic stresses towards anaerobic co-digester with the expectation that the microbial communities will become more tolerant after being exposed repeatedly.

CHAPTER 2 LITERATURE REVIEW

2.1 Anaerobic Digestion Overview

Anaerobic digestion is a biological process in which complex particulate organic matter is converted into methane, carbon dioxide, and other constituents in the absence of oxygen as the terminal electron acceptor. The anaerobic treatment process requires the presence of a diverse and closely dependent group of microorganisms to achieve complete conversion of complex substrates into final products (McCarty and Smith, 1986).

Anaerobic digestion has been widely used to accomplish the task of reducing solids and organic concentration of wastes while at the same time producing energy in the form of biogas. In the United States, there are 1351 wastewater treatment facilities (WWTFs) (43% out of total WWTFs), operating with influent flow rate within range of 1-200 MGD, that employ anaerobic digestion to process biosolids (US EPA CWNS, 2008). There are also currently 176 digesters for livestock manure installed in dairy farms, and more anaerobic digester projects are planned (US EPA AgSTAR, 2011). Moreover, anaerobic technology has gained popularity in continental Europe as well. Holm-Nielsen (2009) reported that anaerobic digestion will have to contribute to at least 25% of total renewable energy sources for European energy demand (20% of total energy) by the year 2020.

Anaerobic digestion is perceived as an appealing choice for treating wastes because it can remove approximately 50-80% of organic content (generally 24-45% of solids) from the influent while producing a collectible stream of biogas that normally consists of 50-80% methane and 30-50% carbon dioxide (depending on the type of feed) (Mendelsohn and

Sweeny, 1994). Theoretically, anaerobic digestion can produce up to 0.35 m^3 per kg Chemical Oxygen Demand (COD) converted at STP condition.

2.1.1 Anaerobic Conversion Process

Anaerobic digestion process can be divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Figure 1 represents a schematic diagram of anaerobic digestion process.

1. Hydrolysis

   This is the first step required for anaerobic digestion, where complex, insoluble particulate organic matter is broken down into simpler soluble compounds to allow their transport through the cell membrane. Three major components of complex organics that usually need to be hydrolyzed are proteins, carbohydrates, and lipids. The process is aided by exoenzymes excreted by facultative fermentative microbes. Proteins are degraded by protease into polypeptides and amino acids. Carbohydrates, (mostly cellulose, xylan, hemicelluloses, and lignin) are converted into simple sugars such as cellobiose, glucose, and xylose with the enzyme cellulase or lignin-modifying enzymes (LME). Lipids, on the other hand, are converted with the help of lipases into their constituent long-chain fatty acids (LCFA) and glycerol groups (Gerardi, 2003)

2. Acidogenesis (Fermentation)

   In this phase, products of hydrolysis such as glucose are mainly converted to ethanol, acetate, other volatile fatty acids (VFA), H_2, and CO_2 by facultative anaerobic bacteria. Peptides and amino acids are fermented to VFA, CO_2, H_2, ammonia and

hydrogen sulfide (Salminen and Rintala, 2002), while products of lipid hydrolysis (fatty acids, glycerol, and galactose) are converted to VFA, CO_2 and H_2.

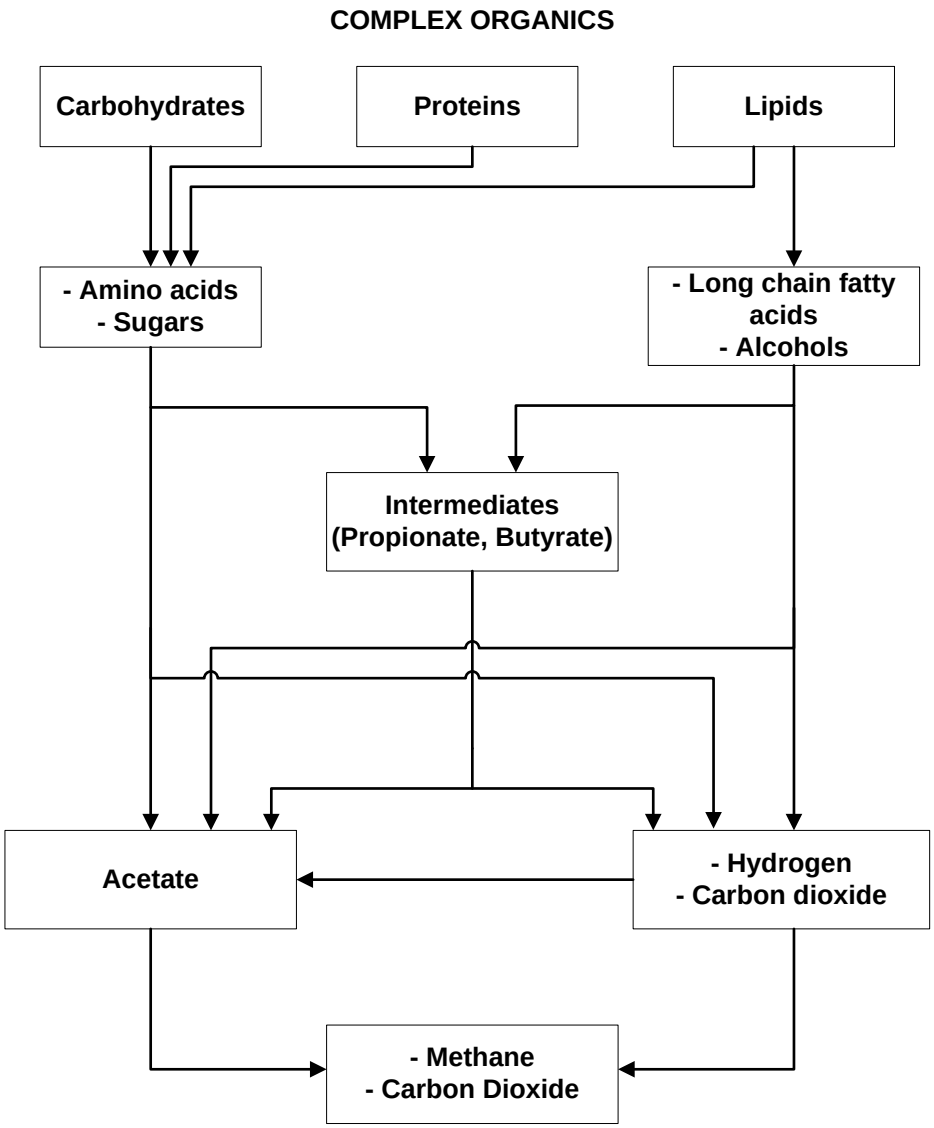

Figure 1 General reaction scheme of anaerobic digestion of complex organic matter (Gujer and Zehnder, 1983)

3. Acetogenesis

   In this step, LCFAs are degraded to shorter-chain fatty acids such as acetate (or propionate) by anaerobic oxidation process called β-oxidation, with the reaction:

   $$(-CH_2\text{-}CH_2) + 2H_2O \rightarrow CH_3COOH + 2H_2$$

   Meanwhile, other short-chain fatty acids such as propionate ($C_3H_6O_2$) and butyrate ($C_4H_8O_2$) are also converted into acetate and H_2.

   H_2 produced during this phase must be continuously removed for the reaction to occur, since most reactions have low or positive change in Gibbs free energy (Madigan et al., 2008)

4. Methanogenesis

   There are two types of methanogenesis occurring during anaerobic digestion: (1) aceticlastic methanogenesis, which is decarboxylation of acetate into methane and carbon dioxide by acetate-utilizing methanogens, which accounts for 70% of methane produced in a digester (Kugelman and McCarty, 1965), and (2) hydrogenotrophic methanogenesis, which reduces CO_2 by hydrogen into methane and accounts for 30% of total methane produced (Hashimoto et al., 1981)

   Methanogenesis is usually the rate-limiting process in anaerobic digestion, while aceticlastic methanogens have the slowest growth rate of all microorganisms responsible for anaerobic degradation and is the focal point of most research.

2.1.2 Parameters of Anaerobic Digestion

This section describes the crucial factors for anaerobic digestion that can either adversely affect or enhance the process.

2.1.2.1 Environmental Parameters

1. Important Nutrients and Feedstock Composition

The growth of microorganisms always relies on the availability of important macro- and micronutrients. Other than carbon source, anaerobic microorganisms need nitrogen for its synthesis of protein, enzymes, and nucleic acids. Gerardi (2003) noted that C/N ratio of 25:1 in feedstock would produce optimum biogas. Furthermore, phosphorus is required by cell to synthesize adenosine triphosphate (ADP) and nucleic acids, while the presence of sulfur is also necessary for metabolic pathways (Madigan et al., 2008).

In addition to macronutrients, microbial growth requires several trace elements as micronutrients and co-factors in lower concentrations. Iron, nickel, cobalt, sulfur, calcium, selenium, zinc, and some other trace nutrients should be present in the digesters or feedstock. Most micronutrients are inhibitory at high concentration to fermentative bacteria and methanogens (Poliafico, 2007; Chen et al., 2008) but can stimulate methane production at low concentrations (Kugelman and Chin, 1971; Krylova et al., 1997).

Feedstock composition can sometimes result in more problematic situations in anaerobic digestion. The presence of recalcitrant compounds (e.g. xenobiotic compounds) may cause issues in anaerobic digesters. LCFAs can also be troublesome since it has the potential to form floating scum and foam.

2. pH and Alkalinity

The pH is an important parameter in anaerobic digestion, especially for microbial metabolism. According to Gerardi (2003), a pH range of 6.2-8 is recommended for methanogenesis to occur, with the optimum pH of 7-7.2. On the other hand, acidogenesis has optimum pH ranging between 5 and 6.

During the acidogenesis and acetogenesis phases, pH will drop due to acids formation. If pH keeps decreasing to low levels with no adequate buffer present for neutralization, severe inhibition of methanogenesis would follow and eventually could lead to digester failure. The presence of alkalinity will assure stability of the digester by preventing undesirable drops or extreme changes in pH. Since anaerobic digesters usually operate at near neutral pH, alkalinity is mainly present in the form of bicarbonates (Gerardi, 2003). Alkalinity can be generated as a result of metabolism of an organic compound or can be added to the system.

3. Volatile Fatty Acids (VFA)

VFAs are present as the intermediate products of anaerobic digestion, and therefore should not be allowed to persist at high concentrations. The most common VFA produced are mainly acetic and followed by propionic acid (Speece, 1996). Butyric and other longer-chain fatty acids can also be present depending on the type of feedstock. The build-up of VFAs inside digesters, as mentioned earlier, will cause the drop in pH and eventually inhibit microbial activity, particularly methanogens (which require pH at normal range to actively grow). High VFA concentration does not necessarily mean that the anaerobic digestion is failing. According to McCarty (1961), anaerobic digesters can still withstand high VFA

concentration (~6000 mg/L) without a decrease in methane production, if the pH can still be maintained at optimum temperature (35-38^{o}C). To assure neutral pH, adequate buffer in form of alkalinity must be provided at concentration of 2000-4000 mg/L in the presence of H_2CO_3 and VFA (Speece, 1996).

Accumulation of VFAs inside the digester can be due to several causes, such as trace metal limitation-caused restriction of metabolism, toxicity impairment of metabolism, kinetic overload, single stage CSTR configuration, mass transfer limitation, hydraulic short circuiting, nitrogen or phosphorus limitation, or thermodynamic impairment of propionate conversion due to elevated H_2 concentration. Zeeman (2005) reported that the toxic effect of total VFA depends on pH and acids composition. Table 1 shows the estimated concentration of acetate and propionate that may cause 50% of methanogenic activity inhibition.

Table 1 Concentration of acetate and propionate to cause 50% methanogenic activity inhibition (Zeeman, 2005)

pH	**50% inhibiting concentration**	
	Acetate (mg COD L^{-1})	**Propionate (mg COD L^{-1})**
5.0	44	13
5.5	100	30
6.0	300	80
6.5	912	241
7.0	2851	745
7.5	8976	2358
8.0	28368	7398

4. Temperature

Temperature is one of the most important parameters affecting anaerobic digestion. There are two optimum temperature regions at which most methanogens are active: mesophilic range of 20-45^{o}C and thermophilic range of 50-80^{o}C (McCarty, 1964; Gerardi, 2003). Anaerobic digesters are usually running in the mesophilic range with optimum temperature at 35-37^{o}C (Angelidaki et al., 2007). On the other hand, thermophilic microorganisms grows optimally at temperature of 52-55^{o}C.

Thermophilic digesters have several advantages over mesophilic condition, some of which are higher substrate degradation and pathogen destruction due to increased growth rate and higher sterilizing effect. However, studies also show that thermophilic microorganisms are more sensitive to environmental conditions which may lead to increased process instability (Dettmer, 2002). In addition, thermophilic digesters require more energy in form of heat generation, which will decrease net energy production.

2.1.2.2 Operational Parameters

1. Retention Time

The hydraulic retention time (HRT) is defined as the average theoretical time that a liquid particle stays in the digester, while the solids retention time (SRT) can be thought as the average time the microorganisms remain inside the digester, and is generally the ratio between mass of biomass in reactor and biomass wasted per day. These two parameters have long been used as major parameters in successful biological treatment, and maintaining adequate SRTs/HRTs is crucial to maintaining a more effective anaerobic system. In

conventional single stage Continuous Stirred Tank Reactor (CSTR), the HRT is equal to the SRT, but in high-rate anaerobic digestion SRT is higher than the HRT. It is also important to note that higher SRT will result in better organic removal, while lower HRT can reduce the financial cost since digester volume can be smaller.

For anaerobic digestion, the typical retention time ranges from 14 to 30 days (Callaghan et al., 2002; Hashimoto et al., 1981; Poliafico, 2007). Dague (1970) also reported that a minimum of 10 days SRT is needed to avoid biomass washout from digester.

2. Mixing

Good mixing is needed for good methane production. A well-mixed digester means enhanced process of substrate, nutrients, and microorganism's distribution as well as temperature homogenization (Gerardi, 2003). Specific benefits of good mixing include eliminating scum build-up, preventing localized temperature pocket or thermal layers, maintaining the chemical and physical uniformity across digesters, and inducing uniformed mass and heat transport between materials and biomass. While a well mixed digester produced more biogas than unmixed digesters, researchers have shown that digester mixing during start-up was not beneficial, as it resulted in lower pH, performance instability, and prolonged start-up time (Karim et al., 2005)

Wang (2013) showed that mixing speed also determines the effectiveness of anaerobic digestion until a certain level. Below the threshold limit, the higher mixing rate results in better anaerobic digestion performance. However, high mixing rate does not necessarily lead to improved anaerobic digestion, since several factors at the molecular level such as shear

stress or mass transfer will also contribute. Additionally, other study showed that continuous recirculation did not improve the digesters performance (Rico et al., 2011)

3. Organic Loading Rate (OLR)

The organic loading rate (OLR) is a measure of organic matter fed into the digester per time, expressed as mass of organic matter over digester volume over time. Typical values of OLR ranges between 0.5-3 kgVS $m^{-3}d^{-1}$ (Gerardi, 2003). OLR is a controllable parameter, which is directly correlated to digester's design and performance, and often an economic issue.

The OLR, influent substrate concentration and HRT are related by following equation

$$OLR\ (\frac{g\ VS/L}{day}) = \frac{S_o(g\ VS/L)}{HRT\ (day)}$$

Where, S_o = concentration of volatile solids (VS) added

2.2 Anaerobic Co-digestion of Lipid-rich Waste with Sewage Sludge

2.2.1 Anaerobic Co-digestion Overview

While anaerobic digestion traditionally utilizes a single substrate in the process, the trend of using several organic sources as co-substrate has become more desirable. Co-digestion of a mixture of two or more substrates simultaneously has been proven to effectively increase the methane yield from the anaerobic process due to positive synergism established in digesters, balance of missing nutrients, enhancement of buffer capacity provided by the co-substrate, as well as optimization of rheological qualities (Mata-Alvarez et al., 2000;

Lehtomaki et al., 2007: Li et al., 2009). The synergistic effect is perceived to result from complementary microbial consortia coming from different wastes (Macias-Corral et. al., 2008). Commonly, the main basic substrate (such as manure or sewage sludge) is co-digested together with minor single substrate or varied additional substrate.

Table 2 Advantages and Disadvantages of Anaerobic Co-digestion

(Mata-Alvarez et al., 2000; Braun and Wellinger, 2003)

Advantages	Disadvantages
• Improved nutrient balance for an optimal digestion and a good fertilizer quality • Produces relatively higher methane yield with increased, steady biogas production throughout the seasons • Additional fertilizer (soil conditioner) • Increases the availability of feedstocks as the option is more varied • Homogenization of particulate, floating, or settling wastes through mixing with animal manures, sewage sludge, or other wastes • Higher income thanks to gate fees for waste treatment • Increased cost-efficiency one plant for several materials) • Dilution of toxic and inhibitive compounds, such as ammonia or sulfide	• Increased digester effluent COD • Additional pretreatment requirements • Increased mixing requirements • High utilization degree required • Decreasing availability and rates • Hygienization requirements • Restrictions of land use for digestate • Practical limitations including transport cost • Difficulties complying with regulations and policies for different types of waste streams

Stabilization of sewage sludge usually aims at the reduction of solids concentration, sludge volume, as well as organic content. Typical sewage sludge comprises of primary sludge separated from wastewater during pre-settling and biological excess sludge from the activated sludge system (Luostarinen et al., 2009). Sewage sludge mainly contains easily biodegradable materials with typical methane concentration around 250-400 m^3/ton organic solids added (Einola et al., 2001; Braun and Wellinger, 2003; Davidsson et al., 2008; Luostarinen et al., 2009). It is also important to note that characteristics from sewage sludge differ from one treatment plant, area, or country to another, due to several determining factors such as water consumption and local industry (Luostarinen et al., 2009).

Other than sewage sludge and agricultural waste, several other organic wastes can also be used as substrates for co-digestion and possess high methane potential. Some of them are easily degradable and do not need major pretreatment prior to digestion (several sources of organic substances for co-digestion with its biogas yields are presented in Table 3). Others, however, can also form inhibiting metabolites during anaerobic digestion which demands dilution with more easily degradable substrates such as manure and sewage sludge.

One source of organic material that has been intensely investigated as substrate for co-digestion due to its promising methane yield potential is lipid-rich wastes. This type of waste, however, still has some limitations as to be used as a sole substrate for anaerobic digesters. Even though lipid-rich wastes are known to have high methane production potential, their degradation products in form of long-chain fatty acids may inhibit methanogens in the process (Luostarinen et al., 2009). Moreover, high concentrations of this organic waste often cause many operational problems in anaerobic digesters due its physical properties.

Nevertheless, barring all the limitations, lipid-rich waste is still considered a more attractive option than carbohydrate or protein wastes as co-substrate for anaerobic co-digestion based on its methane potential.

Table 3 Organic wastes and by-products for co-digestion with their approximate biogas yields in m^3/ton organic solids (Braun and Wellinger, 2003)

Materials	**m^3/t**
Harvest Residues	
Straw, stems, sugar beet toppings, fibrous material	375
Animal Manures	
Food industry waste	200-500
Dough, confectionary waste, whey	400-600
Yeast and yeast-like products	
Yeast and sludge from breweries, wine making, distilleries	400-800
Slaughterhouse waste	
Flotation sludge, animal fat, blood	550-1000
Wastes from plant and animal fat production	
Plant oil, oil seed, fat, bleaching earth	1000
Pharmaceutical wastes	
Proteinacious wastes, bacterial cells, and fungal mycelium	1000-1300
Biowaste from source separated collection	400-500
Market waste	500-600
Sewage sludge	250-350

2.2.2 Lipid-rich Wastea as Co-digestion Substrate

Lipids, characterized either as fats, oil or greases (FOG), are one of the major organics in food wastes, domestic, or industrial wastewaters, and account for 25-40% of total COD of the raw wastewater or approximately 15-20% of total solids in sewage sludge (Mackie et al., 1991; Quemeneur and Marty, 1994; Chipasa and Medrzycka, 2006; Cirne et al., 2007; Noutsopoulos et al., 2013). Considerable amounts of lipid-rich wastes are being produced every year from various types of industries such as food processing, slaughterhouses, wool and leather, edible oil processing industry, dairy products, or olive oil mills (Sasaki et al., 2002; Cirne et al., 2007). However, the main sources of lipids in domestic wastewater are kitchen liquid wastes (14-36% of total lipids content) and human feces (4-23% of total lipids content) (Quemeneur and Marty, 1994; Noutsopoulous et al., 2013). Lipids in wastewater mainly consist of neutral fats, such as triglycerides, and free long-chain fatty acids (LCFA) (Wan et al., 2011; Noutsopoulos et al., 2013).

In these complex biological reactions, methanogenesis appears to be the rate limiting step in the anaerobic digestion of lipids while the first step occurs in a rather fast process (Heukelekian and Mueller, 1958; Novak and Carlson, 1970; Hanaki et al., 1981; Broughton et al., 1998; Li et al., 2002). Moreover, the physical processes of dissolution and mass transfer of insoluble lipids can also limit the overall conversion rate (Novak and Carlson, 1970; Hanaki et al., 1981; Li et al., 2002). Due to lipids' recalcitrance to biological treatment and tendency to float and form aggregate or scum, several physical parameters must be maintained during anaerobic digestion to keep or even enhance the effectiveness of lipid degradation. These parameters include: (1) homogeneous dispersion of lipids in the feed

(uniformity), and (2) sufficient mixing to maintain good contact between microorganisms with lipids in the digesters (Heukelekian and Mueller, 1958; Rinzema et al., 1993; Li et al., 2002).

Lipid-rich wastes, or FOG wastes, along with food scraps, brewery, or dairy waste, are considered high-strength organic wastes due to its high organic content along some of inhibiting compounds, such as Long Chain Fatty Acids (LCFAs) they contain. As previously mentioned, lipid-rich wastes are produced from various sources of industry, such as restaurants or other food service establishments, dairy industry, food processing industry, slaughterhouses, palm oil industry, or even brewing plant. Table 4 displays typical FOG characteristics from several sources that were used as co-substrates in previous research.

In general, the composition of FOG in wastes varies from 2-20% total solids (TS), of which 80 to 99% is volatile (VS) (Kabouris, 2008), depending on the type of industries the wastes are discharged from. The term FOG alone generally describes the layer of lipid-rich material of wastes formed during cooking and food processing. Additionally, there is also petroleum-based FOG that contains non-polar FOG and is commonly generated from industries that utilize petroleum product derivatives (oil and grease) such as automotive-related facilities. This type of FOG, however, is not suitable for biological treatment since it is not easily biodegradable.

Table 4 Characteristics of various lipid-rich waste sources

Component	Total Solids (TS), %	VS/TS, %	Chemical Oxygen Demand (COD), g/L	Total Nitrogen, g/L	Total Phosphorus, g/L	pH	Reference
Restaurant Interceptor FOG Waste	1.8-21.9	88.9-98.6	nd	nd	nd	4.3-4.8	Bailey et al., 2007
Biodiesel glycerin	14.7	95.2	1,160	nd	0.128	8.4	Parry et al., 2009
Polymer Dewatered FOG	42.4	96.5	1,211	5.4	0.67	4.0	Kabouris et al., 2009
Lime Dewatered FOG	49.1	76.5	1,030	nd	nd	6.5	Kabouris et al., 2007
Palm Oil Mill Effluent	11,5-79[a]	85	15-100	180-1400	nd	3.4-5.2	Ahmad et al., 2011
FOG from receiving facility	3.2	93.9	nd	nd	nd	4.2	Wan et al., 2011
Grease trap sludge from meat processing plant	25.4	99	nd	nd	nd	5.1	Luostarinen et al., 2009
FOG from slaughterhouses	15.5-93.5	89-100	410-1680	nd	nd	nd	Battimelli et al., 2010

[a]Unit in g/L

In many municipalities, discharging FOG into collection system is illegal since it can cause serious problem in sewer lines. Accumulation of FOG on pipes can stimulate the formation of hardened deposits via chemical reactions or a physical aggregation process which eventually leads to a reduction of conveyance capacity of sewer systems and ultimately causing overflow problems (He et al., 2011; Long et al., 2012). To prevent financial loss due to aforementioned risks, many municipalities implement pretreatment processes to remove the oil and grease especially from kitchen waste streams. One pretreatment is accomplished by installing grease abatement devices called "grease interceptors" or "grease traps". Since polar FOG are primarily generated from food processing or food service establishments, the majority of grease interceptors are installed adjacent to restaurants or commercial kitchens, outside the building and below the ground.

As part of GI/GT periodic maintenance, regular external pumping is usually performed to prevent the FOG from entering the collection system and sewer lines. Common practice is to separate the FOG layer from the wastes and then transported to a special treatment plant. Given the recent trends, however, the utilization of GIW as co-substrate for anaerobic co-digestion is considered to be more attractive choice and beneficial than other alternatives (Long et al., 2012).

2.2.3 Grease Interceptor Wastes (GIW)

This section reviews GIW as potential co-substrate for anaerobic digestion including the characteristics, issues, and advantages of using GIW as the organic source.

Grease interceptor (GI), or grease trap (GT), is an engineered device designed to capture spent Fats, Oils and Grease (FOG) and related solid wastes from the flow of wastewater discharged by food service establishments (FSEs).

Generally, GI is not designed to work as a wastewater treatment device. It functions as grease abatement and gravitational separation device to retain suspended grease and food solids by providing the necessary time for flotation and sedimentation of the influent waste (Aziz et al., 2011; Gallimore et al., 2011; Long et al., 2012). As a result, inside the GI, three layers are formed; a bottom layer where food particles and debris whose masses are greater, a middle layer of wastewater, and top layer that contains floating FOG. These three components make up what is called grease interceptor waste (GIW).

Typically, GIs can hold 1,000-2,000 gallons in capacity and are installed outside FSEs, below the ground, while GTs are significantly smaller in size (50 gallons) and located just beneath the kitchen sink inside the building (Long et al., 2012). Figure 2 below displays a typical schematic of a grease interceptor installed by FSEs.

As can be seen from the Figure 2, GI is typically divided into 2 chambers, partially separated with a baffle wall to prevent floating FOG scum staying from entering the sewer system. As time progresses, the FOG layer and food particles will accumulate and the build-up will occur. Therefore, to prevent regulation violation, every FSEs with GI installed must perform regular cleaning and maintenance of their GIs.

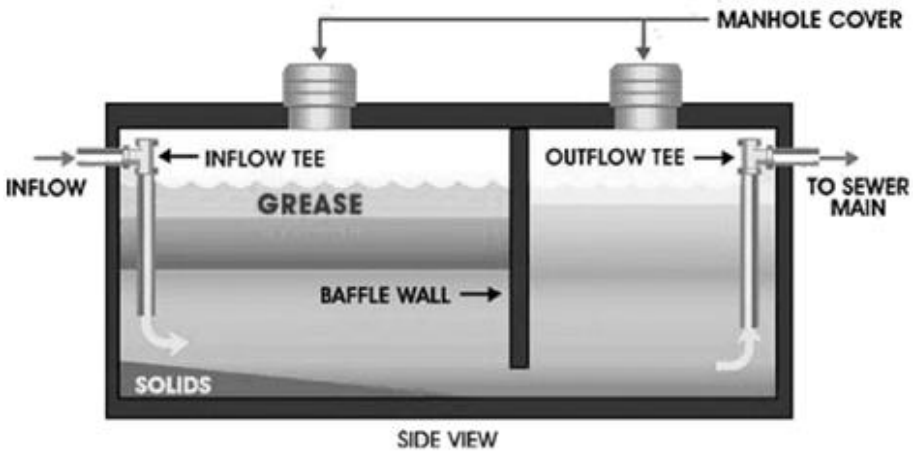

Figure 2 Grease Interceptor Schematic Design

(Source: Hogan Plumbing Co., accessed June 2013)

There are several methods to handle GIW and reduce the accumulation of FOG and food particles. Some factors that need to be considered when deciding the method: the availability of services, disposal and recycling opportunities adjacent to FSE, and ultimately cost. The most common methods are source control, mechanical cleaning, and grease control products (Occiano et al., 2008). The common practice for restaurant owners is hiring professional pumping service to remove waste from GI via mechanical cleaning. Some service companies will transport GIW to a commercial industry where FOG can be utilized as raw materials for soap or cosmetic plants, or to municipal solid waste treatment plants for landfilling, incineration, or composting, while several others may transport them to WWTFs for biodiesel synthesis or biogas production via anaerobic co-digestion process (Wang et al., 2013).

2.2.3.2 Characteristics of GIW

Characterization of GIW can be challenging due to several reasons: (1) use of various types and designs of FSEs, (2) variability of influent flow in each of GIs/GTs, and (3) variability in configuration and maintenance frequency of GIs/GTs (Lesikar et al., 2006; de los Reyes and He, 2009). There are studies reporting complete assessment of physical and chemical characteristics of GIW. Aside from total solids and pH, other parameters such as COD, alkalinity, total volatile acids (TVA), carbohydrate, protein, total phosphorus, total nitrogen, and ammonia have been rarely reported.

Generally, GIW from FSEs is a mixture of vegetable- and animal-based oils, lipids, greases, food solids, and possibly soaps and detergent, as well as other trace materials (Wang et al., 2013). Compared to FOG wastes from food-based industries or food processing facilities, GIW from FSEs had the lowest total solids concentrations (Davidsson et al., 2008; Luostarinen et al., 2009; Battimelli et al., 2010). The typical range of solids concentration of GIW is 1-20% for TS, of which 88-99% is volatile (Johnson et al., 2011). Davidsson (2008) reported that GIW from FSEs contained 17.3% TS of which 98% is volatile. Within the GI, the pH is below 7, with typical values between 3 and 6. This low pH is an indication the presence of various fatty acids as intermediate products formed by catabolism of protein and carbohydrate under anaerobic conditions.

2.2.2.2 Issues with Co-digestion of GIW

Similar to other anaerobic technologies treating lipid-rich wastes, there have been problems limiting full scale application of anaerobic co-digestion of GIW. Although

maximum bioenergy production by anaerobic co-digestion of GIW is desirable, research has shown that there is a limit the amount of that GIW can be introduced to anaerobic digesters. Studies have indicated that 70% of lipids are mainly absorbed onto the sludge and not further degraded, and only 25-30% of the remaining lipids are converted to methane (Petruy and Lettinga, 1997; Mouneimne et al., 2004). Wang (2013) reported that GIW can only be added as co-substrate to anaerobic digestion of thickened waste activated sludge (TWAS) at a maximum concentration of 65.5% (w/w) of VS added before the digester fails.

Excessive amount of GIW added to the system can cause inhibition to methane production, and if this continually occurs will lead to digester failure upset. The inhibitory effect of lipid-rich wastes, such as GIW, on methanogenesis during anaerobic digestion can be primarily attributed to the accumulation of long-chain fatty acids (LCFA) (Hanaki et al., 1981). As intermediate products, the presence of LCFA in a system is not desirable as they need to be converted into final products. When there is an overload of GIW, a rapid increase in LCFA will occur. Combined with the low pH of GIW, the pH inside the digester will decrease; resulting in undesirable environment for methanogens, as methanogenesis requires pH in the range of 6.5-8. Finally, as the accumulation of untreated fatty acids becomes more severe and pH drops, it will eventually inhibit the conversion of acetate to methane. Consequently, the accumulation of methane along with other previous fatty acids will further create more adverse conditions that eventually lead to failure.

LCFAs, in general, can be categorized as inhibitory substances in anaerobic co-digestion process. The adsorption of LCFA onto cell wall of organisms eventually results in substrate and nutrient transport limitations while acute toxicity will inhibit biological activity of both

aceticlastic and hydrogenotrophic methanogens (Noutsopoulos et al., 2013). LCFA can also cause the surfactant effect that reduces the membrane surface tension which interferes with proton transport and energy flow, and could eventually lead to cell damage (Long et al., 2012). Studies also reported that anaerobic digestion of high strength lipid wastes may develop operational problems such as sludge flotation, digester foaming, clogging of gas collection and handling systems, and blockage of pumps and pipes (Long et al., 2012).

It is important to note that the inhibition mechanism by LCFAs towards anaerobic microorganisms is not understood and further complicated by a heterogeneous mixture of substances such as GIW. As GIW contains a very high concentration of organics, if excessive amounts of GIW are being fed to a previously stable anaerobic co-digester, problems may arise. A stable anaerobic digester would experience some stress due to organic shocks coming from GIW, and the system equilibrium will then be affected.

2.3 Functional Stability of Anaerobic Co-Digestion

2.3.1 Ecological Stability Overview

Anaerobic digesters offer a controlled environment consisting of a complex ecological system with many microbial players and processes. As in any other bioreactor system, the nature of microbial diversity and population dynamics are consistently associated with their impacts in these systems, and understanding these mechanisms will give insights into system performance. An investigation connecting the microbial communities and their dynamics to process stability must also deal with basic ecological issues.

According to Holling et al. (1995), from the ecology perspective, there are several key features of ecosystems structure and function:

- Ecosystems do not have single equilibrium. Rather, destabilizing forces far from equilibrium, multiple equilibria, and disappearance of equilibria define functionally different states, and movement between states maintains structure and diversity.
- Instead of being continuous and gradual, ecological change is episodic with accumulation of biomass or nutrients, as the result of internal or external natural processes or human-imposed calamity.
- Spatial attributes are not uniform or scale invariant. Rather, productivity and textures are patchy and discontinuous at all scales (Holling, 1992)
- Policies and management that apply fixed rules for achieving constant yields, independent of scale, lead to systems that gradually lose resilience and suddenly break down in the face of disturbances that previously could be absorbed (Holling, 1986)

Stability Definition

Stability can be defined as the ability of a system to return to equilibrium or to a state reasonably close to its original state in the presence of influential perturbation (Margalef, 1968; Walter, 1980; Botton et al., 2006). A more stable system means that it can bounce back to its previous undisturbed state more rapidly or with the less fluctuation. According to Tilman (1999), functional stability is defined in ecology as resistance (ability to withstand

immediate disturbance), resilience (rate of recovery after disturbance), and temporal stability (sameness of the identity of community biomass over temporal scales)

An ecosystem property can be expressed as a 'stability property' if it associates with one of the following blocks of properties (Grimm et al., 1992): (1) Staying essentially unchanged; (2) Returning to referential state or dynamics after temporal external disturbance has been applied; (3) persisting through time. To quantify the stability properties, one can use stability measures which consist of several parameters contributing to different aspects of stability. The table below presents the most important stability properties with their respective concepts and measures:

Table 5 The most important stability properties, stability concepts and stability measures in ecology (Grimm et al., 1992)

Stability Property	Stability Concept	Related Measures
Staying essentially unchanged	Constancy	Standard deviation, annual variability
Staying essentially unchanged despite the presence of external disturbances	Resistance	Sensitivity, buffer capacity
Returning to the referential state (or dynamics) after a temporal external disturbance has been applied	Resilience	Return time, size of the domain of attraction

Each property, as presented in Table 5, can claim the term of stability, however in this research study, the parameters that are used in the experiment will only include resistance and resilience.

Significantly, stability concept can be applied to two components of an ecosystem: the structural biotic component and the process component (Botton et al., 2006). These components are not necessarily related, and can be paradoxical. According to McMahon (2004), stable conversion of organic substrates and successful treatment in anaerobic treatment require the stable function of a complex, interdependent microbial community and its dynamics inside the reactor system. However, how these factors can contribute to the system stability is still a challenging yet evolving issue (Fernandez et al., 1999; McCann, 2000; Briones and Raskin, 2003). Researchers have indicated that microbial diversity does not necessarily imply functional stability (Fernandez et al., 1999; Fernandez et al., 2000; Hasham et al., 2000; Briones and Raskin, 2003; Botton et al., 2006). However, for the ability of microbial community to adapt to non-optimal situations, a minimal diversity must still be maintained to achieve acceptable level of stability (Briones and Raskin, 2003; Dearman et al., 2006; Riviere et al., 2009; Carballa et al., 2011; Vrieze et al., 2013).

2.3.2 Functional Stability Parameters in Anaerobic Digestion: Resistance and Resilience

In anaerobic digestion, stability of digester function can be defined as the capacity to achieve efficient pollutant reduction under varying environmental conditions including stress thus resulting in stable methane production (Speece, 1996; Vrieze et al., 2013). Stress, in this case, is described as suboptimal condition where the anaerobic system could not perform

well due to disturbances, often external, that are causing inhibition to the biological pathways of the anaerobic process. In a complete system, disturbance is defined as everything that is not contained in the referential system and its dynamics, which usually is investigated by spatial and temporal scales (Grimm et al., 1992). Stress due to perturbation can last temporarily or for longer periods (Sousa, 1984; Pickett and White, 1984; Remmert, 1988; Grimm et al., 1992).

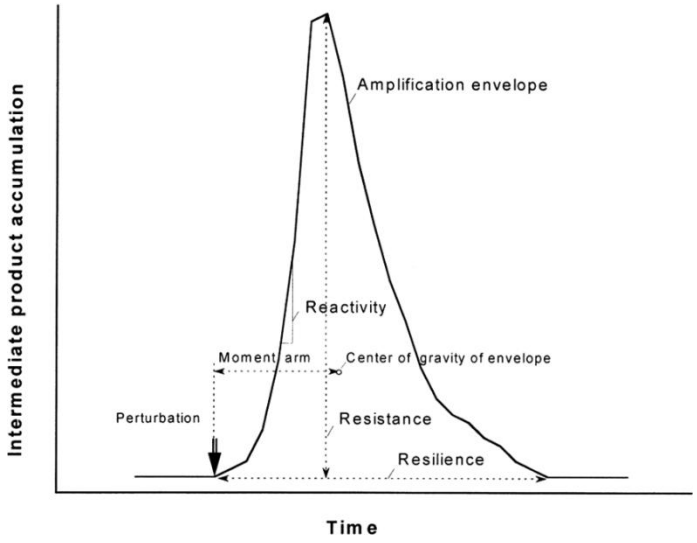

Figure 3 Ecological parameters of functional stability (Hashsham et al., 2000)

In response to perturbation, an unstable bioreactor will face inhibition that eventually leads to build-up of metabolites. The pattern of the accumulation of intermediate products, characterized as amplification envelope (Figure 3), can be utilized to quantify various

parameters that can be adopted to measure the functional stability (Hashsham et al., 2000; Botton et al., 2006). From Figure 3, two main parameters can be obtained: resistance and resilience.

Resistance is a measure of the buffering capacity of the community with respect to the corresponding intermediate product (Hashsham et al., 2000). It is the degree to which microbial composition remains unchanged in the face of disturbance (Allison and Martiny, 2008). An ecological system is resistant if it is similar across variety of environmental conditions, as it is difficult to perturb from its equilibrium state. Resistance is defined as the maximum accumulation of intermediate product; therefore a higher value indicates lower system resistance, hence lower functional stability.

Resilience, in the ecological theory, can be defined in two different ways: (1) engineering resilience, (2) ecological resilience (Holling, 1996). Engineering resilience focuses on efficiency, constancy, and predictability, thus maintaining the efficiency of function (Holling, 1996). It concentrates on stability near an equilibrium steady state, where resistance to disturbance and speed of return to equilibrium are used to measure the property (Tilman and Downing, 1994). In one study, it is defined as the time taken by the accumulated intermediate product to return to its referential state (Hashsham et al., 2000; Botton et al., 2006). Ecological resilience, on the other hand, focuses on persistence, change, and unpredictability, thus maintaining existence of function. It concentrates on conditions far from equilibrium steady state, where instabilities can flip a system into another regime of behavior, that is, to another stability domain (Holling, 1973). Ecological resilience is measured from the magnitude of disturbance that can be absorbed before the system changes its structure. It is

related to the mechanistic understanding of ecosystem behavior under changing conditions, thus it is not always a positive attribute and not necessarily desirable (Botton et al., 2006). Microbial communities are considered to be resilient if, when composition changes, it will recover rapidly, either by growth, physiological or genetic adaptation.

2.3.3 Improving Functional Stability

As previously mentioned, functionally stable ecosystem is a desirable environment, especially in an engineered system such as anaerobic reactors. Stable anaerobic reactor will result in more reliable methane production and better recovery times following stress and perturbation. Therefore, an effort to improve functional stability would be beneficial move to make more practicable an ecosystem prone to instability such as anaerobic co-digesters more practicable.

In the research of improving ecological stability, the reaction of ecological systems to disturbance is the main topic. Disturbance can be a crucial factor facilitating the persistence of many species in the system (Sousa, 1984; Pickett and White, 1984; Remmert, 1988). A disturbance can be defined as everything not contained in the referential system and its dynamics. A referential system can mean an equilibrium steady state, more or less regular oscillations, or chaotic fluctuations (Grimm et al., 1992). In this study, however, a referential system is defined as an equilibrium steady state. It is important, however, to decide if something to be considered as a disturbance or as an inherent part of the system (Grimm et al., 1992). Disturbance in many studies is positioned as external force to push the system out

of its current equilibrium condition, usually in the form of perturbation by toxic, pH, organic loading, or other shocks.

Strong fluctuations in organic loading generally will have a negative impact on the performance of the anaerobic treatment processes due to the slow rate of growth of the methane producing bacteria (McCarty, 1964; van Lier et al., 2001; Cuppens et al., 2012). However, it has been also observed that during the recovery process of anaerobic microorganisms after its exposure to stress such as overloading, a community shift may occur (Delbés et al., 2000), which later suggested that anaerobic communities can withstand the disturbance while at the same time improve its stability in some ways, for instance by using multiple metabolic pathways. Fernandez (2000) and McMahon (2004) also showed that there is a close link between the dynamics of the microbial community and the stability of function, supported by the claim that digester whose community structure had experienced more profound changes due to stress in the past had better functional stability than the one which did not struggle.

Several studies have examined the response of anaerobic systems to environmental perturbations, especially changes in organic loading. These types of strategies include: (1) pulse feed experiments in which a substrate pulse is injected suddenly into an anaerobic reactors; (2) step feed experiments in which the reactor organic or hydraulic loading rate is suddenly increased to a higher level for a period of time, then returned to the steady-state condition; (3) period feed experiments in which the influent substrate concentration is periodically changed in short term or long term (Cohen et al., 1981; Smith and McCarty, 1989; Hickey and Switzenbaum, 1991; Gupta et al., 1994; Xing et al., 1997).

In this study, the proposed strategy is to apply pulse feed experiment to the reactor. The rationale for this strategy is the fact that an anaerobic digester is usually subject to transient perturbation, while the time taken for the system to recover following the perturbation is not well understood and documented. By applying this strategy, an improved functional stability and more robust community of anaerobic microorganisms hopefully can be achieved, thus resulting in a better anaerobic digester system in treating strong lipid-rich wastes and producing higher capacity of methane.

CHAPTER 3 MATERIALS AND METHODS

3.1 Experimental Set-up

The goal of this study is to investigate the induction of functional stability of anaerobic co-digestion process by applying controlled perturbation as the training method. To accomplish this, two anaerobic reactor systems were used. The reactors were identical and semi-continuously operated at mesophilic condition (~37^{o}C) with feeding and decanting volumes resulting in a Hydraulic Retention Time (HRT) of 20 days. Both reactors were used in a previous study (Wang et al., 2013), but were modified during the experiment to meet the requirements of this study. Figure 4 displays the configuration of the experimental set-up. The following sections will discuss the specific designs and major components of the reactors systems.

3.1.1 Reactor System

Two identical 8 L Plexiglas reactors (digester A and B) were used to perform the experiments in this study. The digesters were constructed by the Precision Machine Research Lab at NCSU's Department of Civil, Construction, and Environmental Engineering. A schematic view of the digesters is provided in Figure 4, while Figure 5 shows a photograph of both digesters.

Each digester had a working volume of 6 L, providing 2 L of a headspace for the biogas, and composed by 2 main parts: body and cover system. The reactor body was made of ¼-inch thick Plexiglas with ID of 6 inches and height of 20. Atop the reactor chamber, a 9-inch

ID flange with ½-inch thick Plexiglas was attached. Along its circumference, twelve holes were drilled to fit 12 of ¼-inch bolts for clamping. A 7.5-inch diameter grove was also drilled in the flange to place an O-ring for sealing the top cover. Additionally, grease was spread over the flange surface, groove, and O-ring to prevent any air leakage. A Plexiglas cover with thickness of ½ inches and diameter of 9 inches was attached to the upper flange. It had four identical openings fitted with ¾-inch durable single-barbed nylon tube fittings. One opening was permanently sealed, and two openings were used for biogas flow line and connected to a foam recycle bottle by 3/8-inch ID of Tygon tubing. The larger tube was fitted to one opening and extended with ½-inch ID Tygon tubing to provide a bigger opening for feeding process. To provide air- and watertight seal, every joint of fitting and tubing was completed with sealing tape and double grip hose clamp.

Four ports, designated for sampling and decanting, were placed along the digester body. They were fitted with ¾-inch nylon single-barbed tube fittings and sealed with a combination of Tygon tubing and hose clamps. The top three ports were completely shut, later used as alternative opening to allow flexibility of operation, while the bottom opening was used for sample decanting. Perpendicular to these 4 ports, two other openings completed with tube fittings were positioned just at the top and bottom of reactor for mixing line connection. The reactor bottom was also made of Plexiglas plate with ½-inch thickness and 9 inches of diameter.

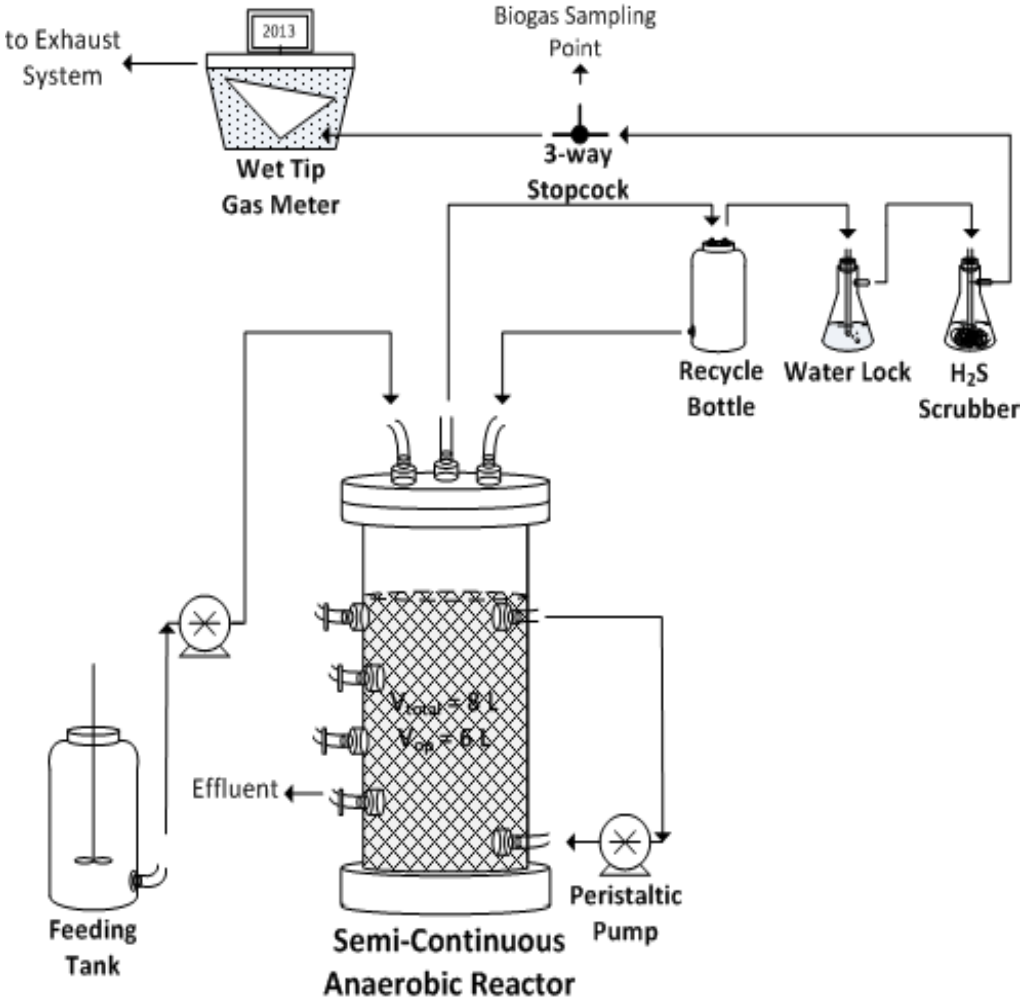

Figure 4 Schematic diagram of anaerobic co-digestion reactor system

3.1.2 Feeding and Decanting System

For the experiment, regular feeding and decanting were simultaneously completed by pumping the feedstock through feeding port on the cover using a peristaltic pump, Masterflex L/S variable speed modular drive with reversible motor and Masterflex L/S easy-load pump heads, and drawing the effluent from decanting port by loosening the clamp. Feedstock was always prepared prior to feeding/decanting process in 1-gal polypropylene container. During the feeding, A Stir-Pak dual-shaft mixer was used to keep the feedstock mixture homogenous with constant mixing. The feeding line was a Masterflex 3/8-inch high performance precision Tygon tubing, connected to the feeding port. For the majority of the experiment, feeding was done every 2 days. Since every cycle, a sludge volume of 600 mL was decanted, the HRT was 20 days.

3.1.3 Biogas Line System

In each reactor system, the two openings fitted with ¾-inch nylon single-barbed tubing at the lid were connected to a recycling bottle of polypropylene, with joint line of 3/8-inch and ¼-inch tubing which was facilitated by a PVDF barbed reducing connector. One connection was to the top side of the recycle bottle, while another connection was to the bottom opening of the bottle. The recycle bottle had also one other opening that led to the rest of biogas system which consisted of water lock bottle, H_2S scrubber, sampling point with 3-way Polymer Luer stopcock, and a wet tip gas meter. Biogas from the headspace was collected through the bottle, while the foamy mixture of liquid, gas, and liquid carried away due to

overloading or nature of the substrate was separated in the bottle with the liquid fraction returned back to the reactor via the bottom opening.

Following the recycle bottle were two Erlenmeyer flasks, which served two different purposes. One flask contained water and functioned to prevent reverse flow of biogas back to reactor as well as to provide a visual check on gas production by bubble formation, or leakage detection by water change level inside the plastic tube inside the bottle. Another flask was placed after the water lock bottle to serve as H_2S scrubber. This H_2S scrubber bottle was filled with steel wool to remove the H_2S from biogas stream when biogas reacted with iron. All apparatus were interconnected by ¼-inch clear Tygon tubing lab and equipped with hose clamps and sealed with parafilm at joints.

From the scrubber, the gas flowed through a 3-way polycarbonate Luer stopcock, which served as sampler to allow removal of biogas by inserting the needle for sampling. Finally, biogas entered a wet tip gas meter to measure the gas volume. One tip of the meter represents 100 mL of biogas being measured. From this gas meter, the biogas finally was discharged by directing it to an exhaust hood.

3.1.4 Mixing System

In this study, mixing was accomplished in both digesters by the sludge recirculation. Sludge at the surface of working volume was pumped out by a Masterflex peristaltic pump with Masterflex L/S easy-load II head, through high performance precision Tygon fuel & lubricant tubing and recirculated back to the reactor in up-flow stream to mix the substrate and release the entrapped biogas to the headspace. Mixing intensity was pre-determined

using the observation from previous research, and was set to run for 30 minutes every hour with controlled speed at 5 of Masterflex peristaltic pump scales. Intermittent turning on and off of the mixing pump was controlled by Chrontrol Timer.

During the experiments, it was observed that several problems occurred with this mixing method, especially when the sludge became thicker due to changing substrate characteristics. Ineffective mixing created several dead zones particularly at the bottom of the reactor. This reduced the available effective reaction volume of substrate and biomass since the mass transfer becomes less efficient. Therefore, to prevent incomplete mixing, once the dead zone was detected, manual force by shaking the reactor was applied, at least once in 2 days.

Other problems occurred with this mixing method mainly were caused by characteristics of sludge that affected the performance of the mixing systems, requiring regular maintenance such as replacement of tubing and clamps, repair of pumps, and modification of the recycle line. The mixing issue is discussed in the Results and Discussions chapter.

3.2. Experimental Procedures

3.2.1 Substrate and Inoculum

This study used active anaerobic sludge obtained from South Durham Water Reclamation Facility as inocula. The sludge was stored for 1 day in a cold room before being seeded into the digesters and placed in a hot room. For co-substrates, two types of feedstock were used for this experiment: thickened waste activated sludge (TWAS) and grease interceptor waste (GIW). TWAS was obtained from North Cary Water Reclamation Facility (NCWRF), while GIW was provided by an American buffet restaurant in Cary, NC and was with the help from

Town of Cary Public Works and Utilities Department. Prior to the start of experiments, GIW was separated into different containers based on its type of wastes: food particles, wastewater, and fats, oil, and grease (FOG). No pretreatment was applied to the wastewater and FOG parts of GIW, however food particles were first blended using food processor to reduce and homogenize particle sizes. After being separated, all substrates were analyzed for their important parameters then stored in a cold room at temperature of 4^{o}C. Table 6 shows the raw feedstock characterization.

Table 6 Raw feed stock characterization

Parameter	**TWAS**	**Grease Interceptor Waste (GIW)**			
		Food Particles (FP)	**Wastewater (WW)**	**Fats, oil, and grease (FOG)**	**Total**[a]
Total Solids (TS, g/kg-wet sample)	34.02 ± 0.23	78.07 ± 0.91	0.99 ± 0.04	625.5 ± 2.0	96.14 ± 4.91
Volatile Solids (VS, g/kg-wet sample)	26.53 ± 0.21	75.94 ± 0.04	0.75 ± 0.04	623.3 ± 2.0	95.07 ± 4.87
VS/TS (%)	78.0	97.3	75.3	99.6	98.9
pH	7.11	4.2	5.2	7.1	4.5

[a]GIW composition: 10%-vol FOG, 40%-vol FP, 50%-vol WW

Figure 5 Photograph of experimental set-up

3.2.2 Feeding Schedule and Pattern

3.2.2.1 Start-up Phases (Phase 1-2)

Both 8L-reactors (reactor A and reactor B-control) ran in a controlled-temperature room, at mesophilic condition (~37^{o}C) with working volume of 6L. Reactor feeding and drawing were done every 2 days, with 600 mL of sludge decanted from reactor while 600 mL of fresh feedstock were fed each time, resulting in a HRT of 20 days.

During phase 1, both reactors (reactor A and reactor B-control) were fed with only TWAS, resulting in an organic loading rate (OLR) of 1.33 g MLVS/L-day. This phase served as adaptation stage for inocula and lasted for 44 days. After phase I, both reactors were fed with the mixture of 70%-VS of TWAS and 30%-VS of GIW, resulting in an OLR of 1.64 g MLVS/L-day. The TWAS-GIW mixture was set at this value based on the observation from previous research showing that it was relatively safe composition for co-digestion. Phase 1 and 2 lasted until both reactors performance were stable, indicated by steady biogas production and methane content.

3.2.2.2 Training Phase (Phase 3)

The purpose of the training phase was to induce functional stability of digester by applying controlled stress to the system. During this phase, different feeding patterns were applied to each reactor. For control reactor B, feeding was constant with the same OLR as in Phase 2. For reactor A, the OLR was changed every 8 days. For 3 feeding cycles (a period of 6 days), the reactor was fed with OLR of 1.64 g MLVS/L-day, but on the 8th day, the composition of TWAS-GIW was changed to result in an OLR of 2.24 g MLVS/L-day. According to previous research, a co-substrate composition of 40%-VS TWAS and 60%-VS was known to start causing some inhibition in performance of digester, indicated by non-increasing yield of methane production as the OLR increased. Therefore, this feedstock composition was labeled as a periodic perturbation, and during this phase, it was applied 4 times every 8 days (in the period of 32 days) to reactor A.

3.2.2.3 Perturbation Tests (Phase 4-5)

Phase 4 is the perturbation test period. Tests were done in 2 stages, with different organic loading rates. After reactor A was trained with periodic minor perturbation to induce functional stability, both reactors were fed high organic concentration substrates with OLR of 2.95 g MLVS/L-day repeatedly 5 times, every 2 days over 10 days period. Major perturbation with OLR of 4.48 g MLVS/L-day then followed as second test only after both reactors showed steady performance. However, due to organic overload being applied, several issues arose. Both reactors showed some signs of failure and their performance decreased rapidly. Therefore, after two feeding times, both reactors were stopped being fed with high OLR and returned to the start-up OLR, thus starting the recovery phase.

3.2.2.4 Recovery Phase (Phase 6)

This final phase functioned as recovery stage. The decision to proceed with recovery phase was solely based on performance data that was showing rapid decline of key parameters such as methane production and pH as well as increasing accumulation of intermediate products. During this phase, both reactors were fed with similar OLR as phase II (= 1.64 g MLVS/L-day), and operated at this condition until they showed improvement and reached steady performance.

In total, for this study, reactors were operated for 168 days. Figure 6 presents the overall loading schedule for the experiments.

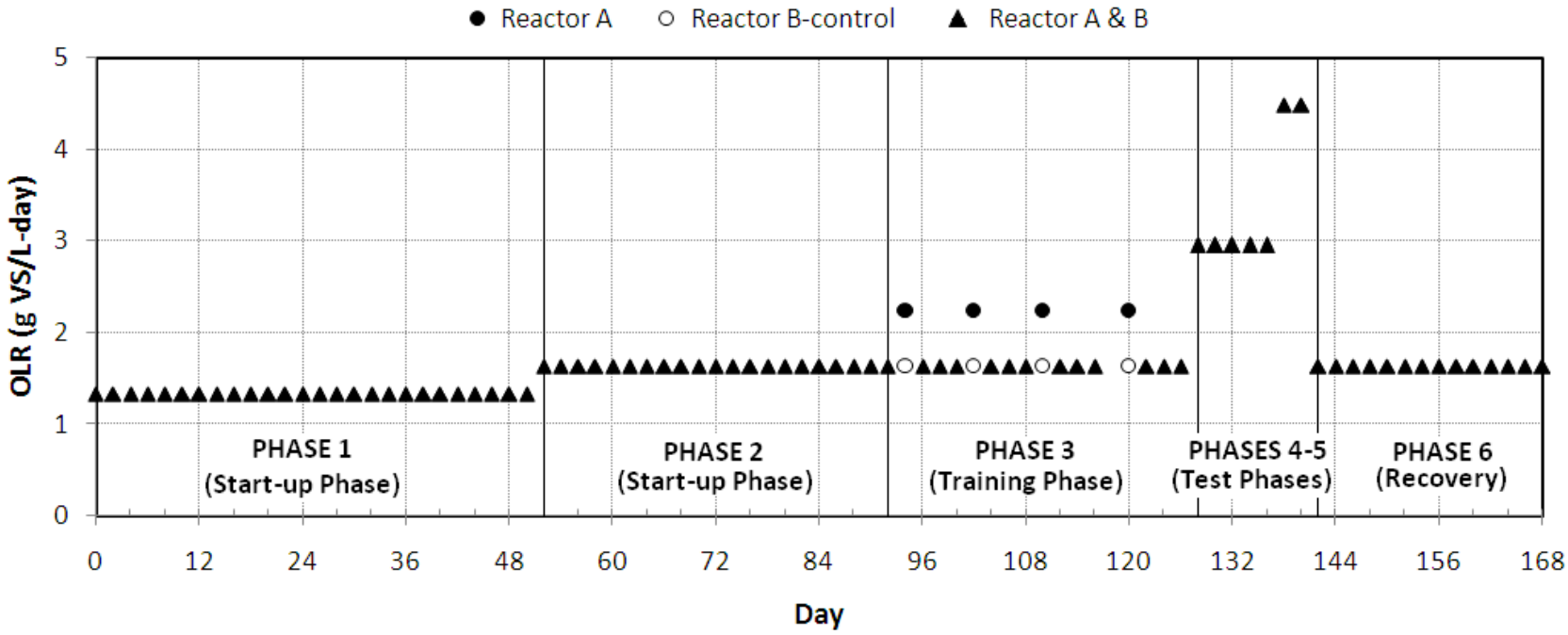

Figure 6 Loading schedule

3.3 Analytical Methods

The performance of anaerobic reactors was monitored by analysis of several parameters. Chemical analytical tests were performed in the lab and measurements were verified in triplicate several times during the course of study to ensure replicability. Table 7 summarizes the analyzed variables and the methods used in this research.

Table 7 Summary of analytical methods used in this study

Parameter		Method	Samples	Frequency
Liquids	pH	pH meter	Effluent	Every 2 days (every 6-12 hours during perturbation test)
	Alkalinity	Titration	Effluent	Every 4-8 days
	VFA	GC	Effluent	Every 6-12 hours during perturbation test
Gas	Gas production	Wet tip gas meter	Biogas	Daily
Solids	Methane content	GC	Biogas	Every 2 days (every 6-12 hours during perturbation test)
	TS and VS	Standard methods	Influent and Effluent	Every 2-4 days

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Experiment Overview

This study was performed for approximately 168 days and divided into 6 experimental phases, with different feeding patterns and organic loading rates (OLR). Two semi-continuous anaerobic reactors (Reactor A and Reactor B-control), were operated at a temperature of 37^{o}C and Hydraulic Retention Time (HRT) of 20 days.

Two early phases functioned as start-up runs where both reactors were fed with only TWAS for phase 1 and mixture of TWAS+GIW (30% [w/w] by VS) for phase 2. The training phase followed after both reactors achieved pseudo-steady state. The goal of the training phase was to introduce controlled transient perturbations to reactor A to induce its functional stability. Both reactors were fed with TWAS+GIW (30% [w/w] by VS) feedstock every two days. However, a mixture of TWAS+GIW (60% [w/w] by VS) was introduced to reactor A every 8 days as periodic organic overload stresses. To confirm the effectiveness of the training method, perturbation tests were performed during phases 4 and 5 with TWAS+GIW (70% [w/w] by VS) and TWAS+GIW (90% [w/w] by VS) respectively. This study was concluded with phase 6 where both reactors were fed back with TWAS+GIW (30% [w/w] by VS). Detailed characteristics of feedstock prepared for this study are presented in Table 8 below.

Table 8 Feedstock characteristics during experiments

	Start-up Phase		**Training Phase**		**Perturbation Test**		**Recovery Phase**
			Phase 3				
	Phase 1	**Phase 2**	*8 days interval for reactor A*	*Other feeding times (A and B)*	**Phase 4**	**Phase 5**	**Phase 6**
Feedstock	100% TWAS	TWAS+GIW[a] (30% by VS)	TWAS+GIW (60% by VS)	TWAS+GIW (30% by VS)	TWAS+GIW (70% by VS)	TWAS+GIW (90% by VS)	TWAS+GIW (30% by VS)
TS, g/kg-wet sample	34.02 ± 0.23	39.57 ± 0.19	50.30 ± 1.51	39.57 ± 0.19	64.23 ± 2.57	92. 59 ± 4.58	39.57 ± 0.19
VS, g/kg-wet sample	26.53 ± 0.21	32.76 ± 0.19	44.87 ± 1.54	32.76 ± 0.19	58.93 ± 2.56	89.54 ± 4.65	32.76 ± 0.19
VS/TS, %	77.99	82.78	89.18	82.78	91.73	96.7	82.78
COD, g/L	84.3	110.8	n.d	110.8	n.d	n.d	110.8

[a] GIW composition: FOG (10% v/v), food particles (40% v/v), wastewater (50% v/v)

4.1.1 Start-up Phases (Phase 1 and Phase 2)

The first two phases were start-up periods to acclimatize and adapt the anaerobic inoculum to both TWAS and Grease Interceptor Waste (GIW). In phase 1, which lasted for 52 days, reactor A and B were identically loaded with 100% TWAS as their feed. The OLR during phase 1 was 1.33 g VS/L-day. After day 10, both reactors seemed to perform steadily, producing around 1.1-3.1 L biogas/d for reactor A and 0.8-3.0 L/day for reactor B. The average biogas production rate during phase 1 for reactor A and B are 2.0 L/d and 1.9 L/d respectively, with average methane content of 54.2% (A) and 57.9% (B). The difference between two reactors in terms of methane content can be traced back to some issues with the biogas line of reactor A that had a leaking problem. However this situation improved over the course of the reactor run as the problem was solved. Both reactors were fed with TWAS which contained 26.53 g/kg-wet sample of VS, resulting in an average methane yield of 0.134 and 0.141 L-CH_4/g VS added for reactor A and reactor B respectively. Analysis of pH and alkalinity for both reactors during phase 1 and 2 also showed good performance. Alkalinity measurements were around 4000 mg $CaCO_3$/L, with the lowest pH at 7, which is in the optimum range for methanogenesis.

After around 2.5 HRT of running both reactors with 100% TWAS, Phase 2 was started by introducing the reactors to GIW as co-substrate to TWAS. A mixture of GIW (30% by weight of VS) with TWAS was fed to both reactors as another acclimatization effort. This composition of GIW and TWAS was later to be used as a basis for feedstock concentration for all experiments. According to the previous study (Wang, 2013), organic load from TWAS+GIW (30%-VS) feed (OLR = 1.64 g VS/L-day) is still in the safe range of the

amount of lipid-rich wastes to be added as co-substrate without resulting in reactor failure. All procedures and analyses were performed similar to Phase 1, with Phase 2 lasting for 40 days.

During Phase 2, an average increase of 210% (2 L/d to 6.2 L/d) in biogas production for reactor A was observed. An increase of 226% (1.9 L/d to 6.2 L/d) of biogas production was also observed in reactor B. Average methane yields for both reactors also were also tripled to 0.39 L-CH_4/gr VS added (for reactor A) and 0.40 L-CH_4/gr VS added (reactor B). As for methane content, both reactors' performance steadily increased and averaged at 62.7% (A) and 63.4% (B).

Figures 7-8 show the parameters being monitored and analyzed during phases 1 and 2 as indicators of reactor performance.

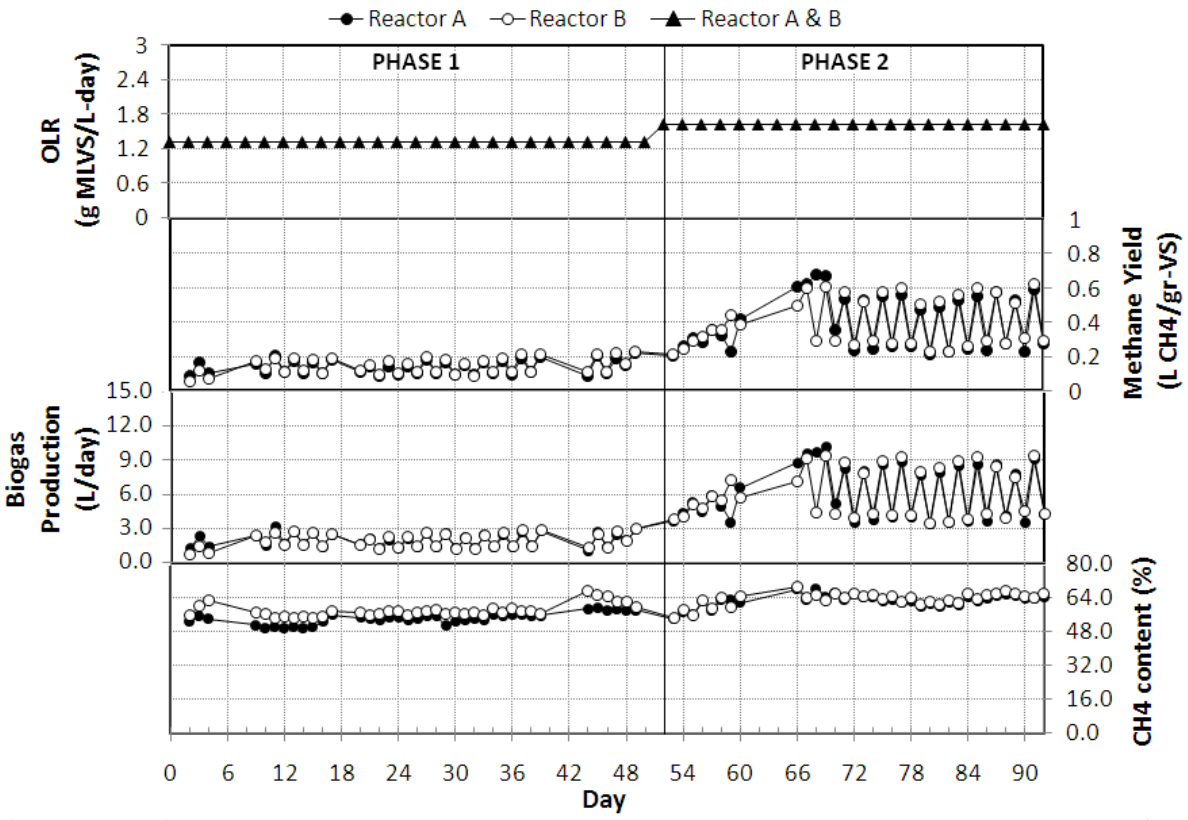

Figure 7 Biogas production, methane content, and methane yield of trained reactor (A) and control reactor (B) during Phases 1-2

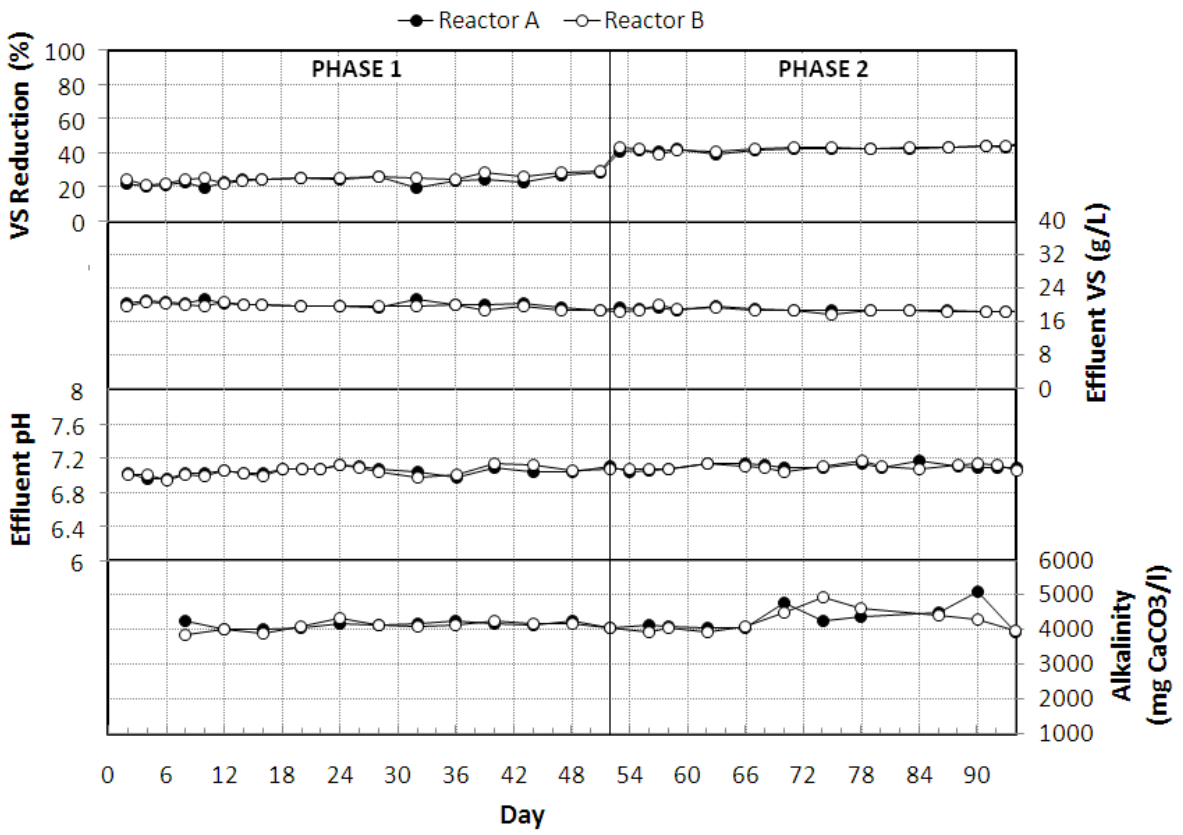

Figure 8 Effluent VS, VS reduction, effluent pH and alkalinity of trained reactor (A) and control reactor (B) during Phases 1-2

4.1.2 Training Phase (Phase 3)

After performance parameters had showed that both reactor A and B were stable treating TWAS+GIW with OLR = 1.64 g VS/L-day during phase 2, the training phase for reactor A began shortly. During this phase, reactor A was treated differently than the control reactor. For most of phase 3, both reactors were loaded with the same OLR (1.64 g VS/L-day). However, on 4 occasions, for every 8 days interval, instead of identical OLR as B, reactor A was introduced to a higher organic load. Feedstock containing TWAS+GIW (60% [w/w] by VS) or an OLR of 2.24 g VS/L-day was fed to reactor A once after 3-4 times of regular feeding, resulting in average OLR of 1.79 g VS/L-day. This periodic spike of organic load was designed to train reactor A with minor perturbation in the form of overload stress to induce its functional stability. The appropriate concentration of organic load used as minor perturbation to reactor A was based on previous research on the threshold limit of GIW addition to anaerobic digestion of TWAS (Wang, 2013). A mixture of TWAS+GIW (60% by weight of VS) was chosen to introduce as perturbation as it was reported to be non-lethal to cause digester failure, just below the threshold limit (65%-VS of GIW added) but was high enough to start pushing the system out of its equilibrium steady state.

The pulse stress was expected to stimulate the ability of microbial communities in the anaerobic digester to keep functioning in a more adverse situation by pushing their equilibrium steady state above the normal level. The hypothesis is that after several perturbations the communities will establish better survival mechanisms against similar type or more of disturbance.

As previously explained, using this pulse feed instead of step feed simulates periodic overloading of anaerobic digesters utilizing lipid-rich wastes from FSEs. Also there was a concern that the time needed for a digester to go back following the perturbation can be uncertain and take a longer time.

During the training phase, the periodic increases in organic loading to reactor A resulted in a fluctuation of performance parameters as expected. Overall, average methane yield and biogas production for reactor A reached values of 0.51 L-CH4/g VS added and 8.6 L/d respectively, obviously due to higher concentration of food for microorganisms to eat. Other parameters such as pH, alkalinity, methane content, and effluent VS for reactor A stayed the same, indicating that the reactor was stable. Table 9 and 10 summarize the average values of parameters for reactors A and B during both start-up phases and training phase.

The training phase lasted for around 36 days, with reactor A periodically stressed with the minor perturbation 4 times. On the 128th day since the reactors started running, the first major perturbation test was performed to both anaerobic digesters.

Table 9 Average values of performance parameters for reactor A during start-up and training phases

Phase	Period (days)	OLR (g VS/L-day)	Effluent VS (g/kg-wet sample)	VS reduction rate (%)	Effluent pH	Alkalinity (mg $CaCO_3$/L)	CH_4 content (%)	Biogas Production (L/day)	Methane yield (L-CH_4/g VS added)
1	52	1.33	20.22	23.81	7.04	4139	54.2	2.0	0.13
2	40	1.64	18.85	42.14	7.09	4326	62.7	6.2	0.39
3	36	1.79[a]	18.13	48.49	7.12	4314	64.2	8.7	0.50

[a]Average OLR (1.64 g VS/L-day for every 2 days, 2.24 g VS/L-day on the eight day cycle)

Table 10 Average values of performance parameters for reactor B-control during start-up and training phases

Phase	Period (days)	OLR (g VS/L-day)	Effluent VS (g/kg-wet sample)	VS reduction rate (%)	Effluent pH	Alkalinity (mg $CaCO_3$/L)	CH_4 content (%)	Biogas Production (L/day)	Methane yield (L-CH_4/g VS added)
1	52	1.33	19.84	25.21	7.04	4102	57.9	1.9	0.14
2	40	1.64	18.75	42.59	7.09	4274	63.4	6.2	0.39
3	36	1.64	18.09	44.44	7.13	4180	64.4	6.5	0.42

4.1.3 Perturbation Test 1 (Phase 4)

The first perturbation test was performed a week after the last training phase. The test basically proposed the same idea of feeding the reactors with TWAS and GIW as co-substrates, only with much higher organic load. An OLR of 2.95 g VS/L-day, or almost twice than the base OLR from previous phases, was continuously applied to both reactors A and B for a total of 5 feeding times during this first perturbation test period.

Rigorous monitoring of parameters was also performed intensively. For the first two feedings, biogas production and methane content were monitored every 6 hours following the first perturbation. In that same time interval, a total volume of 15-30 mL of anaerobic sludge was also drawn from both reactors, to measure its pH and volatile fatty acids (VFA), as well as DNA extraction for future molecular studies. The pH was measured immediately right after collecting the effluent, while sludge for VFA samples were pre-treated first and collected in batch before being analyzed with the help of David C. Black using Shimadzu GC-FID in Environmental Engineering Lab. This sample collection, however, eventually resulted in the decrease of sludge volume in the reactor, though the volume removed every time samples were drawn was insignificant. During the test, the volume inside reactors fluctuated between 5.9 and 6 liters depending on the frequency of sample collection. The next feeding time would then compensate the deficit of sludge volume inside the reactor by adding extra amount of the same feedstock.

As shown in Figures 9 and 10, during this first perturbation test, both reactors performed similarly with increased biogas production and methane content, hence higher methane yield. However, at the same time, pH and alkalinity in both reactors gradually decreased, reaching

6.5 and 2000 mg/L respectively. This reduction in pH and alkalinity is because the intermediate products of anaerobic metabolism in the form of volatile fatty acids had started to accumulate. The reactor was overload by organic substances while its utilization rate was lower due to lack of parallel substrate processing communities or suspected inhibition. Since the first perturbation test utilized higher percentage of GIW added as co-substrate, the concentration of lipid-rich wastes increased, along with its high LCFA content.

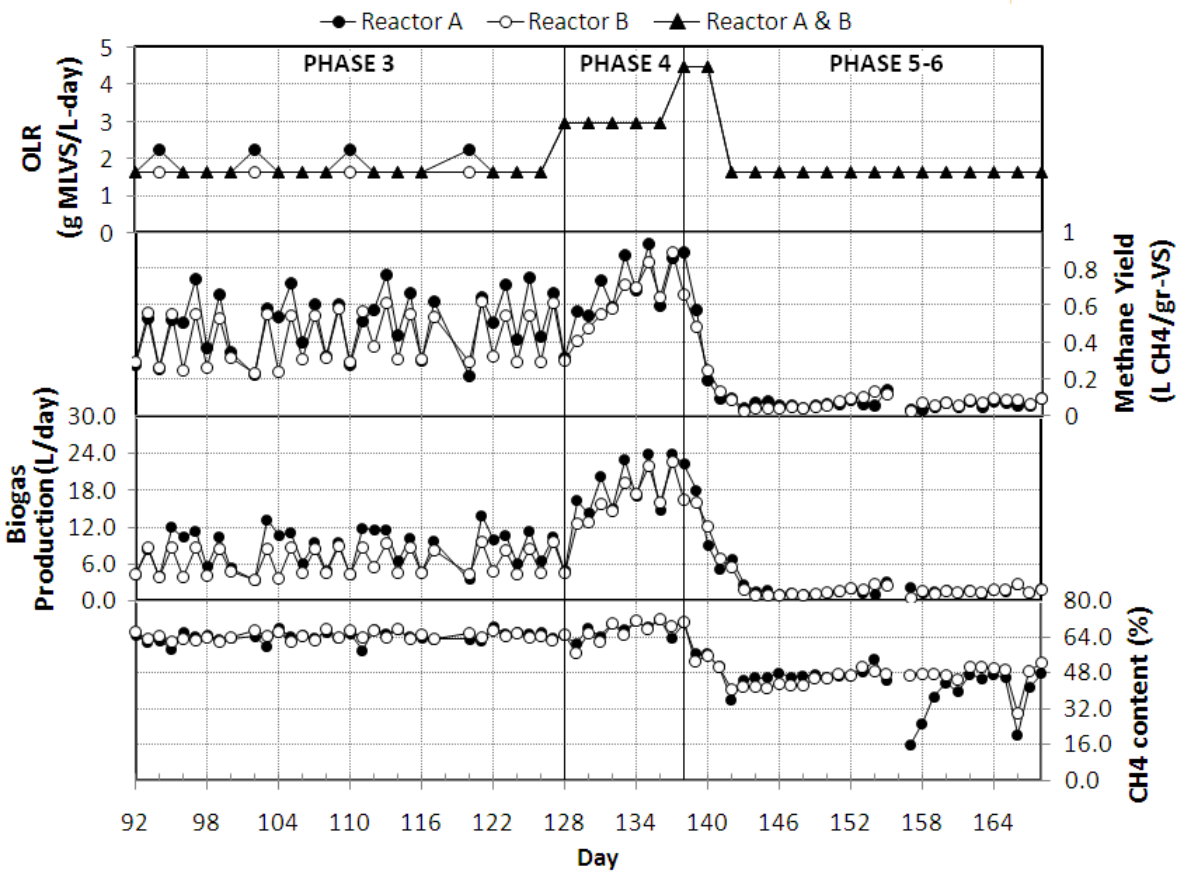

Figure 9 Biogas production, methane content, and methane yield of trained reactor (A) and control reactor (B) during Phases 3-6

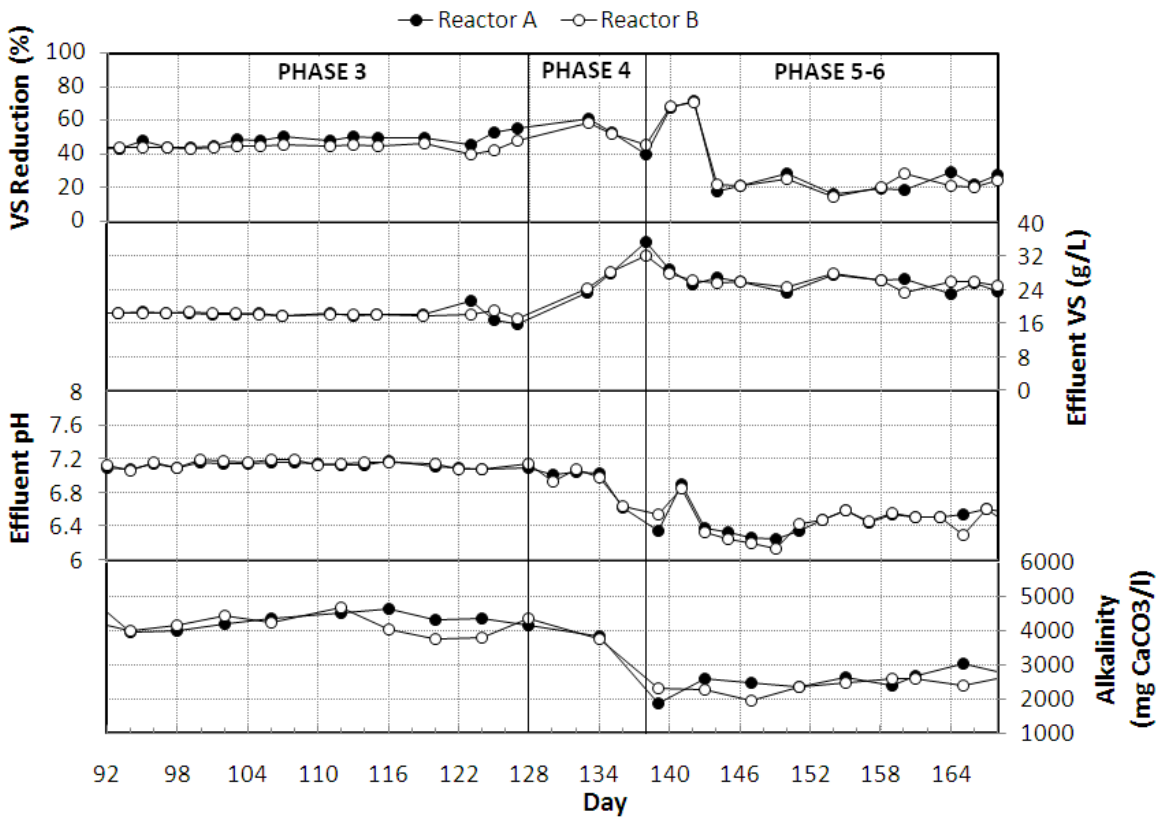

Figure 10 Effluent VS, VS reduction, effluent pH and alkalinity of trained reactor (A) and control reactor (B) during Phases 3-6

As previously described, LCFA can inhibit anaerobic metabolism if absorbed by microbial cells, thus limiting transfer of mass and energy between biomass and organic matters. However, the biogas production and methane yield were still desirable, since both kept increasing. During the first perturbation test, the average of methane yield for reactor A was 0.69 ± 0.18 L CH_4/g VS added while reactor B had 0.60 ± 0.17 L CH_4/g VS added. Figure 11 showed the methane yield and effluent pH during perturbation tests.

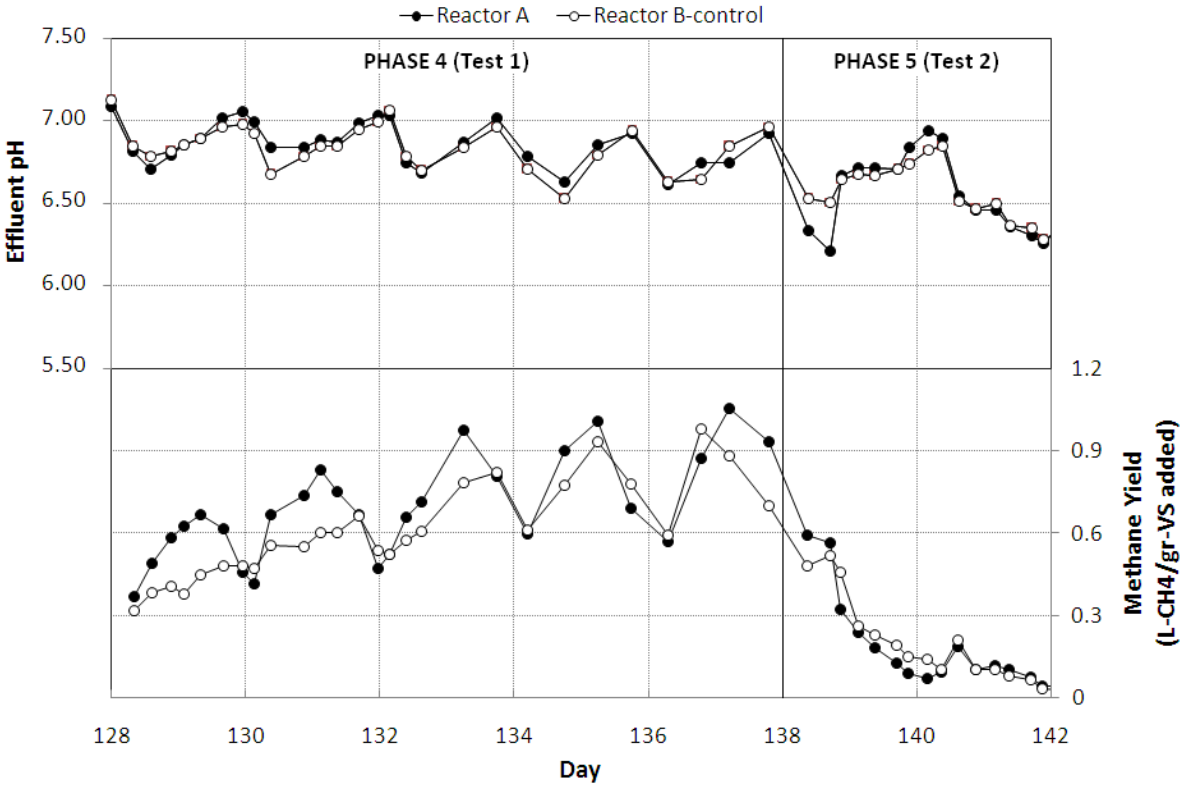

Figure 11 Methane yield and effluent pH during perturbation tests

Perturbation tests were performed to prove whether the strategy of pulse feeding applied during training phase was effective to induce functional stability of anaerobic digesters or not. The main component of this test was the investigation on the dynamics of key intermediate products (volatile fatty acids, VFA) inside the reactor. The pattern of accumulated products in response to perturbation was analyzed for various measures of functional stability, including resistance and resilience. Key intermediate products of anaerobic metabolism were acetate, propionate, butyrate, and including valerate. The results of VFA analyzed from effluent drawn periodically during the perturbation tests are shown in Figure 12.

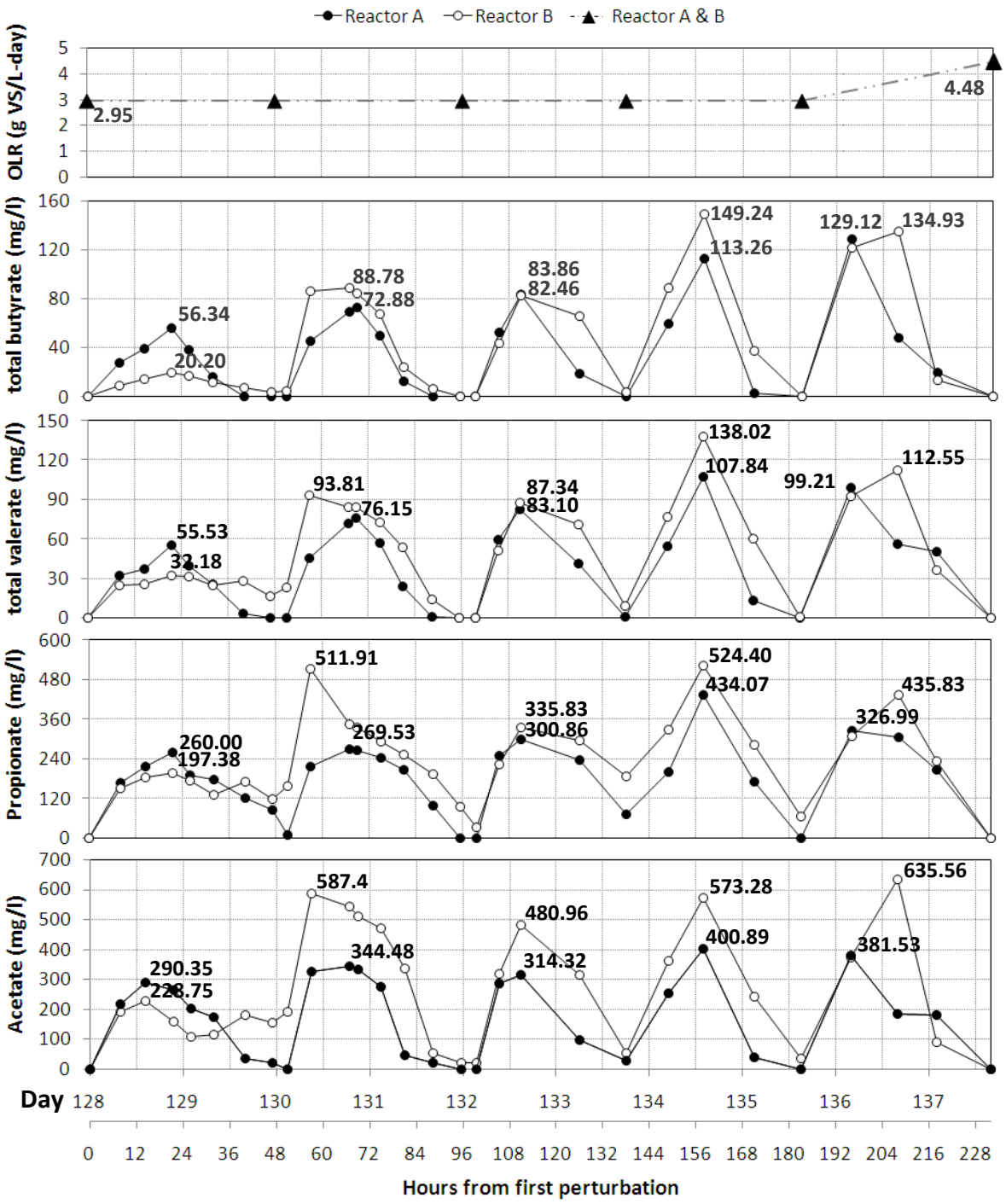

Figure 12 VFA concentrations following the first perturbation test

From Figure 12, for the majority of perturbation test, VFA accumulation was greater in reactor B compared to reactor A, except for the first feeding. Following the first feeding, the accumulation of all fatty acids in reactor A was greater than B. However, reactor A was also faster to return to its previous state where VFA accumulation equaled zero. Accumulation of acetate and propionate in reactor A were diminished within 48 hours since the first feeding was introduced, while total butyrate and total valerate (which include both iso- and n-isomers) required 36 hours to completely be removed from the system. On the other hand, reactor B showed less accumulation of VFAs, but did not recover within 48 hours. Unfortunately, the time required for reactor B to completely remove VFAs following the first perturbation cannot be further determined, as the next feeding cycle ensured that it will not return to zero.

After the second feeding, there was an increase of VFAs accumulation in both reactors, but this time the accumulation in reactor B was greater than in reactor A. Paired with longer return time, it can be concluded that reactor B had weaker performance compared to reactor A. The trend continued in the next 3 feedings, as the maximum accumulation of each VFA climbed up due to substrate overload. However, both reactors always returned back to their reference state within 48 hours, meaning that both reactors still performed well. Based on this condition, it can be said that overall, the first perturbation test did not create adverse effect to the reactors, as both reactors still worked well. The difference between the two reactors in terms of maximum accumulation of VFAs and the return time was also small. It was also observed that through the first perturbation reactor B was getting better over time, in other words, it was also getting trained. As for the reason on why during the first feeding, the

maximum accumulation of reactor B was lower than reactor A, if we look at the graph showing methane yield and pH for both reactors during perturbation (Figure 11), the methane yield of reactor B showed lower value than reactor A. It appeared that, the microbial communities inside reactor B was not yet active in metabolizing the organics in feedstock compared to communities in reactor A, thus resulting in less production of intermediate product and subsequently its accumulation. The communities in reactor B, however, seemed to finally respond towards the organic matter, and start to use them as substrate, in the next feedings.

4.1.4 Second Perturbation Test and Recovery Phase (Phase 5 and Phase 6)

As the first perturbation was deemed as insufficient to push the stability of system out of its equilibrium and considered to be a weak perturbation to cause immediate effects, feeding with organic loading rate of 4.48 g VS/L-d was introduced to push the reactors even further. There have been no previous successful experiments reported that used this high level of lipid-rich waste as co-substrate (90% by VS added) in anaerobic digestion. During the second test, rigorous monitoring was also performed with the same schedule as in the first perturbation test.

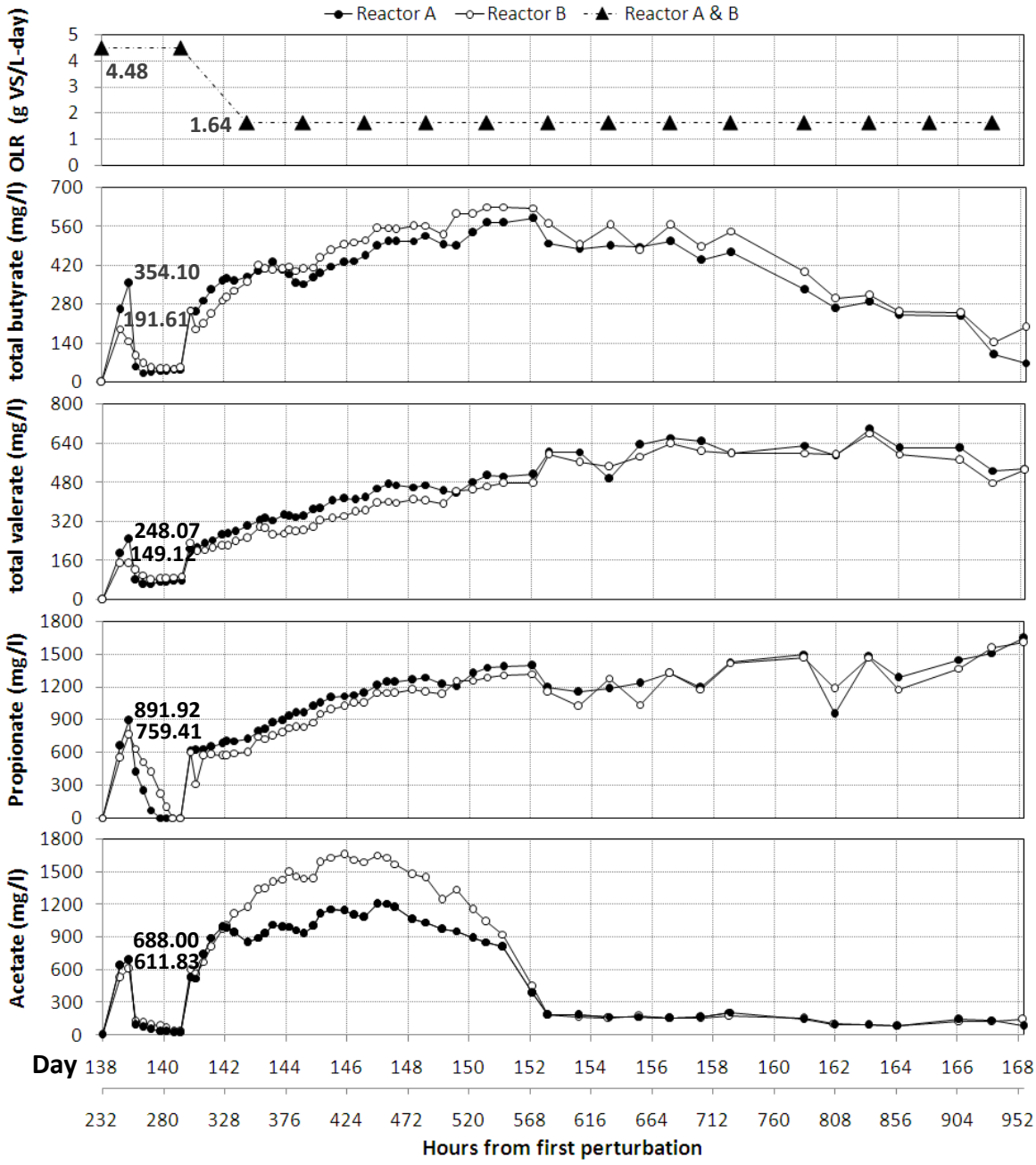

Figure 13 VFA concentrations following the second perturbation test

As displayed in Figure 13, the result of second perturbation test, however, produced an unfavorable outcome. The test lasted only for 4 days, for two feedings, before it the tests were terminated. The first feeding followed a similar pattern as the first feeding of previous perturbation test. Both reactors appeared to run well, with reactor A having a greater maximum accumulation of VFAs but faster return time compared to B. The second feeding, though, resulted in the failure of both digesters.

Following the second perturbation, both reactors started to show signs of failing. Biogas production, methane content, and methane yield kept decreasing, while effluent pH of reactor A and B dropped until it reached below 6 (see Figure 11). This occurrence was also verified by the GC analysis showing that VFA was accumulating without any degradation, meaning that methanogenesis was severely inhibited, thus building up the amount of fatty acids inside the reactors, supported with the decrease in solids reduction. The visual monitoring of sludge inside both reactors also showed that the foam formed in a thick layer and changing color, as depicted in Figure 14. By the end of phase 4, the methane yield for reactor A was 0.043 g VS/L-day and for reactor B, 0.027 g VS/L-day, and methane contents were at 45% and 44% accordingly.

The second perturbation test was deemed unsuccessful since both reactors failed, and recovery phase later followed. Both reactors were fed with OLR of 1.33 g VS/L-day in an effort to regain back the performance of both reactors, and were run for another 24 days.

After over 24 days of recovery, both reactors did not show major improvement. Methane yield stayed at 0.06 g VS/L-day for reactor A and 0.07 g VS/L-day for reactor B. Other parameters such as effluent pH and VS reduction also remained low, averaging at 6.45 and

22.5% for A, and 6.41 and 22% for B. Operational problems also started to appear, and both reactors were eventually terminated.

4.2 Discussion

4.2.1 Functional Stability Analysis

To measure functional stability, key parameters from the amplification envelope were measured. The two parameters from this envelope of key intermediate products in response to a perturbation, as shown by Hashsham et al. (2000) that were used in this study are resistance and resilience. Resistance of a community with respect to an intermediate product is defined as the maximum accumulation of product, while resilience is defined as the time taken by the accumulated intermediate product to return to its reference state (Neubert and Casswell, 1997; Hashsham et al., 2000).

In this study, functional stability analyses were performed using the data obtained during two perturbation tests conducted on two semi-continuous anaerobic digesters. Higher numerical values for both maximum accumulation of product and return time of accumulated intermediate product indicate lower resistance and resilience respectively.

For this analysis, comparisons between two reactors were made based on their performance during perturbation tests. Reactor A was previously trained by transient minor perturbation to induce its functional stability prior to the test. Reactor B, on the other hand, was a control reactor, with no training period before the perturbation test.

The intermediate products were the volatile fatty acids acetate, propionate, iso- and n-butyrate, and iso- and n-valerate. A reference state for this analysis was determined to be the

initial condition before the perturbation test, where no accumulation of VFAs was detected in both reactors.

For the first perturbation test, the accumulation of corresponding VFA in response to perturbation is shown in Figure 12. From this figure, resistance and resilience were computed by analyzing the amplification envelopes of four different fatty acids. The values of corresponding resistance and resilience are presented in the following table:

Table 11 Resistance of anaerobic digester A and B in response to first perturbation test corresponding to different VFAs

	Acetate (mg/L)		**Propionate (mg/L)**		**Total Butyrate (mg/L)**		**Total Valerate (mg/L)**		**Total VFA (mg/L)**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Feeding 1	290	229	260	197	56	20	56	32	662	478
Feeding 2	344	587	270	512	73	89	76	94	763	1282
Feeding 3	314	481	301	336	84	82	83	87	782	986
Feeding 4	401	573	434	524	113	149	108	138	1056	1384
Feeding 5	382	636	327	436	129	135	99	113	937	1320

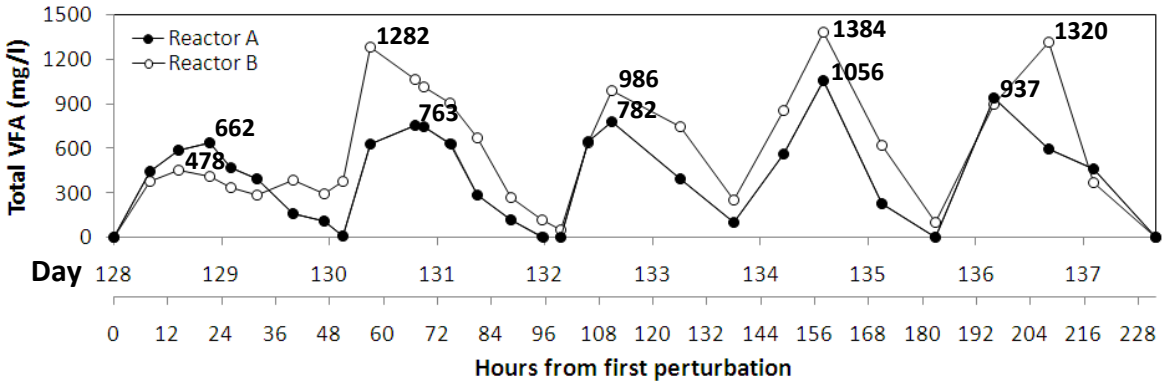

Figure 14 The amplification envelop of accumulated total VFA for each feeding time during first perturbation test

From the table and graphs above, reactor B appears to have a greater value of maximum accumulation than reactor A, and is hence less resistant, for the majority of perturbation test, with the exception of the first feeding. In the first feeding, reactor A appeared to be less resistant compared to B. However, if we look at their resilience data, reactor A needed less time to return to its reference state than B, therefore is more resilient.

Quantifying the resilience of reactor B can be problematic since after two out of 5 feeding times, the accumulated VFAs in reactor A did not return to its reference state. Instead, estimations were performed by projecting the trend of the graph to represent the behavior of its data points; therefore these values can be over- or underestimated.

Table 12 Resilience of anaerobic digester A and B in response to first perturbation test corresponding to different fatty acids

	Acetate (hr)		Propionate (hr)		Total Butyrate (hr)		Total Valerate (hr)	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Feeding 1	48	N/A	48	N/A	36	46	36	N/A
Feeding 2	45	48	45	48	38	47	38	49
Feeding 3	39	40	39	48	39	39	39	39
Feeding 4	36	57	48	49	36	48	48	48
Feeding 5	40	40	48	56	48	48	48	48

From the table above, it seems that the difference in resilience between reactor A and B significantly occurred during the first two feeding. After feeding 3 through 5, both reactors required relatively the same period of time to reach its equilibrium after being perturbed. Within the first two feeding span, reactor A appeared to be more resilient than reactor B, but as more feeding being applied, the difference started to fade. After being perturbed a couple of times, it seems that both reactor were adapting to their new condition and eventually responded in almost identical behavior. The advantage reactor A had before vanished after multiple perturbations had been applied. It appears that both reactors were acclimating.

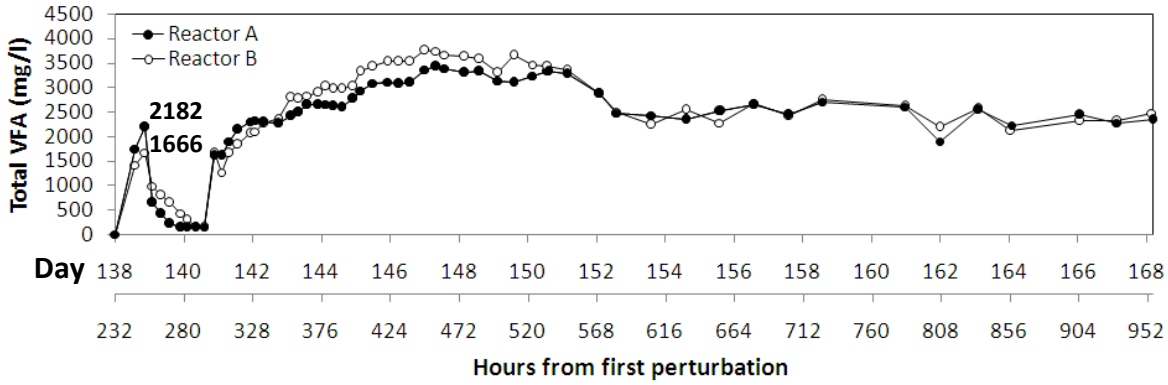

Figure 15 The amplification envelop of accumulated total VFA during second perturbation test

For the second perturbation test, the analysis of functional stability can only be done for the first feeding, since the second feeding led to digester failure for both reactors. After the first feeding of second test was introduced to reactors, the maximum accumulation in reactor A reached a greater value than reactor B. However, the accumulated VFAs in reactor A returned back to its reference system faster than B, as in the previous test.

4.2.2 Effect of Perturbation on Microbial Communities

Anaerobic digestion of lipid-rich materials is often limited by inhibitory effect of LCFAs. LCFAs show acute toxicity towards anaerobic consortium by adsorption onto cell wall/membrane, interference with transport and protective function (Rinzema et al., 1993). Methanogens (mainly aceticlastic methanogens) can be inhibited by LCFAs due to their cell wall, which resembles gram-positive bacteria (Zeikus, 1977).

When acetate-utilizing methanogens are inhibited by LCFAs, other groups of microorganisms will take over obtaining energy from oxidation of acetate to H_2 and CO_2, resulting in high H_2 partial pressure which inhibits the degradation of VFAs, hence VFAs accumulation.

There were four different fatty acids focused on in this study: acetate, propionate, iso- and n-butyrate, iso- and n-valerate. Acetate is mainly produced from degradation of LCFAs, carbohydrates and other short-chain fatty acids; propionate is produced from degradation of glycerol, amino acids, carbohydrates, and n-valerate; butyrate is produced from degradation of carbohydrates and amino acids; and valerate is produced mainly from degradation of amino acids.

In this study, if we look at Figure 12, during the first perturbation test, LCFAs did not increase to inhibiting concentrations to hamper the methanogenesis in both reactors. The majority of VFAs accumulated following the feeding were soon converted into biogas within 2 days in reactor A and B. It appeared that the rate of hydrolysis of lipids to LCFA and glycerols and eventually to VFAs can still be covered with the utilization rate of VFAs by methanogens, resulting in no significant build-up.

However, during first perturbation test, degradation of acetate by aceticlastic methanogens and other fatty acids by respective acetogens (synthropic oxidizing bacteria) occurred in different rate between reactor A and B, and is assumed due to difference treatment during training phase. The trained reactor (A) appeared to have higher utilization rate of VFAs compared to B. This condition is credited to two possible explanations: (1) Acclimation occurred in reactor A during training phase resulting in larger number of

methanogens and synthropic bacteria population, hence more microorganisms to consume the substrate, and (2) Acclimation improved the production of enzyme, hence better substrate utilization rate. Molecular analysis, however, is further required to confirm this.

In phases 5 and 6 (Figure 13), after the second feeding resulting in adverse effect towards microbial communities in both reactors was followed by recovery phase, the degradation of each VFA occurred in rather different rates. Even though being previously inhibited by organic overload, aceticlastic methanogens recovered faster than other groups, as Figure 13 shows that the accumulated acetate needed less time to return back compared to other VFAs. On the other hand, the fact that propionate kept accumulating after recovery loading being applied gives possible explanation that Synthropic Propionate Oxidizing Bacteria (SPOB) were the most severely affected (sensitive) by the second perturbation, resulting in inhibition or even decrease in its microbial population.

Some propionate increase could also originate from the degradation of n-valerate, as it yielded both acetate and propionate. However the decrease of valerate (637 to 537 mg/L for A) was several times smaller than the increase in propionate (1324 to 1642 mg/L for A). Other plausible explanation for the increase in propionate is the product inhibition of SPOB by acetate and hydrogen. Also, the almost simultaneous increase in propionate and the reduction of butyrate could indicate a substrate competition between propionate and butyrate. As the results in Figure 13 shows, the performance of both reactors in respond to second perturbation test, indicated by their VFAs accumulation, appeared to be similar, with the exception for acetate. Maximum acetate accumulation in trained reactor A has lesser value than reactor B, meaning that the previous training method had greater impact on aceticlastic

methanogens than other microbial groups. Other synthropic oxidizing bacteria, on the other hand, appeared to be not affected by the training phase that was previously being applied as both reactors showed similar trend in propionate, butyrate, and valerate accumulation.

4.2.3 Digester Foaming and Operational Problems

After the second feeding of second perturbation tests, it was observed that the excessive foaming problem started to occur. A thick scum layer of lipids was also formed at the surface of reactors. An excessive foaming can cause several problems since it can escape the digester and enter the biogas line. The foam at the surface also made the volume reading becomes more difficult.

Figure 16 Problem with layer of foam and scum on the surface inside reactor (left) and the compressed recirculation tubing (right)

On the other hand, the formation of thick sludge due to high FOG concentration could result in mixing problem. The sludge became too viscous thus making it difficult for the peristaltic pump to recirculate the sludge. Additionally, recirculation pump gas binding problem also occurred. The Tygon tubing often compressed and wore out, thus requiring regular replacement of new tubing, an action that affected the reading of biogas production and its content.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATION

Anaerobic co-digestion of waste activated sludge with lipid-rich wastes is a promising technology in enhancing the energy production and organic degradation by a waste treatment process. However, the limitation on how much lipid-rich waste can be added as co-substrate has prevented this type of treatment process to be effectively utilized due to possible microbial inhibition towards anaerobic communities. The adsorption of LCFA from lipid-rich waste onto cell wall of organisms eventually results in substrate and nutrient transport limitations while acute toxicity will inhibit biological activity of both aceticlastic and hydrogenotrophic methanogens, causing the instability to digesters.

In anaerobic co-digestion of lipid-rich waste and sewage sludge, to achieve process stability can still be a challenge. This study was conducted to investigate the effectiveness of a specific strategy to employ that may induce greater functional stability of an anaerobic system. The strategy was to apply repeated transient perturbation as a training method to induce stability parameters thus enhancing the performance of anaerobic digesters in treating lipid-rich or high-strength wastes.

The training method appeared to have positive impacts towards the stability of anaerobic digesters when tested by rather high perturbation. The trained reactor seemed to be relatively more resilient and resistant in response to stress in the form of high organic compared to control reactor. However, when tested with a really high organic loading rate, the training method did not give promising results as the perturbation was deemed too severe, and both reactors eventually failed due to severe inhibition and other operational problems. Although,

after the second perturbation test, the performance of reactor A, shown by the trend in acetate accumulation, indicated that aceticlastic methanogens were the most affected by the training method, compared to synthropic fatty acids oxidizing bacteria.

There are several strategies utilizing different feeding patterns that can be used to induce functional stability, and one can argue, based on the finding in this study, the pulse feed training method has the potential to be further developed. The important part of this training method is to carefully select the proper organic loading rate to be used as transient minor perturbation as well as the length of training period. This was the first trial of utilizing pulse feed transient perturbation to induce the stability of a system treating lipid-rich waste, therefore there were some imperfect procedures employed during experiments.

The result shows that Synthropic Propionate Oxidizing Bacteria (SPOB) was the most sensitive of all microbial groups present during perturbation of high organic loading rate. Additionally, propionate could present at low inhibiting concentration. Therefore, for future reference, the training phase of reactor by pulse feeding of propionic acid could also be implemented to induce more stability specifically toward SPOB.

Regarding the implementation of pulse feed training phase in this study, one issue of the experimental plan was the lack of comparison with other strategies. Incremental step feeding or period feed experiments can also be investigated at the same time to benchmark which method may result in greater functional stability.

The result of this study would also be comprehensive if complemented with the molecular analysis to investigate the dynamics of microbial populations that determine the stability of function from anaerobic digestion system.

CHAPTER 6 FUTURE WORKS

This study provides some insights toward creating more robust and functionally stable anaerobic co-digestion systems treating lipid-rich wastes. However, more in-depth research and further investigation on several issues are needed to prepare for future encounter. Future work on the effort to induce functional stability of an anaerobic co-digestion system should consider:

- Microbial community analysis and the population dynamics.
- The application of other training or acclimation methods simultaneously to compare the response of treated anaerobic systems to perturbation
- Identification and characterization of FOG layer and food particles from GIW in correlation with inhibition mechanisms.
- The use of multiple and identical reactors to ensure replicable data
- Prediction of transient amplification in a perturbed environment using ecological projection model

REFERENCES

Allison, Steven D. and Jennifer B.H. Martiny. (2008). "Resistance, Resilience, and Redundancy in Microbial Communities." *PNAS*, 105(1): 11512-11519.

Angelidaki, I., A.H. Sorensen, and J.E. Schmidt. (2007). "Notes for the Course 12133: Environmental Biotechnology." Technical University of Denmark, Lyngby, Denmark.

Aziz, T. N., L.M. Holt, K.M. Keener, J.W. Groninger, and J.J. Ducoste. (2011). "Performance of Grease Abatement Devices for Removal of Fat, Oil, and Grease." *Journal of Environmental Engineering (ASCE)*, 137(1): 84-92.

Battimelli, A., M. Torrijos, R. Moletta, and J.P. Delgenes. (2010). "Slaughterhouse Fatty Waste Saponification to Increase Biogas Yield." *Bioresource Technology*, 101(10): 3388-3393.

Botton, S., M. Van Heusden, J. R. Parsons, H. Smidt, and H. Van Straalen. (2006). "Resilience of Microbial Systems towards Disturbances." *Critical Reviews in Microbiology* 32: 101-112.

Bougrier, C., C. Albasi, and J.P. Delgenès. (2006). "Effect of Ultrasonic, Thermal and Ozone Pre-treatments on Waste Activated Sludge Solubilisation and Anaerobic Biodegradability." *Chemical Engineering and Processing*, 45: 711-718.

Braun, Rudolf, and Arthur Wellinger. (2003). "Potential of Co-digestion." *IEA Bioenergy: Task 37 – Energy from Biogas and Landfill Gas*. <http://www.IEA-Bioenergy.net>.

Briones, Aurelio, and Lutgarde N. Raskin. (2003). "Diversity and Dynamics of Microbial Communities in Engineered Environments and Their Implications for Process Stability." *Current Opinion in Biotechnology* 14(3): 270-276.

Broughton, M.J., J.H. Thiele, E.J. Birch, and A. Cohen. (1998). "Anaerobic Batch Digestion of Sheep Tallow." *Water Research*, 32: 1423-1428.

Callaghan, F.J., D.A.J. Wase, K. Thayanithy, and C.F. Forster. (2002). "Continuous Co-digestion of Cattle Slurry with Fruit and Vegetable Wastes and Chicken Manure." *Biomass and Bioenergy*, 27: 71-77.

Carballa, M., M. Smits, C. Etchebehere, N. Boon, W. Verstraete. (2011). "Correlations Between Molecular and Operational Parameters in Continuous Lab-scale Anaerobic Reactors." *Applied Microbiology and Biotechnology*, 89: 303-314.

Chen, Y., J.J. Cheng, and K.S. Creamer. (2008). "Inhibition of Anaerobic Digestion Process: A Review." *Bioresource Technology*, 99: 4044-4064.

Chipasa, K.B., and K. Medrzycka. (2006). "Behavior of Lipids in Biological Wastewater Treatment Processes." *Journal of Industrial Microbiology and* Biotechnology, 33: 635–645.

Cirne, D.G., X. Paloumet, L. Björnsson, M.M. Alves, and B. Mattiasson. (2007). "Anaerobic Digestion of Lipid-rich Waste – Effects of Lipid Concentration." *Renewable Energy*, 32: 965–975.

Cohen, A., B. Distel, A. van Deursen, J.G. van Andel. (1981). "Role of Anaerobic Spore Forming Bacteria in the Acidogenesis of Glucose: Changes Induced by Discontinuous or Low-rate Feed Supply." *Water Resource*, 15: 59-68.

Cuppens, A., I. Smets, and G. Wyseure. (2012). "Definition of Realistic Disturbances as a Crucial Step during the Assessment of Resilience of Natural Wastewater Treatment Systems." *Water Science & Technology*, 65(8): 1506-1513.

Dague, R.R., R.E. McKinney, and J.T. Pfeffer. (1970). "Solids Retention in Anaerobic Waste Treatment Systems." (*Journal) Water Pollution Control Federation*, 42(2): R29-R46.

Davidsson, A., C. Lovstedt, J.I.C. Jansen, C. Gruvberger, and H. Aspegren. (2008). "Co-digestion of Grease Trap Sludge and Sewage Sludge." *Waste Management*, 28(6): 986-992.

De los Reyes III, F. L. and Xia He. (2009). "Effects of Biological Drain Products on Grease Interceptors: Microbiological and Chemical Characterization." *Final report submitted to the Consumer Specialty Products Association (CSPA).*

De Vrieze, Jo, Willy Verstraete, and Nico Boon. (2013). "Repeated Pulse Feeding Induces Functional Stability in Anaerobic Digestion." *Microbial Biotechnology* 6(4): 414-424.

Dearman, B., P. Marschner, and R.H. Bentham. (2006). "Methane Production and Microbial Community Structure in Single-stage Batch and Sequential Batch Systems Anaerobically Co-digesting Food Waste and Biosolids." *Applied Microbiology and Biotechnology*, 69: 589-596.

Delbés, Celine, René Moletta, and Jean-Jacques Godon. (2000). "Monitoring of Activity Dynamics of an Anaerobic Digester Bacterial Community Using 16S rRNA Polymerase Chain Reaction-Single-Strand Conformation Polymorphism Analysis." *Environmental Microbiology*, 2(5): 506-515.

Dettmer, K. (2002). "A Discussion of the Effects of Thermal Remediation Treatments on Microbial Degradation Processes." *US EPA Office of Solid Waste and Emergency Response*, Technology Innovative Office. <http://www.clu-in.org/download/remed/dettmer.pdf>

Einola, J.K.M, S.A. Luostarionen, E.A. Salminen, and J.A. Rintala. 2001. "Screening for an Optimal Combination of Municipal and Industrial Wastes and Sludges for Anaerobic Co-digestion." *In: Proceedings of 9th World Congress on Anaerobic Digestion, Vol.1*. Antwerpen, Belgium, 357-362.

Fernandez, A., S.Y. Huang, S. Seston, J. Xing, R. Hickey, C. Criddle, and J.M. Tiedje. (1999). "How Stable is Stable? Function Versus Community Composition." *Applied Environmental Microbiology*, 65: 3697–3704.

Fernandez, Ana S., S.A. Hashsham, S.L. Dollhopf, L. Raskin, O. Glagoleva, F.B. Dazzo, R.F. Hickey, C.S. Criddle, and J.M. Tiedje. (2000). "Flexible Community Structure Correlates with Stable Community Function in Methanogenic Bioreactor Communities Perturbed by Glucose." *Applied and Environmental Microbiology*, 66(9): 4058-4067.

Gallimore, E., T.N. Aziz, Z. Movahed, and J.J. Ducoste. (2011). "Assessment of Internal and External Grease Interceptor Performance for Removal of Food-based Fats, Oil, and Grease from Food Service Establishments." *Water Environment Research*, 83(9), 882-892.

Gerardi, Michael H. (2003). *The Microbiology of Anaerobic Digesters.* New Jersey: John Wiley & Sons, Inc.

Grimm, Volker, Eric Schmidt, and Christian Wissel. (1992). "On the Application of Stability Concepts in Ecology." *Ecological Modeling*, 63: 143-161.

Gujer, W., and A. J. B Zehnder. (1983). "Conversion Processes in Anaerobic Digestion." *Water Science & Technology*, 15(8-9): 127-167.

Gupta, A., J.R.V. Flora, M. Gupta, G.D. Sayles, and M.T. Suidan. (1994). "Methanogenesis and Sulfate Reduction in Chemostats. 1. Kinetic Studies and Experiments." *Water Resource*, 28: 781-793.

Hanaki, K., T. Matsuo, and M. Nagase. (1981). "Mechanism of Inhibition Caused by Long Chain Fatty Acids in Anaerobic Digestion Process." *Biotechnology and Bioengineering*, 23: 1591–1610.

Hashimoto, A.G., Y.R. Chen, and V.H. Varel. (1981). "Theoretical Aspects of Methane Production: State-of-the-Art". *Livestock Waste: A Renewable Resource, Proceedings of the Fourth International Symposium on Livestock Wastes-1980*, 86-91 and 95.

Hashsham, Syed A., A.S. Fernandez, S.L. Dollhopf, F.B. Dazzo, R.F. Hickey, J.M. Tiedje, and C.S. Criddle. (2000). "Parallel Processing of Substrate Correlates with Greater Functional Stability in Methanogenic Bioreactor Communities Perturbed by Glucose." *Applied and Environmental Microbiology*, 66(9): 4050-4057.

He, X., M. Iasmin, L.O. Dean, S.E. Lappi, J.J. Ducoste, and F.L. de los Reyes III. (2011). "Evidence for Fat, Oil, and Grease (FOG) Deposit Formation Mechanisms in Sewer Lines." *Environmental Science Technology*, 45(10): 4385-4391.

Heukelekian, H., and P. Mueller. (1958). "Transformations of Some Lipids in Anaerobic Sludge Digestion." *Sewage Industrial Wastes*, 30: 1108-1120.

Hickey, R.F., and M.S. Switzenbaum. (1991). "The Response and Utility of Hydrogen and Carbon Monoxide as Process Indicators of Anaerobic Digesters Subject to Organic and Hydraulic Overloads." *Journal of Water Pollution Control Federation*, 63: 129-140.

Hogan Plumbing Company. *Grease Interceptor Installation*. Accessed June 2013. <http://www.hoganplumbingco.com/grease-interceptor-installation-grease-trap-laguna-niguel>

Holling, C. S. (1973). "Resilience and Stability of Ecological Systems." *Annual Review of Ecology and Systematics*, 7: 1-23.

Holling, C.S. (1986). "Resilience of Ecosystems: Local Surprise and Global Change." *Sustainable Development of the Biosphere*, Cambridge: Cambridge University Press, 292-317.

Holling, C.S. (1992). "Cross-scale Morphology, Geometry and Dynamics of Ecosystems." *Ecological Monograph*, 62(44): 447-502.

Holling, C. S. (2000). "Engineering Resilience Versus Ecological Resilience." *Engineering within Ecological Constraints*, National Academy of Sciences, 31-43.

Holling, C.S., D.W. Schindler, B. Walker, and J. Roughgarden. (1995). "Biodiversity in the Functioning of Ecosystems: An Ecological Primer and Synthesis." *Biodiversity Loss: Ecological and Economic Issues*, Cambridge: Cambridge University Press.

Holm-Nielsen, J.B., T. Al Seadi, and P. Oleskowicz-Popiel. (2009). "The Future of Anaerobic Digestion and Biogas Utilization." *Bioresource Technology*, 100: 5478–5484.

Johnson, T., T. Shea, D. Gabel, and B. Forbes. (2011). "Introducing FOG to Solids." *Water Environment and Technology magazine*, April 2011.

Kabouris, J.C., U. Tezel, S.G. Pavlostathis, M. Engelmann, J. Dulaney, R.A. Gillette, and A.C. Todd. (2007). "The Ultimate Anaerobic Biodegradability of Municipal Sludge and FOG." *Proceedings of the Water Environment Federation*, WEFTEC 2007, 6776-6792.

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

Kabouris, J.C., U. Tezel, S.G. Pavlostathis, M. Engelmann, J. Dulaney, A.C. Todd, and R.A. Gillette. (2009). "Mesophilic and Thermophilic Anaerobic Digestion of Municipal Sludge and Fats, Oil, and Grease." *Water Environment Research*, 81(5): 476-485.

Karim, Khursheed, R. Hoffmann, K.T. Klasson, and M. H. Al-Dahhan. (2005). "Anaerobic Digestion of Animal Waste: Effect of Mode of Mixing." *Water Research*, 39: 3597-3606.

Krylova, N.I., R.E. Khabiboulline, R.P. Naumova, and M.A. Nagel. (1997). "The Influence of Ammonium and Methods for Removal during the Anaerobic Treatment of Poultry Manure." *Journal of Chemical Technology & Biotechnology*, 70: 99-105.

Kugelman, I.J., and P.L. McCarty. (1965). "Cation Toxicity and Stimulation in Anaerobic Waste Treatment." *Journal of Water Pollution Control Federation*, 37: 97-116.

Kugelman, I.J., and K.K. Chin. (1971). "Toxicity, Synergism, and Antagonism in Anaerobic Waste Treatment Processes." *Anaerobic Biological Treatment Processes, American Chemical Society Advances in Chemistry Series*, 105: 55-90.

Lehtomäki, A., S. Huttunen, and J.A. Rintala. (2007). "Laboratory Investigations on Co-digestion of Energy Crops and Crop Residues with Cow Manure for Methane Production: Effect of Crop to Manure Ratio." *Resources, Conservation & Recycling*, 51: 591-609.

Lesikar, B., O. Garza, R. Persyn, A. Kenimer, and M. Anderson. (2006). "Food Service Establishment Wastewater Characterization." *Water Environment Research*, 78(8): 805-809.

Li, Y.Y., H. Sasaki, K. Yamashita, K. Seki, and I. Yamigochi. (2002). "High-rate Methane Fermentation of Lipid-rich Food Wastes by a High-solids Co-digestion Process." *Water Science and Technology*, 45(12): 143-150.

Li, R., L. Chen, X. Li., J.S. Lar, Y. He, and B. Zhu. (2009). "Anaerobic Co-digestion of Kitchen Waste with Cattle Manure for Biogas Production." *Energy & Fuels*, 23: 2225-2228.

Long, J. H., T.N. Aziz, F.L. de los Reyes III, and J.J. Ducoste. (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.

Luostarinen, S., S. Luste, and M. Sillanpaa. (2009). "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(1): 79-85.

Macias-Corral, M., Z. Samani, A. Hanson, G. Smith, P. Funk, H. Yu, and J. Longworth. (2008). "Anaerobic Digestion of Municipal Solid Waste and Agricultural Waste and the Effect of Co-digestion with Dairy Cow Manure." *Bioresource Technology*, 99: 8288-8293.

Mackie, R.I., B.A. White, and M.P. Bryant. (1991). "Lipid Metabolism in Anaerobic Ecosystems." *Critical Reviews in Microbiology*, 17: 449-497.

Madigan, M.T., J.M. Martinko, P.V. Dunlap, and D.P. Clark. (2008). *Brock Biology of Microorganisms*. Benjamin Cummings. 12th edition.

Margalef, Ramón. (1968). *Perspective in Ecological Theory*. Chicago: University of Chicago Press.

Mata-Alvarez, J., S. Mace, and P. Llabres. (2000). "Anaerobic Digestion of Organic Solid wastes. An Overview of Research Achievements and Perspectives." *Bioresource Technology*, 74: 3-16.

McCann, K.S. (2000). "The Diversity-Stability Debate." *Nature*, 405: 228-233.

McCarty, P.L. (1964). "Anaerobic Waste Treatment Fundamentals, Part Two: Environmental Requirement and Controls." *Public Works*, 95(10): 123-126.

McCarty, P.L., and R.E. McKinney. (1961). "Salt Toxicity in Anaerobic Digestion." *Journal of Water Pollution Control Federation*, 33: 399-415.

McCarty, P. L., and D.P. Smith. (1986). "Anaerobic Wastewater Treatment: Fourth of a Six-part Series on Wastewater Treatment Processes." *Environmental Science and Technology*, 20(12).

McMahon K, D. Zheng, A. Stams, R. Mackie, L. Raskin. (2004). "Microbial Population Dynamics during Start-up and Overload Conditions of Anaerobic Digesters Treating Municipal Solid Waste and Sewage Sludge. *Biotechnology and Bioengineering*, 87: 823-834.

Mendelsohn, R., and K. Sweeny. (1994). "Biomass in the Energy Cycle Study." *Energy Research and Development Corporation*, Canberra, Australia.

Mouneimne, A.H., H. Carrère, J.P. Bernet, J.P. Delgenès. (2004). "Effect of the Addition of Bentonite on the Anaerobic Biodegradability of Solid Fatty Wastes." *Environmental Technology*, 25(4): 459-469.

Neubert, M.G., and H. Caswell. (1997). "Alternatives to Resilience for Measuring the Responses of Ecological Systems to Perturbation." *Ecology*, 78: 653-665.

Noutsopoulos, C., D. Mamais, K. Antoniou, C. Avramides, P. Oikonomopoulos, and I. Fountoulakis. (2013). "Anaerobic Co-digestion of Grease Sludge and Sewage Sludge: the Effect of Organic Loading and Grease Sludge Content." *Bioresource Technology*, 131: 452-459.

Novak, J.T., and D.A. Carlson. (1970). "The Kinetics of Anaerobic Long Chain Fatty Acid Degradation." *Journal of Water Pollution Control Federation*, 42: 1932-1943.

Occiano, V., A. Pai, I. Hireish, R. Sacro, M. Smoczynski. (2008). "Do Grease Control Products Really Work?" *WE&T*, 74-82. <http://www.wef.org/magazine>

Palatsi, J., M. Laureni, M.V. Alves, X. Flotats, H.B. Nielsen, and I. Angelidaki. (2009). "Strategies for Recovering Inhibition Caused by Long Chain Fatty Acids on Anaerobic Thermophilic Biogas Reactors." *Bioresource Technology*, 100: 4588–4596.

Petruy, R., and G. Lettinga. (1997). "Digestion of a Milk-Fat Emulsion." *Bioresource Technology*, 61(2):141-149.

Pickett, S.T.A., and P.S. White (Editors). (1984). *Natural Disturbance: The Patch Dynamics Perspective*, Academic Press: New York.

Poliafico, M. (2007). "Anaerobic Digestion: Decision Support Software." *Master's Thesis, Department of Civil, Structural and Environmental Engineering*, Cork Institute of Technology: Cork, Ireland.

Quemeneur, M., and Y. Marty. (1994). "Fatty Acids and Sterols in Domestic Wastewater." *Water Research*, 28(5): 1217–1226.

Remmert, H., (1988). "Gleichgewicht durch Katastrophen: Stimmen Unsere Vorstellungen von Harmonie und Gleichgewicht in der Ökologie noch?" *Aus Forschung Med. (Berlin)*, 3: 7-17.

Rico, C., J.L. Rico, N. Muñoz, B. Goméz, and I. Tejero. (2011). "Effect of Mixing on Biogas Production during Mesophilic Anaerobic Digestion of Screened Dairy Manure in a Pilot Plant." *Engineering in Life Sciences*, 11(5): 476-481.

Rinzema, A., A. Alphenaar, and G. Lettinga. (1993). "Anaerobic Digestion of Long Chain Fatty Acids in UASB and Expanded Granular Sludge Bed Reactors." *Process Biochemistry*, 28(8): 527-537.

Riviere, D., V. Desvignes, E. Pelletier, S. Chaussonnerie, S. Guermazi, J. Weissanbach, *et al*. (2009). "Towards the Definition of a Core of Microorganisms Involved in Anaerobic Digestion of Sludge." *ISME Journal*, 3: 700-714.

Salminen, E., and J. Rintala. (2002). "Anaerobic Digestion of Poultry Slaughtering Wastes." *Environmental Technology*, 20(1): 21-28.

Smith, P.D., and P.L. McCarty. (1989). "Energetics and Rate Effects on Methanogenesis of Ethanol and Propionate in Perturbed CSTRs." *Biotechnology and Bioengineering*, 34: 39-54

Sousa, W.P., (1981). "The Role of Disturbance in Natural Communities." *Annual Review of Ecology, Evolution, and Systematics*, 15: 353-391.

Speece, R.E. (1996). *Anaerobic Biotechnology for Industrial Wastewaters*. Archie Press: Nashville, USA.

Tilman, D. (1999). "The Ecological Consequences of Changes in Biodiversity: A Search for General Principles." *Ecology*, 80 (5): 1455-1474.

Tilman, D., and J.A. Downing. (1994). "Biodiversity and Stability in Grasslands." *Nature*, 367: 363–365.

US EPA. (2008). *Clean Watersheds Needs Survey (CWNS) 2008 Report to Congress*. <http://www.water.epa.gov/scitech/datait/databases/cwns/upload/cwns2008rtc.pdf>

US EPA. (2011). "US Anaerobic Digester Status: A 2011 Snapshot." *AgSTAR*. <http://www.epa.gov/agstar/documents/2011_digester_update.pdf>

Van Lier, J.B., A. Tilche, B. K. Ahring, H. Macarie, R. Moletta, M. B. Dohanyos, L. W. Hulshoff Pol, P Lens, and W. Verstraete. (2001). "New Perspective in Anaerobic Digestion." *Water Science and Technology*, 43(1): 1-18.

Walter, G.G. (1980). "Stability and Structure of Compartmental Models of Ecosystems." *Mathematical Biosciences*, 51: 1–10.

Wan, C., Q. Zhou, G. Fu, and Y. Li. (2011). "Semi-continuous Anaerobic Co-digestion of Thickened Waste Activated Sludge and Fat, Oil and Grease." *Waste Management*, 31(8): 1752-1758.

Wang, Ling, T.N. Aziz, and F.L. de Los Reyes III. (2013). "Determining the Limits of Anaerobic Co-digestion of Thickened Waste Activated Sludge with Grease Interceptor Waste." *Water Research*, 47(11): 3835-3844.

Xing, J., C. Criddle, and R. Hickey. (1997). "Long-term Adaptive Shifts in Anaerobic Community Structure in Response to a Sustained Cyclic Substrate Perturbation." *Microbial Ecology*, 33: 50-58.

Zeeman, G. (2005). *Lecture Notes: Water Treatment: Anaerobic Wastewater Treatment. Subdepartment of Environmental Technology*. Wageningen University.

Zeikus, J.G., (1977). "The Biology of Methanogenic Bacteria." *Bacteriology Reviews*, 41: 514-541.

APPENDIX

Appendix A Raw Data of Performance Parameters

A.1 Biogas Production, Methane Content, and Methane Yield

Days	Date	T(K)	P (in.)	Corrected Biogas (L/d)		Methane Content (%)		Methane Yield ($L\text{-}CH_4/g$ VS added)	
				A	B	A	B	A	B
2	**9/24/12**	311	29.71	1.3	0.8	52.7	55.4	0.083429	0.052615
3	**9/25**	311	29.71	2.4	1.5	55.3	60.3	0.163992	0.114516
4	**9/26**	311	29.73	1.5	0.9	54.0	62.4	0.098724	0.067097
9	**10/1**	311	29.43	2.4	2.4	50.9	56.7	0.153529	0.171028
10	**10/2**	311	29.38	1.6	1.8	49.9	56.1	0.098084	0.124908
11	**10/3**	311	29.61	3.1	2.7	50.3	54.5	0.197436	0.182242
12	**10/4**	311	29.70	1.7	1.6	49.8	55.0	0.104745	0.109988
13	**10/5**	311	29.57	2.7	2.7	50.5	54.2	0.173159	0.186087
14	**10/6**	311	29.43	1.6	1.6	49.7	54.8	0.100393	0.110691
15	**10/7**	311	29.54	2.6	2.6	50.0	54.3	0.163035	0.177033
16	**10/8**	311	29.60	1.4	1.4	52.5	54.8	0.095038	0.09915
17	**10/9**	311	29.62	2.5	2.5	55.8	57.9	0.175121	0.181853
20	**10/12**	311	29.77	1.6	1.6	54.5	56.7	0.109583	0.114063
21	**10/13**	311	29.88	2.1	2.1	53.8	55.9	0.14159	0.146988
22	**10/14**	311	29.63	1.3	1.3	53.7	56.4	0.084782	0.088925
23	**10/15**	311	29.34	2.0	2.3	54.8	57.3	0.136864	0.168224
24	**10/16**	311	29.44	1.3	1.3	54.7	57.5	0.0911	0.095684
25	**10/17**	311	29.50	2.1	2.2	53.2	55.7	0.138629	0.157256
26	**10/18**	311	29.33	1.5	1.5	54.0	57.0	0.098873	0.104372
27	**10/19**	311	29.25	2.6	2.6	55.6	57.8	0.178316	0.191943
28	**10/20**	311	29.37	1.4	1.5	55.4	58.2	0.094745	0.10664
29	**10/21**	311	29.63	2.5	2.5	50.6	56.6	0.161814	0.180875
30	**10/22**	311	29.68	1.3	1.2	52.6	56.9	0.087254	0.087648
31	**10/23**	311	29.65	2.2	2.2	53.7	56.6	0.146796	0.15467
32	**10/24**	311	29.63	1.3	1.2	54.0	57.1	0.087627	0.084926
33	**10/25**	311	29.64	2.4	2.4	53.3	55.4	0.163642	0.16989
34	**10/26**	311	29.52	1.4	1.5	56.2	58.9	0.097503	0.109052
35	**10/27**	311	29.23	2.5	2.6	55.1	57.2	0.170127	0.187119
36	**10/28**	311	29.13	1.3	1.4	55.8	58.6	0.09439	0.106741
37	**10/29**	311	28.88	2.7	2.9	55.9	57.5	0.186624	0.206043
38	**10/30**	311	29.03	1.5	1.5	55.3	57.7	0.104828	0.109263
39	**10/31**	311	29.21	2.8	2.9	55.4	56.1	0.196033	0.206044
44	**11/5**	311	29.57	1.1	1.3	58.8	67.3	0.081253	0.109847
45	**11/6**	311	29.54	2.7	2.5	59.1	65.4	0.200792	0.205738
46	**11/7**	311	29.40	1.4	1.4	58.2	64.8	0.099327	0.110433

Days	Date	T(K)	P (in.)	Corrected Biogas (L/d)		Methane Content (%)		Methane Yield ($L\text{-}CH_4/g$ VS added)	
				A	B	A	B	A	B
47	**11/8**	311	29.54	2.5	2.7	58.3	62.1	0.184762	0.213961
48	**11/9**	311	29.79	2.0	2.0	58.1	62.1	0.14354	0.153427
49	**11/10**	311	29.82	3.0	3.0	58.0	59.4	0.217446	0.222645
53	**11/14**	311	29.94	3.7	3.8	53.8	54.7	0.202703	0.211517
54	**11/15**	311	29.79	4.3	4.1	57.5	58.1	0.252806	0.241859
55	**11/16**	311	29.83	5.3	5.1	56.0	55.9	0.302182	0.290973
56	**11/17**	311	29.97	4.5	4.8	62.2	62.9	0.282911	0.309227
57	**11/18**	311	29.90	5.9	5.8	57.9	58.7	0.349724	0.349007
58	**11/19**	311	29.76	5.0	5.5	63.2	63.8	0.319066	0.354588
59	**11/20**	311	29.61	3.6	7.2	62.7	59.6	0.227122	0.438273
60	**11/21**	311	29.65	6.6	5.8	61.5	64.8	0.410586	0.38004
66	**11/27**	311	29.70	8.8	7.1	68.1	68.9	0.607093	0.496893
67	**11/28**	311	29.85	9.6	9.2	63.3	63.9	0.616004	0.597329
68	**11/29**	311	29.91	9.7	4.4	68.5	65.3	0.673975	0.290561
69	**11/30**	311	29.84	10.1	9.4	64.6	62.8	0.666857	0.599911
70	**12/1**	311	29.91	5.2	4.3	66.4	66.2	0.349394	0.289163
71	**12/2**	311	29.80	8.3	8.8	63.3	63.9	0.53155	0.57347
72	**12/3**	311	29.80	3.5	3.9	65.7	66.1	0.235613	0.262207
73	**12/4**	311	29.73	8.1	7.9	64.1	64.9	0.525959	0.519868
74	**12/5**	311	29.70	3.7	4.3	64.5	65.5	0.244128	0.286512
75	**12/6**	311	29.77	8.7	8.9	62.3	63.1	0.550201	0.569929
76	**12/7**	311	29.57	4.0	4.2	63.1	64.5	0.257212	0.274312
77	**12/8**	311	29.54	8.8	9.3	61.6	62.4	0.552377	0.591731
78	**12/9**	311	29.57	4.0	4.2	62.6	64.2	0.257033	0.274012
79	**12/10**	311	29.25	7.7	8.0	60.0	61.1	0.470369	0.49743
80	**12/11**	311	29.60	3.4	3.5	60.8	62.2	0.211364	0.222209
81	**12/12**	311	29.83	7.9	8.3	59.9	60.9	0.483284	0.515381
82	**12/13**	311	29.85	3.6	3.6	61.7	62.9	0.223685	0.227888
83	**12/14**	311	29.77	8.5	8.9	60.4	61.1	0.52303	0.553788
84	**12/15**	311	29.66	3.6	3.9	64.4	65.8	0.238862	0.260054
85	**12/16**	311	29.46	8.6	9.2	62.5	63.2	0.546494	0.593541
86	**12/17**	311	29.21	3.6	4.3	63.7	65.3	0.233342	0.284505
87	**12/18**	311	29.38	8.7	8.5	64.8	65.8	0.572449	0.568767
88	**12/19**	311	29.55	4.1	4.0	65.9	67.5	0.273806	0.273587
89	**12/20**	311	29.05	7.9	7.6	65.1	66.0	0.520852	0.507082
90	**12/21**	311	29.30	3.5	4.5	63.7	64.8	0.22568	0.299889
91	**12/22**	311	29.61	9.1	9.5	63.4	64.1	0.585572	0.616548
92	**12/23**	311	29.56	4.2	4.3	64.6	66.0	0.275654	0.288635

Days	Date	T(K)	P (in.)	Corrected Biogas (L/d)		Methane Content (%)		Methane Yield (L-CH_4/g VS added)	
				A	B	A	B	A	B
93	**12/24**	311	29.44	8.4	8.7	61.9	63.0	0.526822	0.559683
94	**12/25**	311	29.60	3.9	3.9	62.5	64.2	0.250672	0.257561
95	**12/26**	311	29.00	12.0	8.8	58.6	62.1	0.521944	0.55341
96	**12/27**	311	29.50	10.4	3.8	65.7	62.9	0.505189	0.245726
97	**12/28**	311	29.59	11.4	8.6	64.1	62.5	0.743478	0.549915
98	**12/29**	311	29.35	5.5	4.0	64.8	63.8	0.364838	0.258012
99	**12/30**	311	29.85	10.3	8.4	62.5	61.8	0.657755	0.529803
100	**12/31**	311	29.88	5.3	4.9	63.2	63.7	0.342028	0.314826
102	**1/2/13**	311	29.71	3.4	3.5	64.2	66.7	0.219737	0.234316
103	**1/3**	311	29.79	13.2	8.5	59.6	64.3	0.585693	0.555328
104	**1/4**	311	29.82	10.7	3.5	68.0	66.0	0.539275	0.236777
105	**1/5**	311	29.82	11.0	8.6	64.1	61.8	0.719369	0.543416
106	**1/6**	311	29.68	6.1	4.6	64.7	64.6	0.402441	0.304067
107	**1/7**	311	29.96	9.5	8.6	63.2	62.7	0.608715	0.546005
108	**1/8**	311	29.93	4.8	4.6	66.2	67.5	0.321574	0.313667
109	**1/9**	311	29.91	9.4	8.9	63.4	63.7	0.607458	0.579506
110	**1/10**	311	29.96	4.1	4.3	65.6	67.2	0.2766	0.294798
111	**1/11**	311	29.71	11.9	8.7	58.0	63.6	0.511268	0.565605
112	**1/12**	311	29.65	11.5	5.5	67.5	66.8	0.577416	0.374824
113	**1/13**	311	29.62	11.5	9.4	65.4	63.9	0.76439	0.611759
114	**1/14**	311	29.64	6.4	4.4	67.2	67.8	0.439888	0.305658
115	**1/15**	311	29.70	10.2	8.6	64.0	63.2	0.666838	0.554492
116	**1/16**	311	29.52	4.6	4.6	63.4	65.1	0.296693	0.304893
117	**1/17**	311	29.61	9.6	8.3	63.0	63.3	0.617877	0.535395
120	**1/20**	311	29.57	3.4	4.4	63.1	65.9	0.218182	0.295134
121	**1/21**	311	29.44	14.0	9.5	62.3	63.8	0.645576	0.619711
122	**1/22**	311	29.76	10.0	4.8	68.2	66.6	0.509209	0.324904
123	**1/23**	311	29.73	10.7	8.3	65.5	64.6	0.714939	0.542157
124	**1/24**	311	30.02	6.1	4.3	65.9	65.9	0.411219	0.289032
125	**1/25**	311	29.70	11.3	8.4	65.1	63.8	0.750193	0.545942
126	**1/26**	311	29.93	6.4	4.5	65.8	64.6	0.431592	0.294131
127	**1/27**	311	30.06	10.3	9.6	63.4	62.7	0.664391	0.613284
128	**1/28**	311	29.81	4.8	4.6	64.7	64.8	0.314382	0.301024
129	**1/29**	311	29.61	16.3	12.6	61.1	56.8	0.564444	0.403601
130	**1/30**	311	29.10	14.3	12.8	67.8	65.9	0.547087	0.476354
131	**1/31**	311	29.97	20.2	15.8	64.3	61.9	0.735506	0.554006
132	**2/1**	311	29.88	15.0	14.7	69.9	70.1	0.593053	0.583918
133	**2/2**	311	29.57	22.9	19.3	67.0	65.2	0.869547	0.71106
134	**2/3**	311	29.49	17.0	17.4	71.0	71.0	0.683457	0.699834

Days	Date	T(K)	P (in.)	Corrected Biogas (L/d)		Methane Content (%)		Methane Yield ($L\text{-}CH_4/g$ VS added)	
				A	B	A	B	A	B
135	**2/4**	311	29.55	24.0	22.0	68.5	67.2	0.930451	0.836349
136	**2/5**	311	29.50	14.8	15.9	71.7	71.7	0.599068	0.646826
137	**2/6**	311	29.77	24.0	22.7	63.2	69.0	0.857777	0.885668
138	**2/7**	311	29.84	22.2	16.6	70.3	70.6	0.884123	0.661344
139	**2/8**	311	29.44	18.1	16.1	56.4	53.2	0.576767	0.485752
140	**2/9**	311	29.60	9.1	12.1	56.5	55.3	0.191326	0.249352
141	**2/10**	311	29.90	5.0	6.8	50.8	50.4	0.095183	0.127788
142	**2/11**	311	29.69	6.7	5.5	36.0	40.4	0.090111	0.083302
143	**2/12**	311	29.53	2.6	1.7	44.9	42.1	0.043715	0.027326
144	**2/13**	311	29.43	1.5	0.9	45.8	42.0	0.069861	0.04006
145	**2/14**	311	29.54	1.6	0.9	45.8	41.5	0.074273	0.03958
146	**2/14**	311	29.47	1.1	0.8	47.9	43.1	0.052791	0.036563
147	**2/16**	311	29.39	1.1	1.0	46.2	42.5	0.05273	0.044752
148	**2/17**	311	29.63	0.9	0.9	46.5	42.3	0.041525	0.037795
149	**2/18**	311	29.84	1.1	1.0	47.2	45.7	0.05209	0.045817
150	**2/19**	311	29.40	1.3	1.3	46.2	45.4	0.059501	0.058451
151	**2/20**	311	29.56	1.4	1.7	46.3	47.4	0.06444	0.080226
152	**2/21**	311	29.67	1.8	1.9	46.9	47.0	0.087117	0.091864
153	**2/22**	311	29.87	1.3	1.9	48.2	50.6	0.06491	0.09752
154	**2/23**	311	29.63	1.0	2.6	54.0	48.5	0.057176	0.130614
155	**2/24**	311	29.36	3.1	2.4	44.7	47.7	0.140839	0.118281
157	**2/26**	311	29.66	2.1	0.5	15.6	46.7	0.033652	0.02462
158	**2/27**	311	29.18	1.3	1.5	25.4	47.5	0.033819	0.072308
159	**2/28**	311	29.24	1.4	1.1	37.2	47.4	0.051336	0.05137
160	**3/1**	311	29.38	1.6	1.5	43.7	46.7	0.070238	0.070749
161	**3/2**	311	29.41	1.2	1.3	39.5	44.9	0.048006	0.05801
162	**3/3**	311	29.45	1.6	1.6	47.2	50.8	0.0764	0.082227
163	**3/4**	311	29.58	1.1	1.3	45.6	50.4	0.050309	0.068445
164	**3/5**	311	29.53	1.6	1.9	47.3	50.1	0.075425	0.094957
165	**3/6**	311	29.21	1.6	1.7	45.8	49.3	0.072429	0.087151
166	**3/7**	311	29.67	2.6	2.8	20.4	29.7	0.054408	0.084742
167	**3/8**	311	29.70	1.3	1.3	41.5	48.5	0.055219	0.064413
168	**3/9**	311	29.80	1.9	1.8	47.8	52.4	0.09005	0.093851

A.2 Volatile Solids (VS) Effluent and Reduction

Day	VS effluent (g/kg-wet sample)		VS reduction (%)		Day	VS effluent (g/kg-wet sample)		VS reduction (%)	
	A	B	A	B		A	B	A	B
2	20.550	19.912	22.54	24.95	**113**	17.648	17.923	50.44	45.29
4	20.963	20.895	20.99	21.24	**115**	17.980	18.182	49.51	44.50
6	20.874	20.552	21.32	22.54	**125**	16.877	18.919	52.60	42.25
8	20.448	20.059	22.93	24.40	**127**	15.830	17.019	55.54	48.05
10	21.279	19.872	19.80	25.10	**133**	23.140	24.261	60.73	58.83
12	20.408	20.741	23.08	21.82	**135**	27.826	28.082	52.78	52.34
14	20.000	20.210	24.62	23.83	**138**	35.367	32.058	39.98	45.60
16	19.960	20.018	24.77	24.55	**140**	28.916	27.957	67.71	68.78
20	19.866	19.880	25.12	25.07	**142**	25.177	26.097	71.88	70.85
24	19.900	19.693	24.99	25.77	**144**	26.756	25.591	18.33	21.89
28	19.555	19.643	26.30	25.96	**146**	25.739	25.724	21.43	21.48
32	21.255	19.756	19.89	25.54	**150**	23.415	24.577	28.53	24.98
36	20.151	19.956	24.05	24.78	**154**	27.455	27.931	16.20	14.74
39	19.938	18.883	24.85	28.83	**158**	26.356	26.153	19.55	20.17
43	20.351	19.623	23.30	26.04	**160**	26.496	23.287	19.12	28.92
47	19.258	18.880	27.41	28.84	**164**	23.030	25.854	29.70	21.08
51	18.900	18.745	28.76	29.35	**166**	25.486	26.016	22.21	20.59
53	19.378	18.532	40.85	43.43	**168**	23.639	24.858	27.84	24.12
55	19.041	18.745	41.88	42.78					
57	19.319	20.015	41.03	38.91					
59	18.889	19.224	42.34	41.32					
63	19.791	19.319	39.59	41.03					
67	19.169	18.794	41.49	42.63					
71	18.828	18.667	42.53	43.02					
75	18.759	17.739	42.74	43.42					
79	18.843	18.876	42.48	42.38					
83	18.856	18.694	42.44	42.94					
87	18.617	18.436	43.17	43.73					
91	18.412	18.282	43.80	44.20					
93	18.501	18.368	43.53	43.93					
95	18.639	18.337	47.66	44.03					
97	18.369	18.320	43.93	44.08					
99	18.428	18.620	43.75	43.16					
101	18.200	18.262	44.45	44.26					
103	18.162	18.215	48.99	44.40					
105	18.431	18.019	48.24	45.00					
107	17.653	17.808	50.43	45.64					
111	18.421	17.999	48.27	45.06					
113	17.648	17.923	50.44	45.29					
115	17.980	18.182	49.51	44.50					

A.3 Volatile Fatty Acids during Perturbation Tests and Recovery Phase

Day	Hours	Acetate (mg/L)		Propionate (mg/L)		Total Butyrate (mg/L)		Total Valerate (mg/L)	
		A	B	A	B	A	B	A	B
128.00	0	0	0	0	0	0	0	0	0
128.33	8	217	191	169.43	151.23	27.76	9.36	32.18	24.79
128.60	14.5	290	228.75	216.36	186.35	39.37	14.87	37.63	26.03
128.90	21.5	263	160.58	260.43	197.38	56.34	20.19	55.53	32.18
129.08	26	201	109.92	192.57	175.67	38.28	17.10	39.75	31.45
129.33	32	173	115.43	179.38	131.53	16.14	12.02	25.44	24.85
129.67	40	37	182.35	121.29	170.66	0	7.75	3.27	28.42
129.96	47	23	157.02	85.19	117.78	0	3.95	0	16.81
130.13	51	0	191.67	9.31	158.34	0	5.02	0	23.58
130.38	57	325	587.48	217.03	511.91	45.39	86.12	45.35	93.81
130.79	67	344	542.31	269.53	346.23	69.43	88.78	71.75	84.22
130.88	69	332	509.67	267.96	334.55	72.88	84.83	76.15	84.61
131.13	75	275	470.07	243.28	294.66	50.30	67.51	57.50	72.76
131.38	81	46	337.82	207.45	254.55	12.51	24.47	23.96	54.09
131.69	88.5	20	52.95	98.91	194.85	0	6.27	1.17	14.65
131.98	95.5	0	20.45	0	97.68	0	0	0	0
132.15	99.5	0	21.97	0	34.67	0	0	0	0
132.40	105.5	286	316.70	250.86	222.92	52.90	43.82	59.41	51.56
132.63	111	314	480.96	300.86	335.83	83.86	82.46	83.10	87.34
133.25	126	97	313.75	237.42	296.01	19.34	66.30	41.42	70.99
133.75	138	30	54.04	74.19	188.75	0	3.52	0.99	9.20
134.21	149	252	361.15	200.13	328.87	59.62	89.31	55.08	76.96
134.58	158	401	573.28	434.07	524.40	113.26	149.24	107.84	138.01
135.13	171	38	241.53	170.81	284.34	3.21	37.23	13.78	60.27
135.63	183	0	35.36	0	67.22	0	0	0	1.00
136.17	196	382	373.37	326.99	309.15	129.12	121.94	99.21	92.65
136.67	208	183	635.56	306.97	435.83	47.88	134.93	56.57	112.55
137.08	218	181	89.78	208.49	234.31	20.05	13.60	50.30	36.60
137.67	232	0	0	0	0	0	0	0	0
138.25	246	638	528.27	658.61	545.58	259.31	191.61	188.38	149.12
138.54	253	688	611.83	891.92	759.41	354.10	146.89	248.07	147.91
138.75	258	91	138.09	418.53	633.57	56.93	93.84	79.97	121.22
139.00	264	75	116.66	246.14	512.59	32.50	66.16	62.89	100.61
139.25	270	52	94.44	71.94	416.61	34.22	51.04	64.33	82.01
139.58	278	35	81.21	0	213.87	39.16	46.77	72.88	86.15
139.75	282	33	64.17	0	99.48	38.92	46.19	72.13	84.79
140.00	288	25	33.35	0	0	42.76	48.48	77.52	91.57
140.25	294	26	30.47	0	0	44.06	51.34	78.80	96.72
140.54	301	525	596.02	608.41	605.26	258.91	257.91	208.39	230.27
140.75	306	524	556.69	619.16	313.51	253.65	191.61	210.94	199.38

Day	Hours	Acetate (mg/L)		Propionate (mg/L)		Total Butyrate (mg/L)		Total Valerate (mg/L)	
		A	B	A	B	A	B	A	B
141.00	312	737	673.59	634.86	569.23	293.67	209.62	228.39	205.22
141.25	318	897	808.04	651.35	575.67	332.74	244.03	245.69	211.54
141.58	326	994	978.43	678.94	571.39	364.15	293.50	263.82	221.13
141.75	330	984	1003.45	700.02	566.87	370.58	303.41	270.91	221.84
142.00	336	944	1113.44	705.41	594.82	363.84	329.44	278.44	238.40
142.42	346	852	1172.90	721.69	596.73	381.07	357.99	300.85	250.48
142.79	355	896	1334.57	790.89	743.23	399.33	418.72	323.30	295.68
143.00	360	933	1344.86	812.56	724.28	408.24	406.98	332.20	290.94
143.25	366	1017	1407.65	872.66	750.30	430.74	403.82	326.44	266.10
143.58	374	994	1431.23	892.40	777.64	402.15	406.04	346.49	271.74
143.79	379	997	1499.53	927.12	824.48	389.23	416.77	341.55	283.81
144.00	384	966	1461.95	960.82	827.60	355.34	400.28	340.06	279.46
144.25	390	933	1443.22	962.02	836.05	353.05	406.54	341.11	283.81
144.58	398	1005	1437.23	1018.03	876.23	376.06	413.04	370.02	296.76
144.79	403	1112	1587.29	1052.95	956.99	390.55	447.51	374.38	324.71
145.17	412	1160	1633.01	1102.66	990.15	416.87	473.51	404.90	333.78
145.58	422	1146	1661.04	1117.54	1020.71	430.43	492.00	412.84	344.32
145.92	430	1102	1613.72	1127.34	1053.95	436.39	503.27	409.30	358.30
146.25	438	1082	1592.87	1142.94	1053.27	453.55	511.63	418.05	363.27
146.67	448	1202	1653.24	1214.36	1147.80	490.14	553.83	456.21	395.92
147.00	456	1209	1635.02	1242.97	1146.91	505.00	554.97	473.44	402.62
147.25	462	1172	1574.71	1240.00	1138.53	505.93	549.66	470.57	397.29
147.83	476	1062	1480.47	1260.15	1172.72	507.64	560.73	460.77	409.82
148.25	486	1036	1447.01	1287.39	1154.81	527.84	562.62	470.07	404.16
148.79	499	975	1244.03	1218.68	1128.43	493.02	528.30	443.86	390.29
149.25	510	958	1342.67	1206.86	1258.67	490.12	605.49	435.19	447.43
149.79	523	891	1154.26	1325.04	1255.75	537.94	605.08	483.36	448.53
150.25	534	856	1040.17	1376.57	1286.64	573.34	629.97	508.75	465.23
150.79	547	810	927.04	1383.75	1308.66	573.78	630	505.60	476.12
151.75	570	383	447.00	1391.75	1317.87	587.79	626.88	512.32	475.55
152.25	582	184	183.64	1189.72	1151.24	499.32	569.12	602.25	593.23
153.25	606	186	161.42	1153.02	1023.01	478.41	494.07	604.58	562.85
154.25	630	169	157.21	1178.32	1275.74	490.97	565.60	495.74	543.57
155.25	654	168	175.34	1228.93	1036.98	487.23	473.36	633.76	587.34
156.25	678	153	151.15	1324.60	1324.20	505.37	564.15	662.76	637.47
157.25	702	167	158.43	1190.47	1176.37	439.51	485.00	648.86	606.97
158.21	725	208	175.61	1428.68	1418.27	465.11	543.60	597.61	596.56
160.63	783	143	150.79	1496.7	1466.29	330.47	393.95	631.88	600.32
161.63	807	92	109.76	950.76	1179.72	263.03	299.59	591.52	594.40
162.75	834	91	97.97	1471.33	1467.41	286.92	311.74	695.37	680.58

Day	Hours	Acetate (mg/L)		Propionate (mg/L)		Total Butyrate (mg/L)		Total Valerate (mg/L)	
		A	**B**	**A**	**B**	**A**	**B**	**A**	**B**
163.71	857	86	89.38	1280.14	1174.97	241.60	252.20	618.92	595.11
165.71	905	142	127.92	1448.02	1368.56	235.96	249.02	620.23	571.76
166.79	931	139	126.91	1506.69	1556.62	98.75	142.48	528.98	479.56
167.81	955.5	86	144.97	1642.89	1606.98	68.26	197.14	537.66	530.67