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Investigating the Inhibitory Effects of Fats, Oils and Grease Addition on Fatty Acids Degradation During Anaerobic Co-Digestion

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INVESTIGATING THE INHIBITORY EFFECTS OF FATS, OILS AND GREASE ADDITION ON FATTY ACIDS DEGRADATION DURING ANAEROBIC CO-DIGESTION

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ABSTRACT

Most of the wastewater treatment plants are being upgraded to serve as water resource recovery facilities, reducing and in some cases eliminating, the dependence on the electricity grid for energy requirements remains one of the preeminent targets. Methane yields from conventional anaerobic digestion systems are not often sufficient to fulfil the requirements of these modern-day resource recovery facilities. Although there are numerous articles on co-digestion of municipal sludge with fats, oils and grease (FOG) waste reporting noticeable increase in biogas production, there are enough reports of inhibition and digester upset to warrant further study. Our present study is focused on characterizing formation and consumption of intermediates at various stages occurring in biochemical methane potential tests. This information is crucial in understanding the nature and the mechanism of inhibition. Preliminary studies were conducted to find a sludge to FOG ratio which enabled us to produce maximum possible biogas and use that ratio for setting up bench-scale digesters for further investigations. Among all the compositions tested, FOG 25 was the only reactor to overcome inhibition after lag phase of 45 days. We also found that FOG-acclimated culture improved the performance of the digester by increasing cumulative methane production by 29.9%, during batch operation. When operated in semi-continuous mode, methane production rate per g-VS added in FOG 25 was 7.5% greater than control and %COD conversion to methane increased by 30.34%. For samples with FOG content higher than 25%, methanogenesis was inhibited, with very little methane produced. These results suggest possible recovery of digesters inhibited after a certain lag phase and contradicts earlier hypothesis of LCFA inhibition to be irreversible. Results obtained from the VFA and LCFA analysis suggest that accumulation of palmitate and stearate at high concentrations can be inhibitory to the methanogens as well as the microbes degrading VFAs and LCFAs. Additionally, absence of fatty acids with carbon chains of $14>n(C)>6$ could be indicative of degradation mechanisms other than β-oxidation.

Keywords: Anaerobic co-digestion, fats, oils and grease (FOG), intermediates, methane, inhibition

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ABBREVIATIONS

APHA	American Public Health Association
AWWA	American Water Works Association
BMP	Biochemical Methane Potential
C/N	Carbon to Nitrogen Ratio
C/P	Carbon to Phosphorous Ratio
CRD	Capital Regional District
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DAF	Dissolved Air Floatation
DDI	Distilled De-ionized
DLVO	Double Layer Theory named after Derjaguin, Landau, Verwey & Overbeek
EPA	Environmental Protection Agency
FAME	Fatty Acid Methyl Ester
FOG	Fat, Oil and Grease
FSE	Food Service Establishment
FW	Fatty Wastewater
GC-TCD	Gas Chromatography with Thermal Conductivity Detector
GC-FID	Gas Chromatography with Flame Ionization Detector
GIW	Grease Interceptor Waste
GSB	Granular Sludge Bed
GTS	Grease Trap Sludge
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
LCFA	Long-Chain Fatty Acids
MIC	Minimal Inhibitory Concentration
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate

PS	Primary Sludge
PVDF	Polyvinylidene Difluoride
ReWa	Renewable Water Resources
sCOD	Soluble Chemical Oxygen Demand
S/I	Sludge to Inoculum Ratio
SRT	Solids Retention Time
SS	Sewage Sludge
tCOD	Total Chemical Oxygen Demand
TS	Total Solids
TWAS	Thickened Waste Activated Sludge
VFA	Volatile Fatty Acids
UASB	Upflow Anaerobic Sludge Blanket
VS	Volatile Solids
WEF	Water Environment Federation
WRRF	Water Resource Reclamation Facility

1. INTRODUCTION

It has long been established that biogas as a source of energy is far more important to the under-developed and the developing countries. However, over the past few decades anaerobic digestion has risen again in popularity in the developed countries as the water quality and waste disposal regulations around the world become more stringent. The Environmental Protection Agency (EPA) reports that there are over 1200 water resource recovery facilities (WRRF) in the United States that have installed an anaerobic digester apart from the 242 operating digesters on livestock farms, with the first ones dating back to the early 1900s (EPA, 2016). Although the primary application of anaerobic digesters in WWRFs is in stabilizing the biosolids generated during the biological treatment of domestic and industrial wastewater, in many cases, sufficient gas is produced to meet most of the energy demands of these plants. This has, in turn, encouraged more WWRFs to employ anaerobic digestion as a treatment technique (Tchobanoglous and Abu-Orf, 2014).

1.1 Anaerobic Co-digestion

Anaerobic digestion is a biochemical process of breakdown of complex insoluble organic matter with the help of a wide range of microbial communities in the absence of oxygen to form methane and carbon dioxide. Like majority of the biological processes, proper functioning of anaerobic digestion depends on several environmental factors such as temperature, pH, availability of nutrients, carbon to nitrogen (C/N) and carbon to phosphorous (C/P) ratios, presence of inhibitory and toxic substances, etc. With so many contributing parameters, impairment of digesters due to minor fluctuations is a frequent occurrence (Mata-Alvarez, Macé and Llabrés, 2000). To overcome this issue, engineers and scientists developed a solution where organic wastes of different origins and characteristics are fed to the same system to abate the influence of fluctuations in environmental conditions. This process is known as anaerobic co-

digestion. Anaerobic co-digestion overcomes many shortcomings of the traditional digestion process such as: (1) produce mixtures with an optimal C/N ratio, (2) dilution of potentially toxic and inhibitory compounds in either of the substrates used, (3) supply of buffer capacity to the mixture, (4) increase the biodegradable content of the waste, and (5) widen the range of bacterial strains taking part in the process. The list of substrates that could be used as feed to the digester consists of sludge from WRRFs, animal manure, harvest residues, organic wastes from agriculture related industries, meat and fish industrial wastes, dairy wastes, food waste, collected municipal organic solid waste from markets and households, reed canary grass, silage, straw, wood shavings and energy crops among others (Moody *et al.*, 2011; Esposito *et al.*, 2012)

1.2 Fats, Oils, and Grease (FOG)

The waste stream generated during cooking and food processing is composed of lipids and fatty acids, collectively termed as fats, oils, and grease (FOG). The release of these streams into the collection system is against the regulations in most of the municipalities since it can accumulate along with the wet wipes and sanitary items, which can cause property flooding or even a city-wide sewer blockage and overflow. FOG that does enter WRRFs, can cause disruption in settlement and clarification facilities apart from affecting the activity of microorganisms and slowing down the degradation process (Wallace *et al.*, 2017). The addition of FOG to the digesters was deemed beneficial because of the high number of carbon and hydrogen atoms in their molecules, which implied a high theoretical methane production (Neves, Oliveira and Alves, 2009). Also, the methane percent in the biogas produced is higher in wastes with fats (66-73%) than with carbohydrates and lipids (50-58%). It has also been suggested that digesters with the combination of sludge and FOG produce higher methane as compared to the ones with sludge alone due the lower (more negative) mean oxidation state of carbon in fats as compared to carbohydrates and proteins (Gujer and Zehnder, 1982).

1.3 Degradation Pathways and Inhibition

In the process of co-digesting multiple substrates anaerobically, the degradation process begins with three major constituents – carbohydrates, proteins and lipids. The steps involved in the formation of methane from these constituents consists of hydrolysis, fermentation, acetogenesis and methanogenesis. Proteins and carbohydrates are degraded by fermentative bacteria into amino acids and sugars during the hydrolysis step, and then into volatile fatty acids and acetate during the fermentation step along with carbon dioxide and hydrogen. Lipids, on the other hand, which consists of triglycerides and long chain fatty acids (LCFA) are degraded to produce free fatty acids and glycerol in the hydrolysis stage. LCFA degradation is believed to occur through β-oxidation to sequentially generate two carbon-unit in the form of acetate with hydrogen and carbon dioxide as byproducts (Pavlostathis and Giraldo-Gomez, 1991; Rasit *et al.*, 2015). However, failures of anaerobic treatment systems have often been reported due to accumulation of LCFAs, since these are toxic to microorganisms above certain concentrations, and tend to adsorb onto the microbial cell surface making the process mass transfer limited. The chain length of LCFA, degree of saturation and its synergistic nature – are all contributing factors that affect the inhibition of methane production (Salminen and Rintala, 2002). The most commonly found LCFAs in sludges and wastewaters are myristate (C12:0), palmitate(C16:0), palmitoleate(C16:1), stearate(C18:0), oleate(C18:1) and linoleate(C18:2). Among these, palmitate and oleate have often been reported as the compounds of primary concern (Alves *et al.*, 2009).

Throughout the years, co-digestion of municipal sludge with FOG waste has shown to noticeably increase biogas production ranging from 13% for a single phase 5-gallon CSTR to 197% for a two-phase CSTR (John C Kabouris *et al.*, 2009; L. Parry *et al.*, 2009; Long *et al.*, 2012). While the purpose for most of these studies was to investigate the biogas production and energy recovery potential, there is very little data regarding the process kinetics for anaerobic co-digestion of primary sludge (PS) and thickened waste activated sludge (TWAS) with FOG waste. On reviewing the literature, we noticed that although every group conducted biochemical methane potential tests (batch tests to evaluate the amount of biogas that can potentially be produced from given composition over a period – described in the following chapter)

information regarding the intermediates formation and consumption at various stages occurring in the duration of the test was lacking. This information is crucial in understanding the nature and the mechanism of inhibition which are not very well understood. The inhibition of aceticlastic methanogens may very well affect the degradation of LCFA themselves, since these LCFAs have been known to degrade via acetate yielding β-oxidation reactions. Among other studies, Koster & Cramer have shown that high concentration of LCFA can have a detrimental effect on the functioning of anaerobic treatment processes (Koster and Cramer, 1987). Also, Lalman & Bagley have suggested that LCFAs like oleate, stearate and linoleate affect not only aceticlastic methanogenesis but also hydrogenotrophic methanogenesis (David and Lalman, 2000; Lalman and Bagley, 2001). The earlier hypothesis of LCFA inhibition to be irreversible is now being contradicted by new findings that suggest possible recovery of digesters inhibited after a certain lag phase (Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994; Pereira *et al.*, 2003, 2004). Therefore, insights into the intermediates formation and degradation can be useful in developing a better understanding of the inhibition process and in turn help develop solutions to overcome operational challenges.

RESEARCH OBJECTIVES

The main objective of this project is to study the anaerobic co-digestion of primary sludge and thickened waste activated sludge with FOG waste and develop a thorough understanding of possible inhibitions to pathways involved in the conversion of the mixed substrate to methane and carbon dioxide. The specific objectives are:

a. To find a primary sludge:thickened waste activated sludge:FOG (PS:TWAS:FOG) ratio which would enable us to produce maximum possible biogas with the help of biochemical methane potential (BMP) tests and also investigate the possibility of recovery from inhibition at high FOG loadings.
b. To identify and quantify the intermediates being formed and keep track of these individual compounds with the help of high performance liquid chromatography (HPLC) by setting up two lab-scale digesters (1 control and 1 optimal PS:TWAS:FOG ratio).
c. To analyze the biogas data collected during the BMP tests and the VFA and LCFA results obtained during operation of the digesters, to study the discrepancies in biogas production and the degradation process believed to be caused by the presence of LCFAs like palmitate and stearate at high concentrations.

2. LITERATURE SURVEY

2.1 Overview of Fats, Oil and Grease (FOG)

The concerns surrounding proper management and disposal of FOG waste has been around for over half a century. Even in 1944, Dawson et al. and Cohn addressed the issues regarding the clogging of the waste lines and entering the wastewater treatment plants due to unregulated disposal of FOG waste from various establishments. They identified the potential of reclaiming the economic value by proper utilization of this lipid-rich waste (Cohn, 1944; Dawson and Kalinske, 1944). It is also estimated that the development of cost-effective technologies to either produce biochemical products or energy from FOG waste can potentially become a significant source of revenue (Wallace *et al.*, 2017). Fats and oils are a mixture of triacylglycerols which are insoluble in water but can solubilize in organic solvents (Beare-Rogers, Dieffenbacher and Holm, 2001). A typical fatty acid composition of common oil sources has been provided in Table 2.1.

Table 2.1 Typical acid composition (%, by weight) of common oil sources (Kincs, 1985)

Fatty Acid	Soybean	Cottonseed	Palm	Lard	Tallow	Coconut
Lauric	0.1	0.1	0.1	0.1	0.1	46.5
Myristate	0.1	0.7	1.0	1.4	2.8	19.2
Palmitic	10.2	20.1	42.8	23.6	23.3	9.8
Stearic	3.7	2.6	4.5	14.2	19.4	3.0
Oleic	22.8	19.2	40.5	44.2	42.4	6.9
Linoleic	53.7	55.2	10.1	10.7	2.9	2.2
Linolenic	8.6	0.6	0.2	0.4	0.9	0.0

Apart from the common long chain fatty acids listed in Table 3.1 which make up large portions of FOG, ester waxes, phospholipids, sterols and sterol esters have been claimed to be present in FOG waste (Husain *et al.*, 2014). The composition is highly dependent on the sources and there are three primary sources of FOG waste (Blanc and Arthur, 2013):

(a) **Domestic Sources:** Although commercial sources are the biggest contributors of FOG waste, domestic sources can contribute a significant amount during festive and holiday seasons where food is the major part of the celebrations. The Capital Regional District (CRD), an area of British Columbia (Canada) with an estimated population of 382,250 reported that 1,000,000 kg of FOG from residential sources enters the wastewater systems every year (Blanc and Arthur, 2013).

(b) **Commercial Sources:** Commercial sources primarily consists of the Food Service Establishments (FSE) such as restaurants, hotels, pubs, convenience stores, etc. The Environmental Protection Agency (EPA) estimates that the annual production of grease trap waste entering the wastewater treatment plants can range between 800 to 17,000 pounds/year per restaurant (EPA, 2007).

(c) **Industrial Sources:** Although the FOG waste generation from industrial sources is usually regulated, it can still be a potential source. Some examples of the sources are food processing industries, rendering plants and abattoirs.

EPA reported that FOG was one of the leading causes of sewer blockages in the United States and accounted for over one out of every five sanitary sewage overflows (USEPA, 2004). Even in the year 2000, over 60% of all sewer blockages in Hong Kong were caused to due excessive grease accumulation (Chan, 2010). It is also reported that developed countries have higher per capita FOG consumption (50 kg/yr) than in comparison with developing or under-developed countries (20 kg/yr) (Williams *et al.*, 2012). It becomes important to understand the FOG deposit formation in sewer lines since spills caused due to blockages tend to have health effects like irritancy (burning eyes, sore throat, coughing), response to odor (headache, nausea, vomiting), toxic effects due to chemical/microbes and psychosocial effects (sleeplessness, loss of appetite) (Bridges, 2003).

Keener et al. (2008) collected 27 FOG deposit samples from 23 locations around the US and reported accumulation rates of 0.10 cm/d and thickness of deposit layer ranging from 0.25 to 0.5 cm. The physical characteristics suggested that most of the samples were formed due to chemical reactions rather than cooling and accumulation. The moisture content was reported to be in the range of 8% to 86% and

such high variability suggested very little influence of moisture content on properties of deposits formed. They found that 84% samples contained greater than 50% lipid content with palmitate being the primary compound. 85% samples had calcium as the primary metal with an average concentration of 4225 mg/L but could not find any correlation between hardness and calcium concentration in FOG deposits. The tendency of FOG deposits to accumulate fat and calcium beyond background levels hinted at a chemical process and based on their study of the physical and chemical properties, saponification was proposed as the possible mechanism during which a free fatty acid formed as a result of hydrolysis of FOG reacts with calcium to form metallic solids. (Keener, Ducoste and Holt, 2008).

The findings published in the year 2011 and 2013 by He et al. shed more light on the mechanism of formation of FOG deposits. Their investigations revealed that palmitic was the primary saturated fatty acid (38-78%) with oleic being the primary mono-unsaturated fatty acid (9-31%) and linoleate being primary poly-unsaturated fatty acid (0.6-15%), which confirmed the results obtained by Keener et al. (Keener, Ducoste and Holt, 2008). Based on their findings, they suggested two processes of FOG deposit formation (He *et al.*, 2011):

(a) Accumulation of calcium around fatty acids due to compression of charged double layer (DLVO theory) due to negative carboxylic ends of free fatty acids, and
(b) Saponification reaction between free fatty acids and calcium to form calcium based fatty acid salts.

They also found that significant leaching of calcium occurred even at neutral pH values and that oil is required for the formation of deposits at water/air or water/concrete interfaces since it acts as the carrier of free fatty acids (He *et al.*, 2013).

In a study conducted in England, nine locations were monitored over 14 months and it was discovered that most fatty acids profiles consisted of C14-C18 acids with higher concentrations of oleic than palmitic were found near the sewer pump station, whereas, higher concentrations of palmitic than oleic were found downstream. This suggested possible biotransformation in the sewers which can possibly affect

the properties of FOG deposits. They also reported links between water hardness and FOG properties which were earlier dismissed by Keener et al. – the calcium concentration in FOG as well as the oil in the solids were found to be directly proportional to the water hardness, thereby affecting physical properties of FOG (Williams, 2012). More studies on the properties of FOG deposits have shown that the properties of the deposits formed are dependent on the calcium source owing to their solubilities along with existing pH and temperature conditions. $CaCl_2$ produced a soft, gel-like structure, whereas, $CaSO_4$ and $Ca(OH)_2$ produced a hard, granulated texture (Iasmin *et al.*, 2014).

The most recent study conducted which ran a total of 128 samples out of which 32 samples consisted only oleate and no fatty acids, did not form hardened FOG deposits, and found that deposits could be formed irrespective of the metal addition through crystallization process. Also, FOG deposit weight was highly dependent on the amount of calcium present (increased with higher concentration). They also found that stearic and palmitic are more readily incorporated into deposits than oleate and tend to compete for inclusion in the deposits (Gross *et al.*, 2017). Due to all the complications related to FOG discharges outlined above, it becomes crucial to avoid it from entering the sewer lines. There are regulations that require the FSEs and industries to install interceptor/collector devices which would separate FOG and also have thes equipments serviced at regular intervals. This management strategy, however, results in collection of concentrated FOG waste which is difficult to treat and/or dispose. Anaerobic co-digestion and production of bio-diesel have found wide application as treatment methods to deal with FOG. Several municipal WWTPs are successfully co-digesting FOG with municipal sludge but digester failures at high FOG loadings have frequently been reported and numerous articles have been published.

2.2 Co-digestion of Municipal Sludge and FOG

As discussed earlier, anaerobic digestion is a process of degrading complex organic matter to produce methane and carbon dioxide. When multiple substrates are fed to the digesters, degradation occurs

through various pathways – main pathways have been outlined in Figure 2.1. Three primary groups of organic compounds consist of lipids, carbohydrates and proteins. During the initial phase of hydrolysis, lipids break down to form long chain fatty acids (LCFA) and glycerol, carbohydrates to form glucose and proteins to form amino acids. LCFA then undergo β-oxidation reactions to eventually form acetate, hydrogen and carbon dioxide, and glycerol is degraded by lipolytic bacteria to form propionate. Glucose-fermenting and amino acid-degrading acidogens form acetate, propionate, butyrate and valerate through various pathways. These VFAs are then oxidized by different acetogens to form acetate which is later consumed by aceticlastic methanogens to produce methane. Apart from aceticlastic methanogens, hydrogenotrophic methanogens employ another pathway to produce methane, i.e. by utilizing hydrogen and carbon dioxide (Rasit *et al.*, 2015).

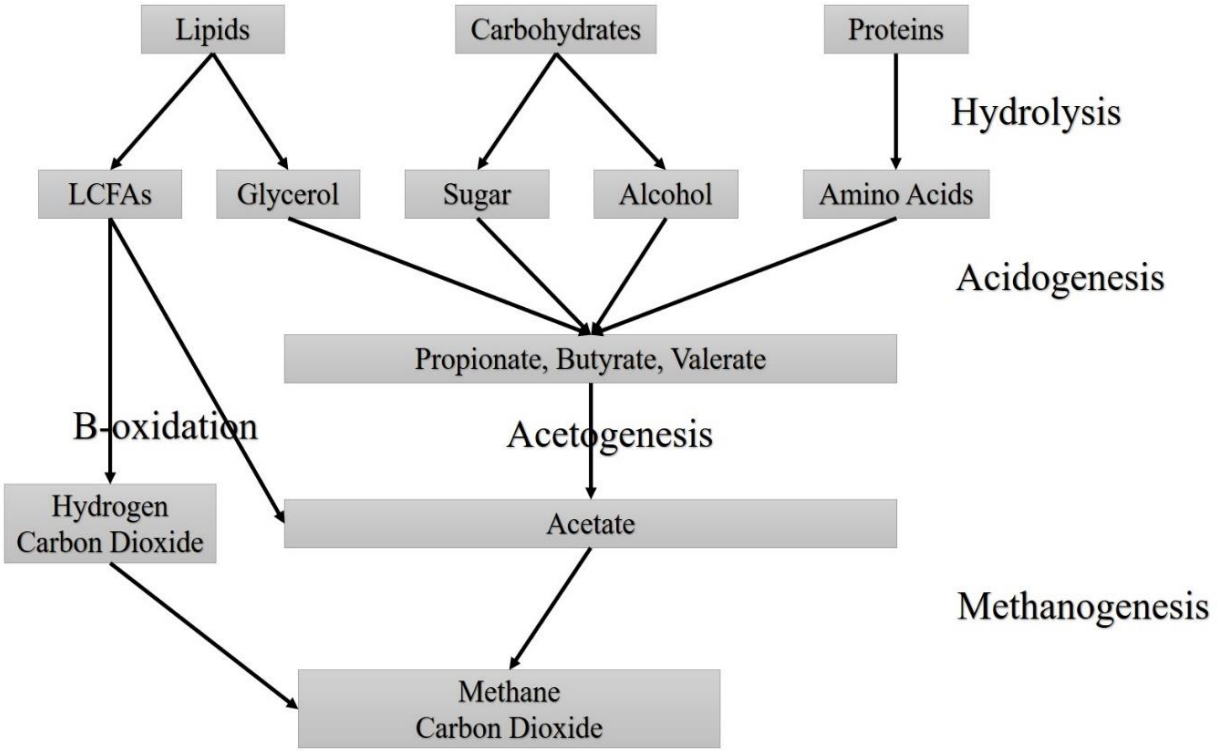

Figure 2.1. Complex Organic Substrate Degradation Pathways

There have been numerous articles published over the last decade showcasing the possibilities and successful implementation of anaerobic co-digestion of municipal sludge and FOG waste. In 2008,

Davidsson et al. were among the first to publish their findings. Batch and continuous reactors were operated at mesophilic conditions with organic loading rates (OLRs) ranging from 2.4-2.7 kg-VS/m^3·d. They found that the addition of grease trap sludge to sewage sludge increased the methane potential and methane yield. In pilot testing, the methane yield increased 9-27% for grease sludge amounts corresponding to 10-30% of total VS added (Davidsson *et al.*, 2008). These findings proved to be the first of numerous articles which propelled the research in the field of anaerobic co-digestion of municipal sludge and FOG waste.

In the same year, another group published their findings based on the use of dewatered FOG waste as a co-substrate along with municipal sludge. They conducted batch and semi-continuous studies at mesophilic and thermophilic conditions with an OLR ranging from 2.2 to 4.35 kg-VS/m^3·d. The results from the biochemical methane potential analysis showed that methane production normalized to g-VS_{FOG} destroyed was highest for the reactor with 18% FOG portion of the COD (1164 mL) although higher VS and COD % destruction (46.9% and 61.5%) was achieved with reactor consisting 58% FOG portion of COD loading. However, once the culture was acclimated over longer time periods, the highest methane was produced by reactor with 58% of FOG portion of COD (909 mL to 1358 mL). The production of VFAs was also very low indicating a very efficient degradation process (Kabouris,* *et al.*, 2008). When the experiments were conducted at thermophilic conditions and OLR of 4.35g-VS/L·d, they achieved higher VS % destruction than at mesophilic conditions (51.2% vs 45.0%). Moreover, addition of FOG increased methane yield by 2.6 (197 mL vs. 512 mL CH_4) times at thermophilic and 2.95 (152 mL vs. 449 mL CH_4) times at mesophilic conditions, respectively (John C Kabouris *et al.*, 2009).

Another variation of the same set of experiments was carried out comparing the impact of having an acid-phase reactor (solids retention time, SRT = 1 d) before the digester (SRT = 11 d), with the regular digester (SRT = 12 d). Using the acid-phase reactor produced no biogas for the first 20 days but later produced 2 mL/g-VS but more importantly, was able to decrease unsaturated fatty acids by 43% and increase soluble COD, VFAs and ammonia by 16%, 26% and 20%, respectively. Also, the methane-phase reactor produced higher methane at thermophilic conditions than mesophilic and achieved 85% fat

reduction compared 78%. It was noteworthy that without the acid-phase reactor, total fat reduction was only 28% and 68% at mesophilic and thermophilic conditions, respectively (John C. Kabouris *et al.*, 2009).

Co-digestion of municipal sludge with grease trap sludge obtained from a meat processing industry has also been investigated at mesophilic conditions (Luostarinen, Luste and Sillanpää, 2009). The OLR ranged from 1.14-2.23 kg-VS/m^3·d (SS:GTS 95:5) and 1.37-4.41 kg-VS/m^3·d (SS:GTS 80:20) and the best results were obtained for grease trap sludge VS loading of 46% of the feed with 16 d SRT and loading rate up to 3.46 kg-VS/m^3·d. Grease trap sludge addition of 55% and 71% of feed VS, decreased methane production due to overloading and LCFA inhibition. Gonzalez et al. took one step further and compared the performance of organic fraction of municipal solid waste (OFMSW) and FOG as co-substrates. They operated their digester at 2 different OLRs (2 kg-VS/m^3·d and 4.5 kg-VS/m^3·d) and SRTs (16 d and 14.5 d). Based on the results obtained from their BMP tests, mixture with 15% FOG in the feed VS was selected to study the semi-continuous operation. Co-digesting with FOG increased biogas production by 72% and methane yield by 46% when compared to OFMSW digestion alone (Martín-González *et al.*, 2010).

Apart from OFMSW, addition of synthetic kitchen waste to the co-digester along with sludge and FOG has also been attempted. BMP tests were conducted at 35 ºC and sludge to inoculum ratio (S/I) ranging from 0.30 to 1.61. The co-digestion of 0.35 g FOG with S/I ratio of 0.46 produced highest methane of 418 mL/g-TVS and low VFA concentrations. However, it was found that increasing the S/I ratio resulted in decreased methane percentage values in the biogas produced (Li, Champagne and Anderson, 2011). Similar results were obtained when restaurant grease was co-digested in a 20 L reactor operated at 20 d SRT. Very stable operation has been reported even with FOG loading of 387% of the control which increase biogas production by 467% and methane yield by 25.2% on VS destruction basis. In this study, PS:TWAS ratio fed to the digester was 75:25 and was maintained at mesophilic conditions (Liu and Buchanan, 2011). Silvestre et al. tried grease waste obtained from the skimming of a dissolved air floatation unit and co-digested it with municipal sludge (PS:TWAS ratio of 70:30). They operated their 5.5 L reactor at 20 d SRT, with OLR ranging from 1.2-1.2 kg-VS/m^3·d and a temperature maintained at of 35 ºC. They reported that

samples with higher fat content (699 g_{fat}/kg-VS) had a higher final methane potential than the other samples. 23% grease waste VS addition of 3.0 kg-COD/m^3·d OLR increased methane yield by and impressive 138%, and the adapted biomass showcased 2.5 and 3.75 times higher acetate and β-oxidation activity than initial inoculum (Silvestre *et al.*, 2011).

Another 2 L semi-continuous digester operated at OLRs ranging from 2.34-3.40 g-VS/L·d produced daily methane yields of 252.5, 598.4 and 614 L/kg-VS_{added}·d for TWAS, FOG(L) and FOG(L)+M. Co-digestion improved methane yield by 137% when compared to TWAS digestion alone. They reported digester malfunctioning for FOG(H)+M which was identified as a result of sharp pH and alkalinity drop to 5.6 and 1000 mg/L, respectively. As an attempt towards recovering the activity of the digester, NaOH was added to increase the pH to 7.72 but it did not prove effective (Wan *et al.*, 2011). In 2012, Neczaj et al. investigated continuous operation of a 5.5 L grease trap waste and sewage sludge co-digester operated at 10 d SRT and OLR ranging from 1.98-3.37 kg-VS/m^3·d. They observed that during the start-up, VS reduction was low (44%) along with high VFA concentration which is typical during the acclimation phase of microorganisms. Stable operation was maintained and confirmed by keeping a track of VFA:alkalinity ratio which was in the range of 0.3-0.6. When this ratio is in the range of 0.4-0.8, some instabilities are to be anticipated but beyond 0.8, the digester is bound to fail and therefore, becomes a very useful indicator to assess the performance of a digester. They also conducted LCFA analysis which showed that palmitate was the most abundant followed by oleate. On the other hand, stearate concentration was always on the lower end (Neczaj *et al.*, 2012).

In order to improve and optimize methane production different types of pretreatment methods have also been investigated. Thermo-alkaline pretreatment was among the first methods attempted which involved pre-treating the samples at 80 ºC or 120 ºC with pH being raised to either 8, 9 or 10. Employing this technique in treating a combination of WAS and fatty wastewater showed increase in methane production rate. Even semi-continuous operation for WAS:FW mixture of 90:10 pretreated at 80 ºC with pH of 8 and 0.14 g-KOH/g-VS lead to 58% increase in methane production. Attempts of conducting

pretreatments at 170 ºC resulted in the formation of recalcitrant compounds (Carrere *et al.*, 2012). Another method of pre-treatment is using disintegration technologies like ultrasound, thermal hydrolysis and enzymatic treatment. For a 5.5 L reactor operated at 20 d SRT and OLR of 2.2-3.6 kg-COD/m^3·d, grease addition was done in steps of 4, 23 and 37% VS in feed. Highest COD removal of 55% was obtained for 23% grease VS addition with no further rise for higher % grease VS addition which suggested HRT limitations. This composition was selected to further investigate the effects of different pretreatment techniques of which ultrasound showed higher biogas production when compared to thermal hydrolysis and enzymatic treatment with an 89.5% rise in comparison to sewage sludge methane production (Bouchy *et al.*, 2012). In another study, enzyme addition to a batch reactor co-digesting grease trap and municipal sludge (PS:WAS ratio of 60/40) was assessed. It was noticed that addition of lipase did not impact methane content of the biogas, however, sharp increase in biodegradability of waste was noticed. For 2%, 5% and 10% of grease trap addition, biodegradable content of the feed increased by 130%, 127% and 78% respectively. It was reported that, lipase when dosed in the range of 0.33-0.83% (v/v) provided the most biodegradable waste content (Donoso-Bravo and Fdz-Polanco, 2013).

Li et al. compared the effect of temperature, SRT and OLR on the biogas production performance of anaerobic co-digestion with FOG waste in semi-continuous flow digesters. They operated a 15 L digester at SRTs ranging from 12 to 24 d. Based on the experiments carried out, S/I ratio of 0.20 and OLR of 2.50 g-TVS/L·d was recommended for optimal operation under both mesophilic and thermophilic conditions with an SRT of 24 d. It was also concluded that, in general, thermophilic conditions facilitated production of more biogas with higher methane content in biogas as compared to mesophilic conditions, with highest biogas production rate of 17.4 L/d and methane content of 67.9% which were 32.8% and 7.10% higher, respectively (Li, Champagne and Anderson, 2013). A similar study conducted using grease interceptor waste (GIW) and TWAS as co-substrates in a 6 L reactor with a 20 d SRT showed that increasing the GIW added to the digester stepwise from 10% (v/v) to 20% (v/v) with a simultaneous increase in OLR from 1.58 to 2.16 kg-VS/m^3·d, increased the methane yield by 317% in comparison to the phase when OLR was 1.24

kg-VS/m^3·d with no GIW addition. On further increasing the GIW addition to 40% (v/v), 16% decrease in methane yield was noticed along with formation of a foam layer on the top which is one of the visual indicator of a malfunctioning digester due to methanogenic inhibition (Wang, Aziz and De los Reyes, 2013).

Over the last couple of years, a wider variety of FOG-related waste are being treated. Yalcinkaya et al. (2015) reported using un-dewatered grease trap waste as the co-substrate in a 5.25 L semi-continuous mesophilic digester operated at 15 d SRT and OLR ranging from 1.74-3.14 g-VS/L·d. They noticed biogas production steadily increased with increase in grease trap waste loading up to 46% but decrease as soon as loading was increased to 70%. Also, methane content increased from 61% to 69%. They noticed the accumulation of VFAs (5466 mg-VFA/L in reactor 1 and 5569 mg-VFA/L in reactor 2) and averaged reduction in the pH from 7.55 to 5.5. The activity of the digester was inhibited, and recovery of the digester was carried out by feeding municipal sludge alone for 22 days (indicator for recovery were rise in pH and biogas production) (Yalcinkaya and Malina, 2015). Another variety of FOG waste co-digested was butcher's fat. The studies were conducted at both mesophilic and thermophilic condition in a 3 L reactor with a retention time of 40 d. Contrary to the results that have been reported earlier, in this study, mesophilic reactor produced cumulatively higher methane than thermophilic reactor (293 L/kg-VS vs. 114 L/kg-VS) and had a higher methane content (62% vs. 51%). Also, in the mesophilic reactor little to no accumulation of VFAs was noticed when compared that in thermophilic reactor. Although they did observe high concentrations of stearic and palmitate, no inhibitory effects were noticed and suggested that this could be due to the entrapment of the LCFAs in the flocculent aggregates which reduced the access of the microbes (Martínez *et al.*, 2016). One of the recent studies investigated the impacts of adding OFMSW to a co-digester treating municipal sludge and FOG. A 52% rise in methane yield was obtained when sewage sludge to grease trap sludge ratio of 30% was used during the second stage of operation. However, addition of OFMSW made a significant impact by increasing the methane yield by 82% and the reasons were increased potential of VS and increased VS loading. Variation in the methane yield during the course of the

experiment were noticed which was probably caused as a result of changing OLR due to variation in VS content in the sewage sludge and substrates content in the feedstock. And like any other digester, high concentration of VFAs were reported in the start-up phase which then dropped to stable value of around 864 mg-VFA/L on average (Grosser and Neczaj, 2017).

2.3 Lipid Inhibition: Problems & Recent Findings

Issues surrounding proper functioning of the digesters have been reported in most of the articles reviewed in the previous section. In most of the cases, lag phases and accumulation of LCFAs and/or VFAs acted as the indicators of possible inhibition. Davidsson et al. observed lag phase during their batch experiments with oleic and stearate when it was compared to the reactor which received sewage sludge as the sole substrate. They suggested that it was primarily caused due to an overload of biodegradable organic matter (Davidsson *et al.*, 2008). Kabouris et al. reported a lag phase of 10 d when the reactor with highest FOG loading produced very little methane after which they noticed an exponential rise. They noticed that all reactors with FOG amendments had a 5-d lag phase during the start-up period (Kabouris,* *et al.*, 2008). Loustarinen et al. found that prolonged lag phases were common irrespective of the source or type of inoculum used and at highest concentration of oleate concentration (10 g-VS/L), they encountered a lag phase of 20 d (Luostarinen, Luste and Sillanpää, 2009). Similarly, in another study dealing with sewage sludge, FOG and OFMSW, 35% FOG-VS in feed resulted in a 2-d lag phase (Martín-González *et al.*, 2010). Li et al. employed linear and non-linear regression in order to estimate lag phases for FOG waste and kitchen waste and concluded that FOG based digestion would result in longer lag phases than kitchen waste. This was primarily attributed to the presence of LCFAs (Li, Champagne and Anderson, 2011).

When FOG skimming from a DAF unit was used as a co-substrate, samples with 699 g_{fat}/kg-VS had the longest lag phase at 5 d. This inhibition was a result of accumulation of VFAs for the first 8 days and was also indicated by hydrogen accumulation (Silvestre *et al.*, 2011). In another study, loss of activity

was observed during the operation of the digester as a result of increased OLR and it took approximately 40 d to recover, during which feeding was completely stopped. However, when the digester resumed stable operation biogas production had dropped to half of its pre-inhibition operation. Two possible reasons behind the inhibition were the acidification and relatively short SRT of 10 d which would washout the microorganisms (Wan *et al.*, 2011). Nazaitulshila et al. reported accumulation of LCFA at S/I ratio of higher than 1.0 resulted in lag phases of 5-7 d (Nazaitulshila *et al.*, 2015). Although a 10-d lag phase was observed, when dewatered and un-dewatered grease trap waste were compared as co-substrates, un-dewatered waste provided more stable operation and reportedly could also reduce the inhibition risk in the co-digesters (Yalcinkaya and Malina, 2015). When thermophilic and mesophilic digester operations were compared, thermophilic digester suffered an initial lag phase which was primarily because inoculum added was obtained from a mesophilic digester and the lag phase was associated to the period of acclimation to higher temperature (Martínez *et al.*, 2016).

A thorough review of the existing knowledge becomes crucial when most of the studies on anaerobic co-digestion of FOG as the co-substrate report prolonged lag phases and inhibition. It has been over half a century when one of the first few papers reporting the inhibitory effects of long-chain fatty acids on the growth of microbes was published. It was found that fatty acid could either promote or inhibit the growth of microbes and was entirely dependent on the concentration. Additionally, inhibition was also reported to be affected by the degree of saturation – antibacterial activity increased with the number of double bonds and cis-forms being more active than the trans-forms. In the same article it was also hypothesized that, fatty acids might adsorb to the surface leading to inhibition when present in sufficiently high concentrations (Nieman, 1954). The investigation into the inhibitory studies picked up in 1980s when effects of LCFAs were being studied on different groups of microorganisms. Hanaki et al. reported that LCFAs caused lag phases that ceased the methanogenic activity and the breakdown of LCFAs as well as n-butyrate (Hanaki, Matsuo and Nagase, 1981). This was followed by a study of effects of caprylic, capric, lauric, myristate and oleates on aceticlastic methanogens. It was found that lauric acid was the most

inhibitory compound with an IC_{50} value of 4.3 mM. Oleate was found to be as inhibitory as lauric acid but caprylic acid was only partially inhibitory (Koster and Cramer, 1987).

Research in the field again picked up pace when Angelidaki et al. reported that low concentration of LCFAs were toxic to the thermophilic digester and ceased the degradation of acetate, propionate and butyrate. Their experiments showed that toxic effects of oleate was higher than stearate with initial inhibitory concentration of 0.1 to 0.21 g/L for oleate and around 0.5 g/L for stearate which confirmed the findings of Nieman back in 1954 (Angelidaki and Ahring, 1992). When experiments were conducted to study the effects of shock loading on the activity of methanogens, occurrence of lag phase was observed which was attributed to as the "adaptation" period as has been shown to be extremely common during the start-up. This study confirmed earlier findings that inhibition was a function of concentration and not LCFA:biomass ratio. It was also reported that only about 0.2% of the acetotrophic methanogens survived when concentration of LCFAs increase beyond the threshold value which would require months to recover or re-inoculation (Rinzema *et al.*, 1994). Since the concept of inhibition was well understood, Hwu et al. investigated the effect of LCFAs on sludges obtained from different source. They found that toxicity varied depending on the source of the sludge and was based on physical characteristics like specific surface area and size distribution – sludges with higher surface area suffered greater toxicity. IC_{50} values ranged from 0.26 to 3.34 mM for sludges of different origins which led them to conclude that biologic factors like sludge origin, methanogenic activity and sludge adaptation to lipids weren't as important in terms of toxicity. Based on their findings, they recommended the use of granular sludges for treating lipid-rich wastewater to reduce the end-products of LCFA hydrolysis (Hwu, Donlon and Lettinga, 1996). They continued their studies by studying the effect of temperature variation on toxicity and found that raising the temperature increased the toxicity for aceticlastic methanogens. 50% inhibition for 55 ºC occurred at 0.35-0.79 mM, for 40 ºC occurred at 0.53-2.27 mM and for 30 ºC occurred at 2.35-4.30 mM. Toxicity increased four times at thermophilic condition as compared to mesophilic. This rise in toxicity was explained by the fact that at

higher temperatures, sludges are loosely open structures and tend to have higher surface areas and previous studies suggested that higher surface areas lead to greater toxicity (Hwu and Lettinga, 1997).

In 2001, Lalman et al. studied the effects of stearic and oleates on aceticlastic and hydrogenotrophic methanogens. They found that oleate degradation was favored by unacclimated cultures whereas stearate degraded particularly slowly. C16 and C14 were found to accumulate when oleate was being degraded but no byproducts were detected during stearic degradation. It was also discovered that shorter chain fatty acid production from oleic and linoleate was energetically more favorable and thus, resulted in β-oxidation directly (Lalman and Bagley, 2001). Alves et al. in their study of impact of gradually changing oleate concentrations reported that acclimatized sludge had a higher tolerance to toxicity and showed higher biodegradability of oleate (Alves *et al.*, 2001). Another study comparing the effects of organic shocks to hydraulic shocks was conducted during the same time. They noticed that during organic shocks (increasing the COD loading from 4000 mg/L to 20000 mg/L) treatment of oleate deteriorated far more than when hydraulic shocks (decreasing the HRT from 16 to 3.2 h). The subdued impact during the hydraulic shock was believed to be the result of reaction between calcium and/or magnesium with oleate which resulted in formation of precipitates which enabled the growth of aceticlastic culture (Cavaleiro, Alves and Mota, 2001). Lalman et al. conducted another set of experiments to study the inhibitory effects of C18 LCFAs individually as well as in mixtures to see if it affected the hydrogenotrophic methanogens. They noticed significant deterioration in acclimated as well as unacclimated cultures responsible for the degradation of butyrate due to LCFAs. Also, hydrogenotrophs were found to be mildly inhibited by saturated C18 and adding mono-saturated C18 only slightly increased inhibition with no further rise with poly-unsaturated C18, which suggested that accumulation of hydrogen is not necessarily a good indicator of inhibition of butyrate degradation (Lalman and Bagley, 2002).

Shin et al. examined the inhibition of propionate and β-oxidation reactions and found that IC_{50} values for palmitate and stearate were greater than 3800 mg/L whereas for oleate and linoleate, it was in the range of 2700-2850 mg/L and 545-615 mg/L, respectively. This helped conclude that double bonds

increased the inhibition effect. Also, β-oxidation was found to be slower than methanogenesis in most of the cases due to the presence of LCFAs as substrates (Shin *et al.*, 2003). While attempting to develop a direct relationship between the amount of LCFA accumulated and the activities of different substrates, Pereira et al. found that activities of acetogenic, aceticlastic and hydrogenotrophic microbes decreased as the accumulation of LCFA increased and the impact was found to be more severe in case of propionate and butyrate degradation. They also observed that the microbes were able to methanize adsorbed LCFA at a rate of 250 mg-COD/g-VS·d even when LCFAs were adsorbed to the surface. This suggests that microbes remain active even when accumulation occurs, which was not well understood prior to these findings (Pereira *et al.*, 2003). In another set of experiments, they also proved that accumulation of LCFA leads to mass transfer limitations. When oleate was fed as the carbon source and degraded to form palmitate, LCFAs encapsulated the sludge. However, when palmitate was fed as the carbon source, sludge remained non-encapsulated which showed higher methanogenic activity. They found that 50 h of lag time was required for the encapsulated sludge to degrade all the adsorbed LCFA and recover activity which was of the same order of magnitude as that of non-encapsulated sludge. This proved the existence of mass transfer limitation in co-digester (Pereira *et al.*, 2005).

Since most of the digesters undergo phases on inhibition, different techniques for recovery and abatement have been attempted. Some of the techniques like changing feeding patterns, dilution and addition of adsorbents was examined. Among these, dilution and addition of adsorbent appeared to be ideal for recovery purposes. Also, acclimatization by repeated LCFA pulses and inhibition in the reactor increased the tolerance of the digester and increased the degradation rates from 0.04 to 0.16 g-COD/g-VS·d. Wu et al. investigated two abatement techniques (bentonite and calcium addition). From their studies they found that addition of either of the two reagents did not improve palmitic degradation. However, addition of calcium decreased the lag time, reduced the reagent required and additionally, lowered the solids concentration in the digestate (Wu *et al.*, 2017). More in-depth information regarding different abatement techniques can be found in the literature.

Most of the co-digestion studies conducted so far have focused on testing the potential to increase biogas production and finding upper limits of FOG loading. On the other hand, most of the inhibition studies have been carried out with pure free fatty acids in either an upflow anaerobic sludge blanket reactor, granular sludge bed reactor or a fixed bed reactor which neither is entirely representative of the FOG waste added to the digesters nor the reactor configuration of a digester. Additionally, precise and comparative analysis of variations in VFA and LCFA degradation processes as a result of FOG addition during the operation of a digester is lacking. In order to fill some of these gaps, a series of experiments were carried out which have been outlined and discussed thoroughly in the following chapters.

3. MATERIALS AND METHODS

3.1 Sample Collection

The samples for Primary Sludge (PS), Thickened Waste Activated Sludge (TWAS), digested sludge (used as inoculum) and FOG were obtained from the Mauldin Rd Wastewater Treatment Plant operated and managed by Renewable Water Resources (ReWa), Greenville, SC. The samples were provided in 1-gallon containers which were brought to the laboratory immediately and stored in the refrigerator at 4 ºC until used.

3.2 Sample Characterization

The following parameters were measure out to characterize the samples obtained from ReWa. The techniques used for analysis are described below as well.

a. Total Solids (TS)
b. Volatile Solids (VS)
c. Total Chemical Oxygen Demand (COD)
d. Ammonia (NH_3)
e. Total Phosphorous (P)
f. pH

3.2.1 Total Solids (TS)

TS in all samples was measured using the Standard Methods for Examination of Water and Wastewater (APHA/AWWA/WEF, 2012). For conducting both TS and VS tests, 75 mL porcelain evaporating dishes were used. These dishes were first ignited at 550 ºC for 1 h and stored in the desiccator

to cool (t.h.e.® Desiccant; 8 mesh, Non-indicating). Once the temperature of the dishes returned to room temperature, they were weighed. A sample volume of 20 mL was chosen for the analysis. A well-mixed sample of this volume was transferred into a pre-weighed evaporating dish using a pipette. The evaporating dishes were then transferred into an oven maintained at 103 ºC and allowed to dry for 2 h. These were then transferred to the desiccator to cool and then weighed. This cycle was continued until there was no variation in the measured weight of the dishes. The total solids were calculated using the following equation:

$$TS\ \left(\frac{mg}{L}\right) = \frac{(W_2 - W_1)\ x\ 1000}{Vol.of\ sample}$$

Where,

W_1 = Weight of dried sample and the dish, mg

W_2 = Weight of the dish, mg

3.2.2 Volatile Solids (VS)

VS analysis is a continuation of the TS analysis explained earlier; the solids produced in the TS analysis were ignited at 550 ºC in a muffle furnace. The temperature of the furnace was brought up to 550 ºC before inserting the evaporating dishes. The ignition was carried out for 30 min and the samples were then transferred to the desiccator to cool. Before placing the samples in the desiccator, the excess heat was allowed to dissipate in air for 2 min. The samples were weighed and ignited again for 30 min and the cycle was repeated until constant weight was obtained (APHA/AWWA/WEF, 2012). The volatile solids were calculated using the following equation:

$$VS\ \left(\frac{mg}{L}\right) = \frac{(W_2 - W_3)\ x\ 1000}{Vol.of\ sample}$$

Where,

W_2 = Weight of dried sample and dish before ignition

W_3 = Weight of dried sample and dish after ignition

Upon completion of the test, the evaporating dishes were thoroughly washed, ignited again at 550ºC and stored in the desiccator.

3.2.3 Total Chemical Oxygen Demand (COD)

COD was measured using Hach Kit TNT 823 (250-15000 mg/L COD, Ultra High Range). The analysis began with preheating the COD reactor to 150 ºC. The sediment present in the vials is brought into suspension by inverting the vial a couple of times. To this suspension, 0.3 mL of sample was added using a pipette. Once the vials were capped, they were inverted gently a few times to ensure proper mixing. The vials were held by the cap since the reaction is highly exothermic and tends to become very hot. The vials were then placed in the COD reactor to heat for 2 h and once complete, the temperature was dropped to 120ºC or less. This was followed by inverting the vials several times while still hot and placing them on a rack to reach room temperature. The vials were then cleaned thoroughly from the outside and then inserted into the photometer. The photometer identifies the barcode and generates an automatic evaluation.

3.2.4 Ammonia (NH_3)

Ammonia (NH_3-N) was measured using Hach Kit TNT 832 (2-47 mg/L NH_3-N, High Range). The analysis began with removing the foil from the cap and adding 0.2 mL sample to the vials with the help of a pipette. After the sample addition, caps were immediately inverted and screwed back on. The contents of the vials were mixed 2-3 times and allowed to sit for 15 min at room temperature and then, inverted 2-3 times before placing the vial into the photometer. The photometer identifies the barcode and generates an

automatic evaluation. In case of “over-range” or “under-range” results, different dilutions for the sample were made.

3.2.5 Total Phosphorous (PO_4)

Total phosphorous (PO_4) was measured using Hach Kit TNT 844 (1.5-15.0 mg/L PO_4, High Range). The analysis began with removing the foil from the cap and adding 0.5 mL sample to the vials with the help of a pipette. After the sample addition, the caps were immediately inverted and screwed back on. The contents of the vial were shaken 2-3 times and placed inside a reactor which was preheated at 100ºC, for 1 hr. The vial temperature was then brought down to room temperature and then, firmly mixed 2-3 times again. 0.2 mL of Reagent B was added to the vial and grey DosiCap^{TM} C was screwed onto the vial. The vial was inverted 2-3 times, allowed to sit for 10 min and then inverted 2-3 times again. The vial was then cleaned thoroughly from the outside and then inserted into the photometer. The photometer identifies the barcode and generates an automatic evaluation. In case of “over-range” or “under-range” results, different dilutions for the sample were tried.

3.2.6 pH

pH measurements were carried out using the Orion Star A211 pH meter by Thermo Scientific. The electrode was stored in ROSS Storage Solution and the calibration was done every time before the measurements were taken. The calibration was done using three buffer solutions with pH values – 4, 7 and 10. After every pH measurement, the electrode was cleaned using DDI water and dried using tissue wipes.

3.3 Biochemical Methane Potential (BMP) Tests

The BMP test were carried out in 160 mL serum bottles. The number of bottles were selected based on the different ratios chosen on volume (in triplicates for each ratio chosen). Sample mixtures based on the composition were poured in beakers and stirred to ensure well-mixed samples. 100 mL volume of different sample mixtures were added to the serum bottle using a pipette. All the serum bottles were prepared after keeping the required number of septa and aluminum seal caps ready. The samples were purged in the serum bottles for 5 minutes to ensure anaerobic environment. After sparging, the septum was immediately placed on the serum bottle and followed by the aluminum cap which was sealed with the help of a crimper. Once all the bottles were sealed, they were placed in the temperature-controlled shaker at maintained at 37 ºC and 130 rpm. (Temperature = 37 ºC @ 130 rpm). The volume measurement for the gas collected was done at the same time every day. To measure the volume, the bottles were placed in a chemical fume hood, and 20 mL or 50 mL frictionless syringes were used along with 23 G disposable needles to collect and measure gas volume. The amount of biogas collected was recorded in a spreadsheet and graphs of daily biogas generation (mL) vs. time (days), and cumulative biogas generation (mL) vs. time (days) were plotted. Two different BMP tests were conducted. Test I was conducted using TWAS and FOG as substrates whereas during test II in addition to TWAS and FOG, PS was added to mimic large scale municipal anaerobic digester feed. The compositions used during both the tests have been provided in the Table 3.1 and Table 3.2, respectively.

Table 3.1 BMP Test I Composition (PS, TWAS and FOG characteristics in Appendix)

Sample	Total Vol.	Inoculum	TWAS	FOG	COD
	mL	*mL*	*mL*	*mL*	*g/L*
Control	100	30	70	0	74.96
FOG 25	100	30	52	18	72.39
FOG 33	100	30	46	24	71.56
FOG 50	100	30	35	35	69.82
FOG 100	100	30	0	70	64.67

Table 3.2 BMP Test II Composition

Sample	Total Vol.	Inoculum	Primary Sludge	TWAS	FOG	COD
	mL	*mL*	*mL*	*mL*	*mL*	*g/L*
Blank	50	50	0	0	0	8.52
Control	100	50	25	25	0	52.88
FOG 25	100	50	18.75	18.75	12.5	56.71
FOG 50	100	50	12.5	12.5	25	60.55
FOG 75	100	50	6.25	6.25	37.5	64.38
FOG 100	100	50	0	0	50	68.22

3.4 Batch and Semi-Continuous Digesters Setup & Operation

Two bench-scale digesters were set-up and operated in batch and semi-continuous mode at mesophilic conditions, the compositions of which have been provided in Table 3.3. The digesters were constructed with 1 L Pyrex bottles, and the temperature was maintained at 35 ºC with the help of a water bath (2 L beaker) placed on hotplate/stirrer. The temperature was monitored with the help of a thermometer. The first digester was setup to act as the control and the other one consisted the composition which resulted in maximum biogas production during the BMP tests. After the addition of samples into the respective digesters, they were purged with nitrogen gas for 45 min and then, immediately sealed with a rubber stopper. Three tubings of varying lengths were inserted in the rubber stopper, among which one was submerged to the bottom of the reactor to enable drawing well-mixed samples, another one just above the sample surface for sample addition and the third one in-line with the bottom of the stopper to allow for the gas flow into a Tedlar bag. A septum was inserted in a T-joint with was attached to the tubing carrying gas from the digester to the Tedlar bag to draw 0.25 mL sample for GC analysis. The gas collected was measured daily using 50 mL disposable syringe. The gas % measurement for quantification of methane was carried out using GC analysis procedure outlined in the following section. Once the results for the batch mode operation was verified with the results obtained from BMP tests, the digester were switched to semi-continuous mode. SRT of 30 days was maintained by withdrawing and adding 47 mL sample every two days. For control this accounted for 47 mL of 50:50 PS:TWAS mixture and for reactor with 25% FOG sample, 25:75

FOG:PS+TWAS mixture. From the 47 mL of volume withdrawn, 20 mL was stored in a separate vial for pH, VFA and LCFA analysis and the remaining was discarded. The details about VFA and LCFA analysis have been provided in the following sections.

Table 3.3 Composition of Lab-Scale Digesters

Sample	Total Vol.	Inoculum	Primary Sludge	TWAS	FOG	COD
	mL	*mL*	*mL*	*mL*	*mL*	*g/L*
Control	700	231	234.5	234.5	0	47.91
FOG 25	700	231	175.875	175.875	117.25	48.88

3.5 GC-TCD for Methane Quantification

The amount of methane generated was measured using a gas chromatography unit equipped with a thermal conductivity detector (Shimadzu GC 2014 with TCD) and a Restek ShinCarbon ST Micropacked Column (1.00mm x 1/16" x 2m). 0.25 mL of sample was taken from the headspace of the serum bottles using a Hamilton Gastight® GC Syringe. Ultra-high purity Argon was used as the carrier gas at 415 kPa pressure and 10 mL/min flowrate. The duration of run for each sample was set at 5 min, with the injector and the detector temperature set at 150 ºC whereas the column at 120 ºC. The area under methane peak in the chromatogram generated was used to calculate the percentage and concentration of methane generated. Graphs similar to daily and cumulative biogas generated were plotted for methane percentage.

3.6 HPLC for VFA Identification & Measurement

An HPLC unit equipped with Aminex® HPX-87H Ion Exclusion Column (300 mm x 7.8 mm; 5 mM H_2SO_4, 0.6 mL/min) at 210 nm wavelength, was primarily used for analyzing the composition of volatile fatty acids that are formed during the operation of the lab-scale batch and semi-continuous digester. The duration of the run for each sample was set at 50 min and the temperature for the column was 30 °C. 1 mL sample was drawn from the 20 mL collected using a 3 mL disposable luer lock syringe. The sample

was filtered using a PVDF syringe filter with a pore size of 0.2 µm. 0.15 mL of the filtrate was taken in a 12x32 mm screw thread clear vial using a pipette and was diluted 10 times to have a total HPLC sample volume of 1.5 mL. An open-top polypropylene screw cap with 8mm Teflon septum was used to seal the vial. The samples were stored in the freezer until the analysis was carried out at the end of the week.

3.7 GC-FID for LCFA Identification & Measurement

The protocol for LCFA analysis was adapted from Ziels et al. (2015) and Burja et al. (2006). The sludge samples taken during the operation of batch and semi-continuous digesters were stored in the freezer and thawed at room temperature prior to extraction. 1 mL of the 20 mL sludge sample withdrawn was taken in a 10 mL glass vial, to which 200 µL of 250 g/L sodium chloride, two drops of 50% sulfuric acid and 2 mL of 1:1 hexane: methyl-tert-butyl ether was added. These samples were sealed using Teflon-lined caps and shaken thoroughly. The samples were then centrifuged at 3000 x g for 12 min. 1.5 mL of the supernatant was transferred into another glass vial to which 2.5 mL methanol, 250 µL hydrochloric acid and 250 µL chloroform was added, vortexed for 10 s and placed in the heating block at 90 ºC for 2 h (Ziels *et al.*, 2015). The sample is then purged with nitrogen gas until all the organic solvent is vaporized and reached room temperature. 1 mL of water was added to the vials and vortexed. Finally, to each vial, 1.6 mL of GC grade hexane and 0.4 mL of chloroform was added and vortexed and was repeated 3 times. The final samples were allowed to sit until clear liquid separation was achieved (Burja *et al.*, 2006). From the organic phase, 1.5 mL sample was withdrawn using a pipette and transferred to 1.8 mL standard crimp autosampler vials which were sealed with 11 mm crimp seal silver caps with PTFE/Tan silicone septum. The measurements were carried out using a gas chromatography unit (Shimadzu GC-2010) equipped with a flame ionization detector (FID). The column was Rt-2560 (100 m x 0.25 mm x 0.2 µm) and helium as the carrier gas. Injector and detector temperature were set at 240 ºC and column was set at 100 ºC for 5 min and increased to 240 ºC at a rate of 3 ºC/min. Supelco 37 component FAME mix purchased from Sigma was used as the FAMEs

standard in this study. Pentadecane (100 µg/mL) and tridecanoic acid (200 µg/mL) were used as the internal standard and for % recovery calculations, respectively.

4. RESULTS AND DISCUSSIONS

4.1 Biochemical Methane Potential Test

4.1.1 Biogas Production

In order to obtain the optimal PS:TWAS:FOG composition ratio, a biochemical methane potential test was carried out. The results shown in Figure 4.1 show that the biogas production for control initiated as expected and produced 541.7 mL of biogas in the first 10 days. All the other reactors with FOG amendments produced significantly lower biogas – with a consistent trend of decreased gas generation with increased FOG volume. After 10 days, FOG 25 (25% (v/v) FOG addition), FOG 50 (50% (v/v) FOG addition), FOG 75 (75% (v/v) FOG addition) and FOG 100 (100% (v/v) FOG addition) produced 110.17 mL, 71.5 mL, 50 mL and 26.67 mL biogas, respectively. After 10 days, most of the readily available COD was consumed in the control and in all the other reactors, a small rise in biogas production was noticed which ceased on day 20. Between days 20 and 45, all the reactors with FOG amendments were inhibited with very little gas production. Over the next 20 days, FOG 25 produced 771.5 mL biogas in contrast to 94.67 mL between days 20 and 45. However, FOG 50 to FOG 100 indicated no signs of recovery. This showed that reactors with FOG loadings can recover from inhibitions for FOG loading up to a certain limit, which in our case was 25% (v/v) and 23% FOG feed VS concentration. FOG feed concentrations ranging from 30% to 60% have been used which maximized gas production indicating that performance of a digester in highly dependent on feed characteristics (Suto *et al.*, 2006; Davidsson *et al.*, 2008; John C. Kabouris *et al.*, 2009). At the end of the BMP test, FOG 25 had generated the highest volume of biogas with 1133.34 mL and lowest for FOG 100 with only 254.67 mL of biogas over the entire duration of 84 days.

4.1.2 Methane Content

The results of methane content of the biogas produced were obtained with the help of GC analysis and have been shown in Figure 4.2. After flushing the reactors with nitrogen initially, it took several days for methane content to reach a steady value. The methane content gradually increased over time as the nitrogen in the headspace was replaced with the methane generated. The control reactor reached its steady state value of 62.73±4.90% relatively early due to the large volume of gas production which replaced the nitrogen inside the reactors sooner.

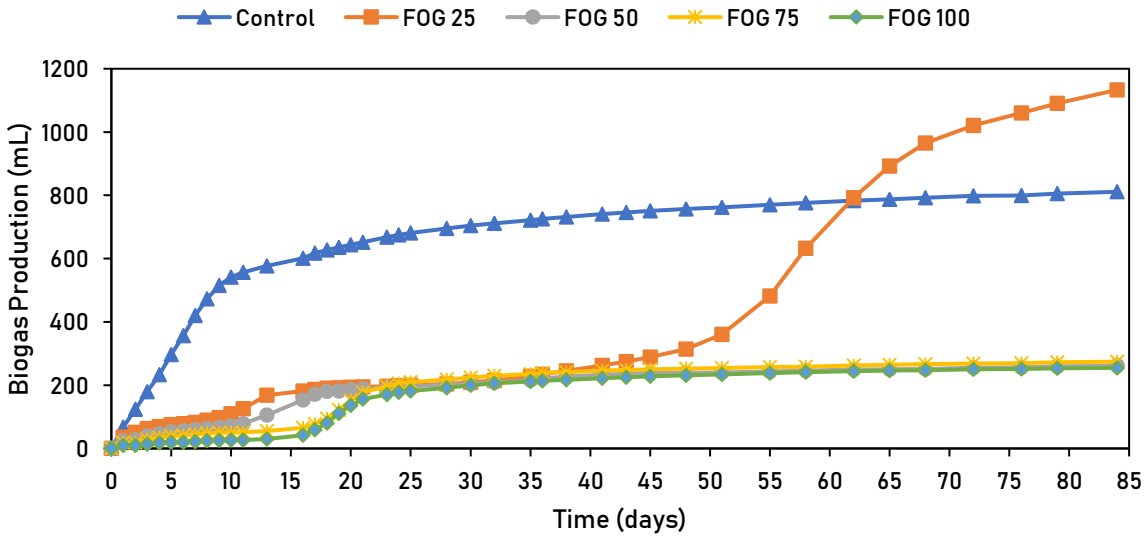

Figure 4.1 Average cumulative biogas production during BMP test with different FOG loadings carried out in triplicates

Methane content was found to decrease with increasing FOG loading. Although the average methane content for FOG 25 was 58.99±4.67%, the values were found to be higher than control in the later stages of BMP test when biogas production increased post-inhibition (59.38±3.53% for control v/s 61.57±4.28% for FOG 25 from day 45 to day 84. Methane % of 54.37±5.04, 50.40±6.11 and 50.72±5.49 were obtained for FOG 50, FOG 75 and FOG 100, respectively. These results are contradictory to high methane % expected from lipids-rich substrates and can be attributed to the fact

that the microorganisms were unable to breakdown LCFA due to its presence at high concentrations which also inhibits methanogens which degrade acetate to produce methane (Alves *et al.*, 2009).

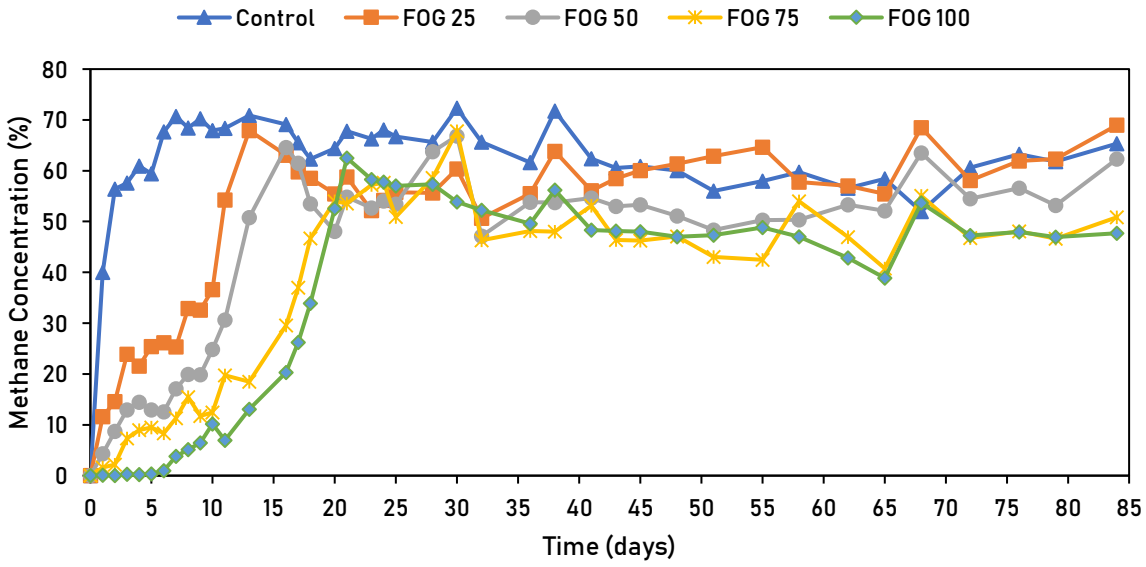

Figure 4.2 Average methane content of biogas during BMP test carried out in triplicates

4.1.3 COD Conversion to Methane

The calculations for % COD converted to methane were carried out using the biogas production values along with the methane content of the biogas. The results have been shown in Figure 4.3. The volume of methane produced was converted to moles of methane which was then used to find the moles of corresponding COD. The results have been plotted as COD conversion vs time in Figure 4.3. After 84 days of operation, 28.78% of COD was converted to methane in the control reactor. Out of the reactors fed with different FOG ratios, 34.65% COD in FOG 25, 8.51% COD in FOG 50, 9.63% COD in FOG 75 and 8.25% COD in FOG 100 was converted to methane. COD conversion values varying from 31.4% to 61.5% for FOG portion of COD loading ranging from 0% to 58% (Kabouris,* *et al.*, 2008). In another set of experiments to find out the ultimate biodegradability of PS, TWAS and PS+TWAS resulted in COD conversion values of 58.5%, 26.3% and 40.0%, respectively over a period

of 120 days (John C. Kabouris *et al.*, 2009). One more study reported COD destruction of 36% with WAS as the sole feedstock (Girault *et al.*, 2012). In our study there are a few important observations:

(a) In control, the first 19.83% out of a total of 28.78% COD was converted in the first 10 days and the remaining 8.95% over a period of 75 days.

(b) Only 7.86% COD in FOG 25 had been converted until day 45 which increased to 25.59% conversion on day 65 and then to 34.65% on day 84.

For different FOG compositions, results for the methane production normalized to COD in the reactor has been given in Table 4.1.

Table 4.1 Methane production normalized to g-COD fed during BMP test

Sample	g-COD	mL CH_4	mL CH_4/g-COD
FOG 0	5.28	604.52	114.32
FOG 25	5.67	727.80	128.32
FOG 50	6.05	178.81	29.53
FOG 75	6.43	202.27	31.41
FOG 100	6.82	173.35	25.41

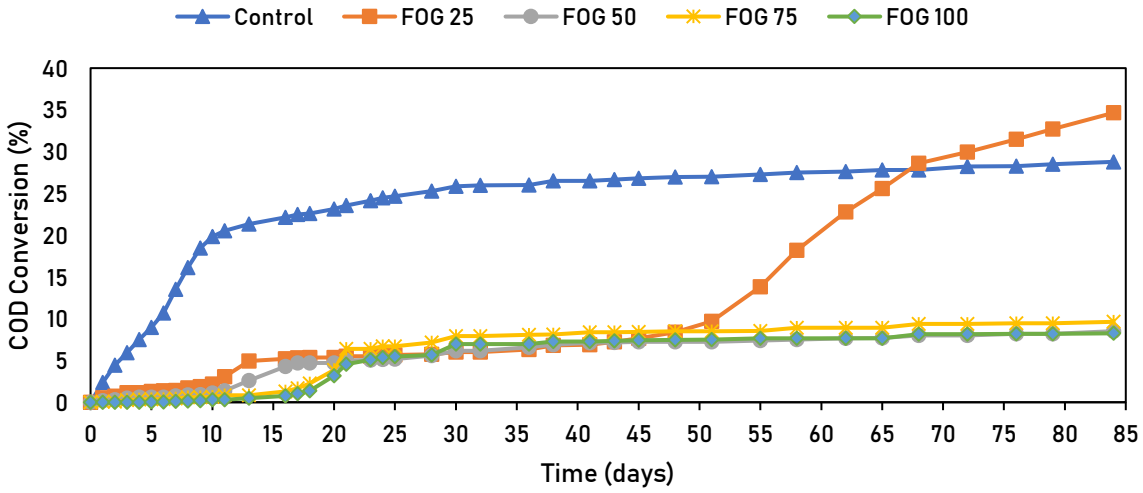

Figure 4.3 Average % COD converted to methane during BMP test carried out in triplicates

4.2 Bench-scale Digester – Batch Mode

4.2.1 Biogas Production

The optimum PS:TWAS:FOG ratio was used to construct the bench-scale digesters and the cumulative biogas production can be studied in Figure 4.4. The results obtained from the BMP tests showed that addition of 25% (v/v) FOG to the digester produces maximum cumulative biogas over a period of time and also, that it needs to overcome a lag phase of 40 days before microbes can start metabolizing as a result of acclimation to FOG. Two bench-scale digesters (1xControl and 1xFOG 25) were set up. The digesters were first operated in batch mode to verify the results obtained from the BMP test. In this case, although FOG 25 experienced a shorter lag phase, it took the same amount of time to produce as much biogas as the Control, as was found during the BMP test. Over a period of 55 days, Control and FOG 25 produced 6625 mL and 6595 mL which is equivalent to 197.55 mL/g-COD added and 192.74 mL/g-COD added of biogas, respectively. On comparison, Parry et al. reported production of 267 mL/g-COD added initially (L. Parry *et al.*, 2009). For FOG 25 two distinct lag phases were observed, first until day 7 and then from day 12 to day 23. After day 25, a sharp rise in biogas production was observed which confirmed acclimation of the microorganisms.

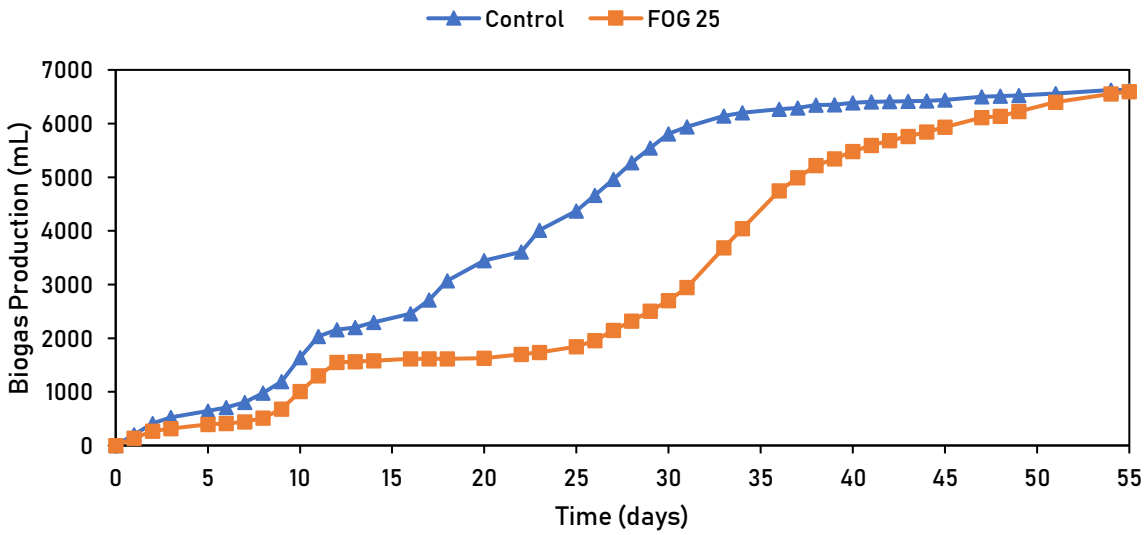

Figure 4.4 Cumulative biogas production in batch operation

4.2.2 Methane Content

During the start-up phase in the batch mode, both the digesters took about 11 days to produce a steady biogas with a fairly consistent methane content which has been outlined in Figure 4.5. The methane content in the digester with no FOG addition between days 11 and 55, averaged at 71.75±6.44%, whereas FOG 25 averaged at 64.84±4.60% which are in agreement with results obtained in other studies (Kabouris,* *et al.*, 2008; Neczaj *et al.*, 2012). Results for the both these digesters are higher than those obtained during the BMP tests.

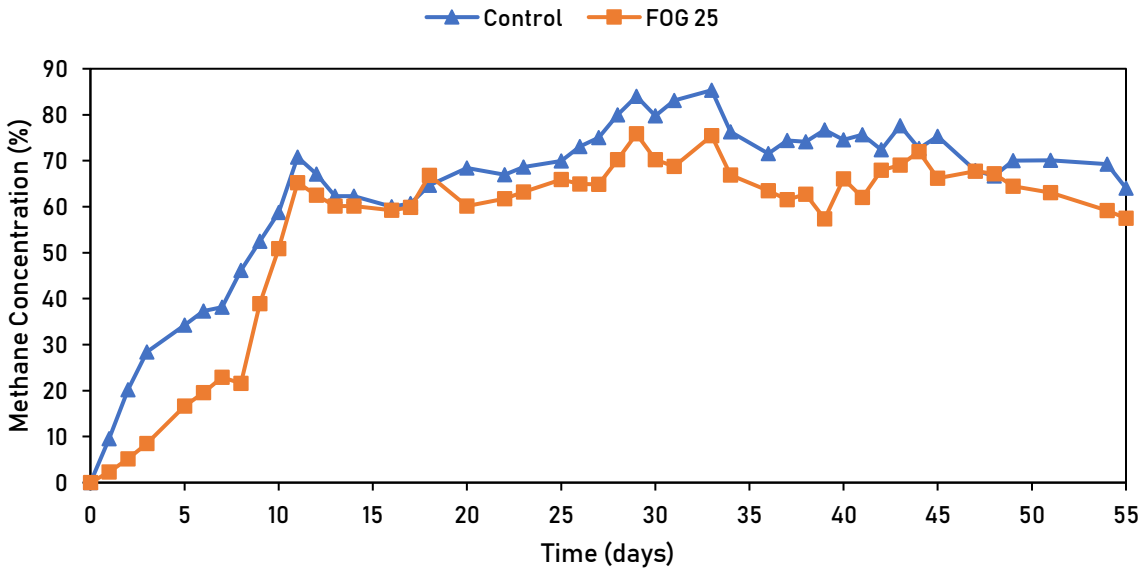

Figure 4.5 % Methane content in biogas during batch operation

4.2.3 COD Conversion to Methane

During the BMP tests, we found that 27.28% of COD was converted to methane in the control reactor after 55 days and only 13.82% conversion was observed in the case of FOG 25. Contrary to those results, for the bench-scale digesters significantly higher conversion % were obtained for both – control as well as FOG 25. Control achieved 35.03% COD conversion to methane whereas FOG 25 achieved 32.04% conversion. As can be seen in Figure 4.6, in case of FOG 25, COD conversion to

methane either ceases to occur or slows down during the lag phases. This could be due to inaccessibility of methanogens to acetate due to mass transfer limitations which prevents methanogenesis (Pereira *et al.*, 2005). Although the trends in batch operations of bench-scale digesters are similar to those during the BMP tests, the reason behind low COD conversion to methane during the BMP test could be: (1) different sludge and FOG samples used during the two tests.

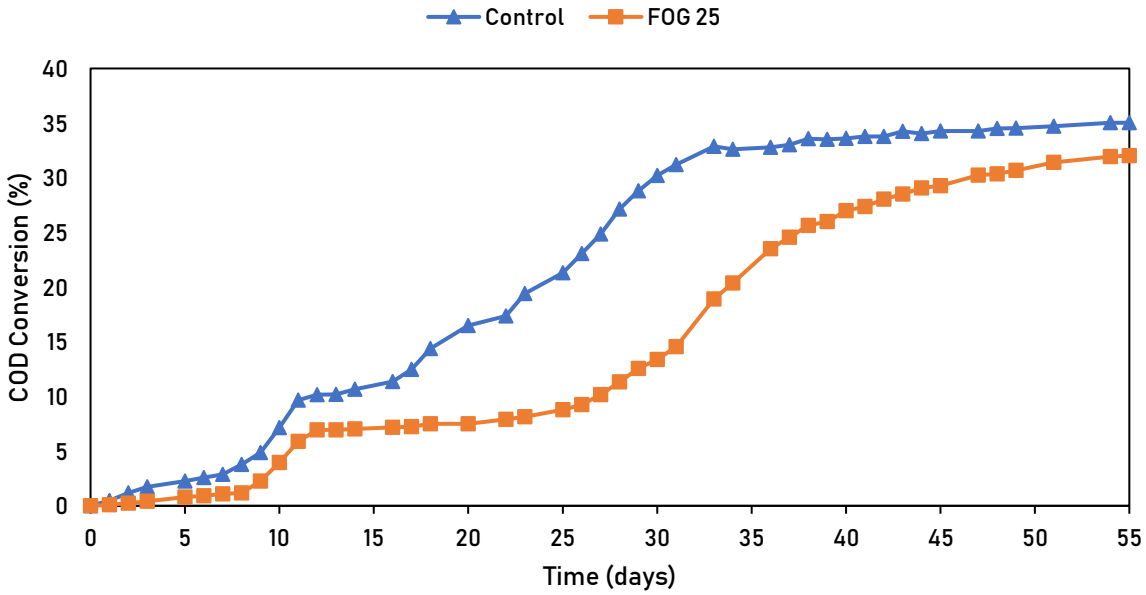

Figure 4.6 % COD converted to methane in batch operation

4.3 Bench-scale Digester – Semi-continuous Mode

4.3.1 Methane Production Rate

During the operation of digester in semi-continuous mode, it is far more beneficial and intuitive to report methane production in terms of rate because new feed, and therefore, volatile solids are being added regularly. Since the organic loading rate is known, the performance of the digester can be judged by finding the amount of methane that is being produced for each g of VS added every day. When the digesters were operated in batch mode, samples were withdrawn once every five days for HPLC analysis which led to reduction in working volume by 95 mL. Therefore, to restore the volume to the

initial conditions, 2.52 g VS and 2.44 g VS was added on day 56 to control and FOG 25, respectively. Beyond this point, to maintain an SRT of 30 d, 47 mL of sample was added every two days which accounted to a loading rate of 0.63 g VS/d and 0.61 g VS/d in control and FOG 25, respectively. The results have been plotted in Figure 4.7 which shows a steady rise in methane production rate. The rates were found to be fairly similar until day 58, and then FOG 25 produced methane at higher rate as compared to the digester with no FOG addition. Methane production rates can be seen to be constantly fluctuating because feeding was done every two days, and the first day post feeding produced higher biogas in comparison to the second day (the first day contributed to 55-60% of the total biogas generated between two consecutive feeding events). This effect has been reported in several studies and is believed to be a result of low working volumes in comparison to the large-scale digesters (Wang, Aziz and De los Reyes, 2013).

For control, the methane production rates were found to fluctuate between 0.05 L CH_4/g-VS·d and 0.65 L CH_4/g-VS·d with an average value of 0.4 L CH_4/g-VS·d, whereas, for FOG 25 it ranged from 0.05 L CH_4/g-VS·d during the initial phases to 0.68 L CH_4/g-VS·d with an average value of 0.43 L CH_4/g-VS·d. Similar results were reported in earlier studies where methane production varied from 0.15 to 0.47 L CH_4/gVS (John C. Kabouris *et al.*, 2009) and 0.32 to 0.68 L CH_4 /gVS (Davidsson *et al.*, 2008). After achieving steady state, average methane production rate for FOG 25 was found to be 7.5% higher than the control. Although the biogas production for FOG 25 was consistently and higher than the control, a fairly small increase in methane production rate is obtained due to fluctuations in the methane yield. Additionally, COD conversion to methane percentages were calculated by converting the average methane produced per day during steady state operation to moles of COD and then g of COD and then dividing it by the daily COD loadings. The average % COD conversion to methane is 43.76% for control and 57.03% for FOG 25 calculated from day 67 to day 95, respectively which corresponds to an increase of 30.34% in FOG 25. Also, the average cumulative methane production

increased by 29.93% on average as a result of FOG addition which also corresponds to 49.44% and 50.96% COD conversion for control and FOG 25, respectively.

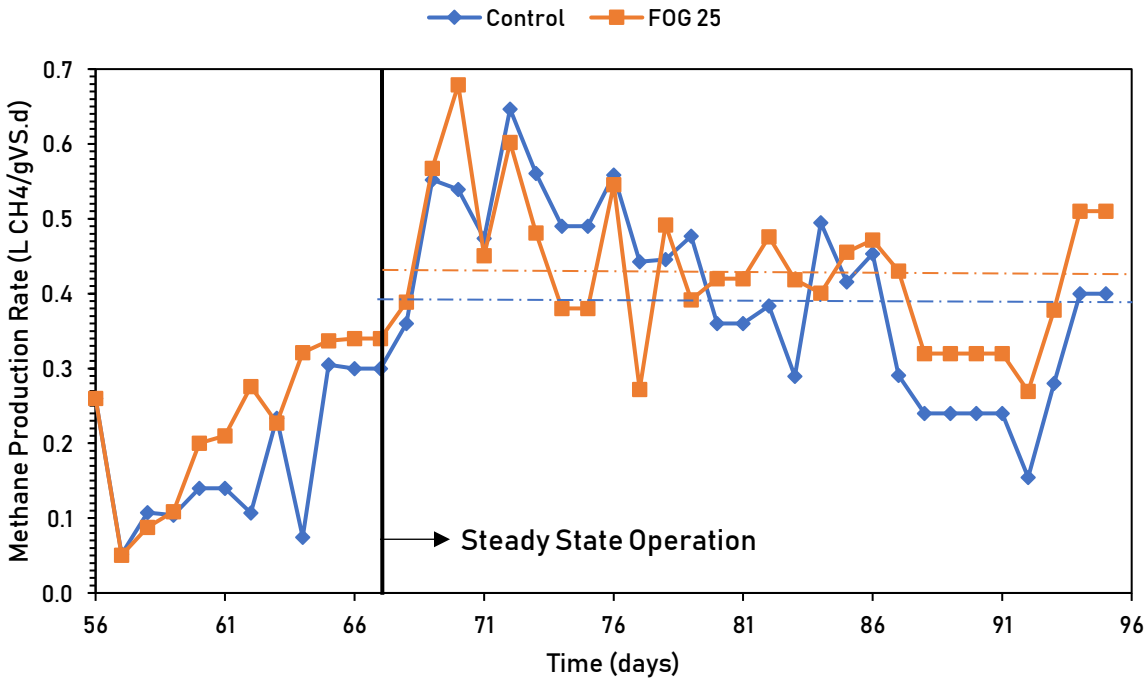

Figure 4.7 Methane production rate during semi-continuous operation

4.3.2 Methane Content

Methane content during semi-continuous operation was found to be on the lower side during the initial phases when the average was 52.28±5.43% for control and 57.24±2.95% for FOG 25. These values later increased to 62.41±4.80% for control and 64.34±3.17% for FOG 25. The rise in methane content can be because of the degradation of lipids as it has been reported that proteins and carbohydrates can be converted to biogas with a 50-58% methane content whereas fats can be converted to biogas with 66-73% methane content (Gujer and Zehnder, 1982). The variation in methane content over the period of semi-continuous operation has been shown in Figure 4.8.

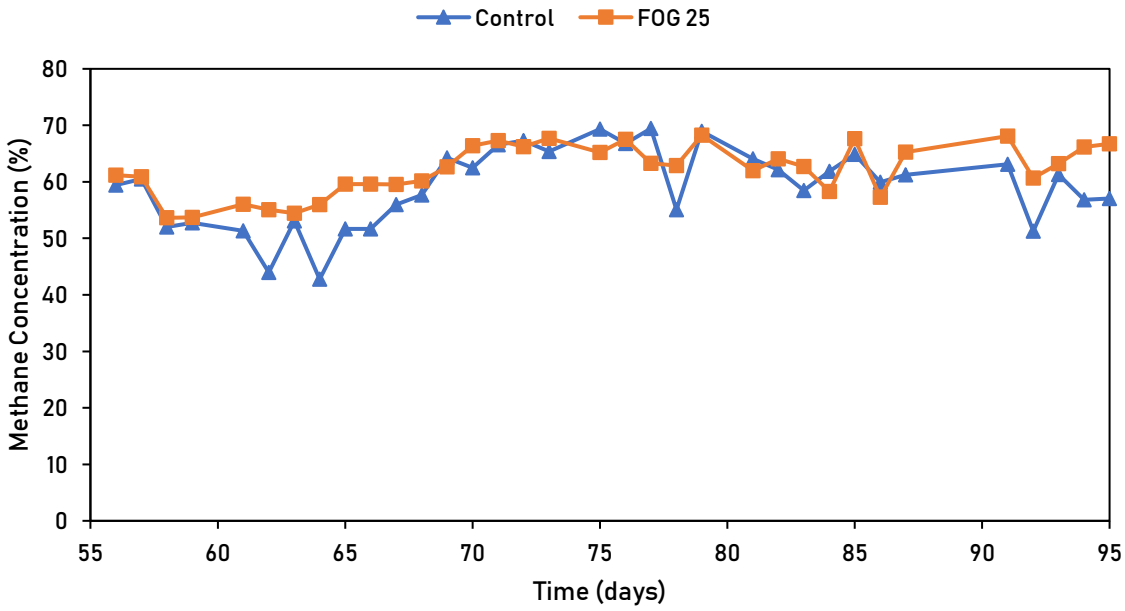

Figure 4.8 Methane content of biogas during semi-continuous operation

4.4 Concentration Profiles of Intermediates during Co-digestion

4.4.1 LCFA Distribution in Feed

LCFA % distribution and concentration distributions have been shown in Figures 4.9 and 4.10, respectively. When the feed sludge (PS+TWAS) and FOG sample was analyzed, wide range of LCFAs were detected. In both the samples, palmitate was found to be the most dominant LCFA (50.86% and 41.03%) followed by oleate (35.23% and 17.51%). In addition to these two in feed sludge, only myristate (5.79%) and myristoleate (4.51%) were detected. On the other hand, FOG mixture consisted numerous LCFAs, some of which were not detected during FOG 25 digester operation – laurate (0.63%), myristate (4.16%), myristoleate (0.35%), palmitoleate (0.87%), heptadecanoate (0.60%), elaidate (6.52%), linelaidate (1.20%), linoleate (6.43%), arachidate (0.50%) among others. During anaerobic treatment, rapid conversion of oleate to palmitate has been shown to thermodynamically favorable over hydrogenation reaction to form stearate (Lalman and Bagley, 2001; Cavaleiro *et al.*, 2016). This explains the presence of high concentrations of palmitate in the digesters rather than a

combination of stearate and oleate. Also, the first LCFA samples for analysis were taken on day 3 and it seems likely that most of the oleate will have completely oxidized to palmitate during the first three days.

The concentration of palmitate and oleate in the FOG sample was found to be 1635.75 mg/L and 698.10 mg/L as compared to 389.40 mg/L and 269.74 mg/L in the PS+TWAS feed sample, respectively. However, stearate was detected only in the FOG sample at 441.69 mg/L. The presence of numerous other LCFAs in FOG mixture which were not detected during the operation of the digesters suggests that most of LCFAs with n(C) < 14 decompose to form short chain fatty acids and although, all unsaturated LCFAs with n(C) = 14 or larger have a natural tendency to undergo β-oxidation (Cavaleiro *et al.*, 2016), they will have an inhibitory impact when present in high concentrations. Based on these findings, it would be interesting to study if the effects of fatty acid with n(C) < 14 at very high concentrations are similar to those of palmitic and oleate leading to inhibition. It might also provide an insight into the underlying mechanism of degradation of such fatty acids. The changes in the profiles of the major LCFAs detected (stearate, palmitate and myristate) during the operation of the digesters have been studied and discussed individually in the following sections.

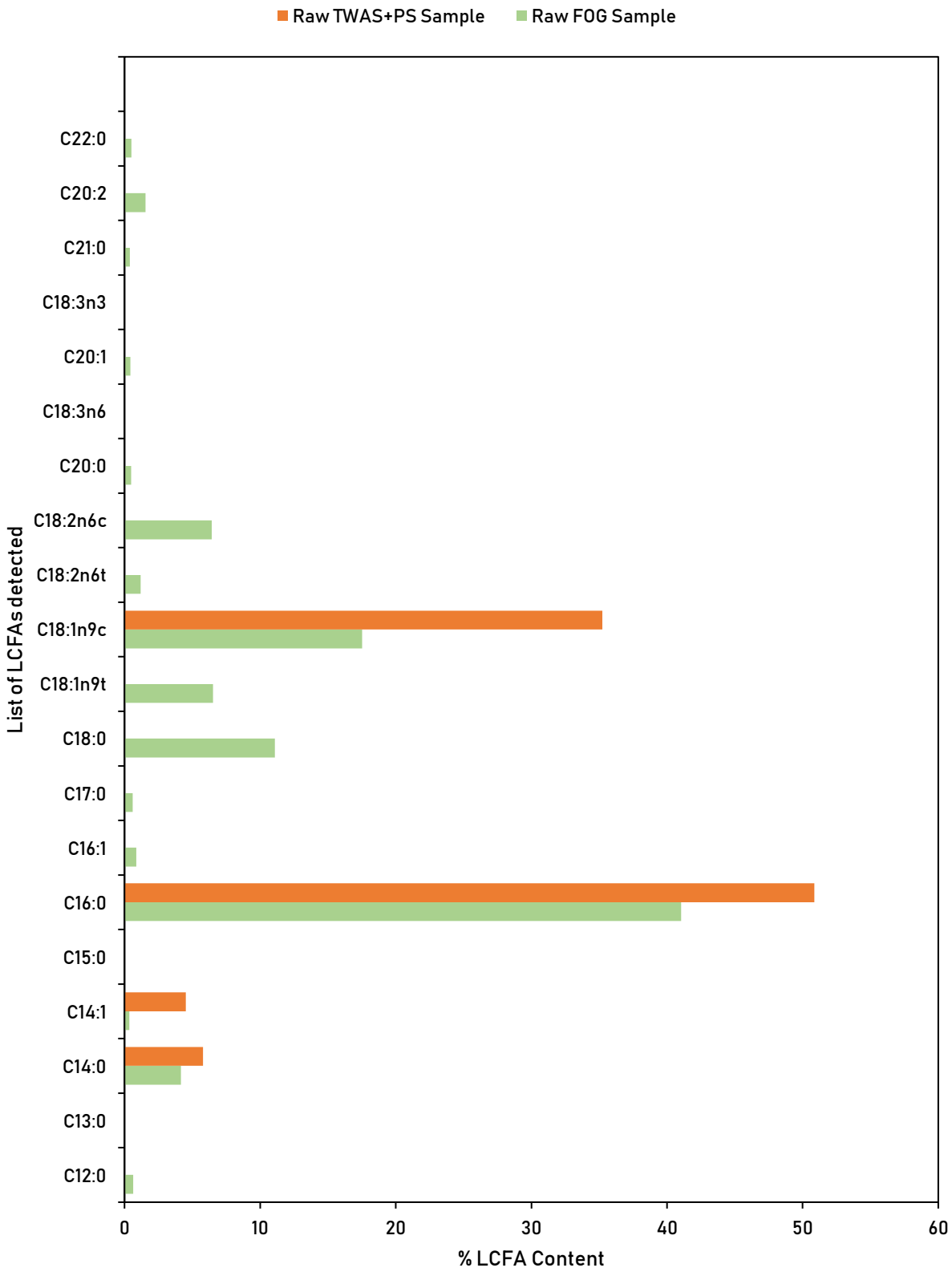

Figure 4.9 % LCFA distribution in feed sludge and FOG mixture

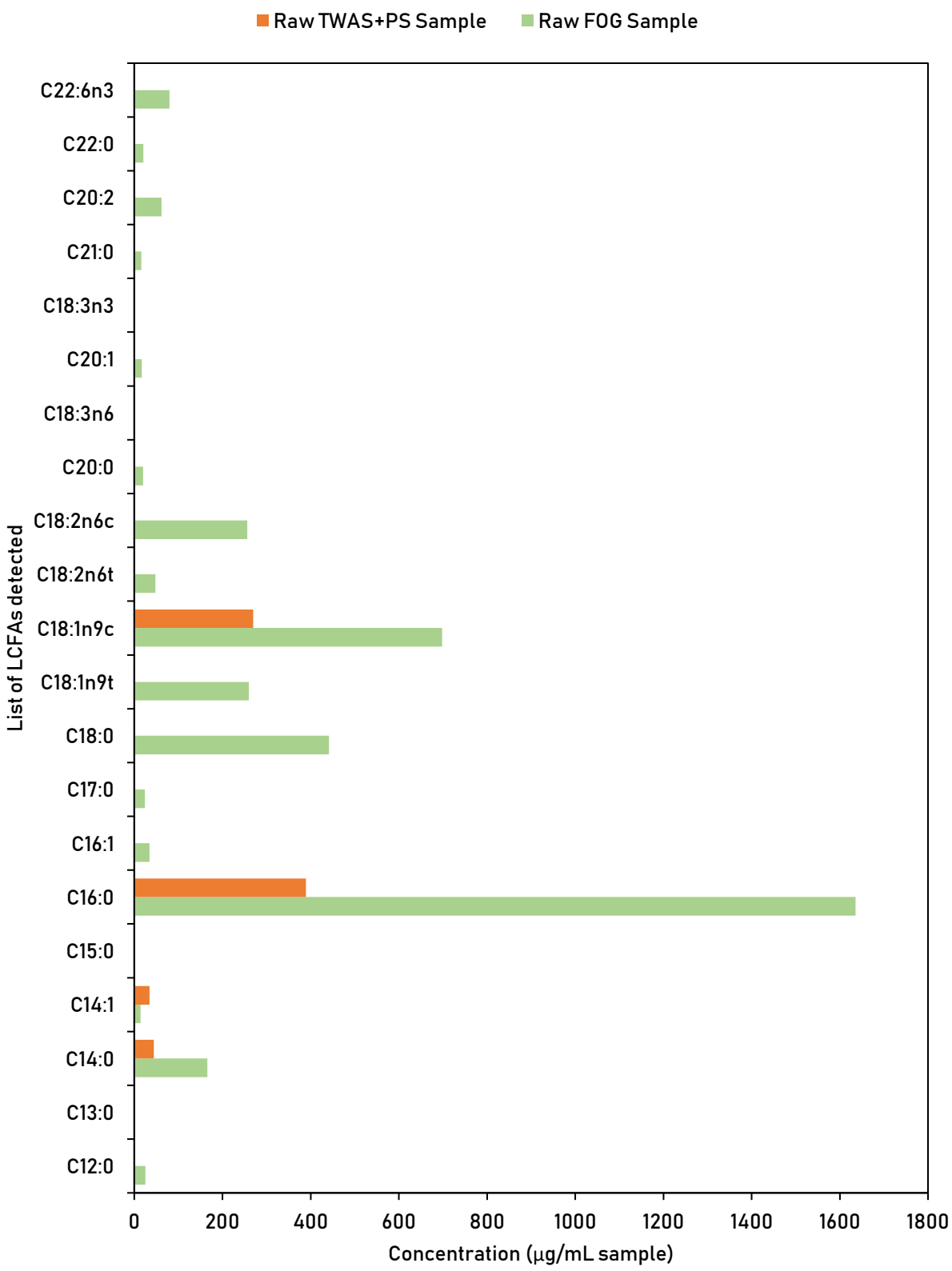

Figure 4.10 LCFA concentrations in feed sludge and FOG mixture

4.4.2 Long Chain Fatty Acids Analysis

Long-chain fatty acid were detected and quantified with the help of gas chromatography which could identify four major fatty acid by-products in the degradation process of lipids in the digester. It is widely known and documented in the literature that lipids breakdown to form long-chain fatty acids and glycerol (Angelidaki, Ellegaard and Ahring, 1999). These LCFAs then undergo degradation via β-oxidation reaction where an LCFA with 'n' carbon atoms degrade to form a fatty acid with (n-2) carbon atoms and acetate as discussed earlier. In both the digesters (Control vs. FOG 25) during the initial phase, stearate (37.4% vs. 22.3%) and palmitate (43.9 vs. 57.0%) were the dominant species with traces of myristate (9.2% vs 2.4%) and myristoleic (5.1% vs. 2.2%) detected. Oleate has been shown to degrade into palmitate, and palmitoleate, which is present in the FOG samples, to form myristate (Cavaleiro *et al.*, 2016). Fatty acid with carbon atoms between 6 and 14 were not detected which suggests contribution of reaction mechanisms other than β-oxidation. The concentration profiles of three important LCFAs have been discussed in the following sections.

4.4.2.1 Stearate

During the initial stages, stearate (C18:0) concentration in the control and FOG 25 is 281.2 mg/L and 235.1 mg/L, respectively. It has been reported that during stearate degradation, no intermediary LCFAs were detected (Ahring, Sandberg and Angelidaki, 1995; Lalman and Bagley, 2001). However, for control, a steady decline in the concentration was observed, whereas for FOG 25, accumulation at several stages was detected with the first occurring around day 10 and the next around day 21, both of which coincide with the lag phase events reported after biogas measurements, which suggests that a reaction mechanism other than β-oxidation might be responsible. In general, stearate concentration was found to be higher in FOG 25 than in control through the entire duration except the start-up phase. After

switching to semi-continuous mode, the average concentration was found to be around 21.6 mg/L and 47.4 mg/L for control and FOG 25, respectively. The profiles have been shown in Figure 4.11.

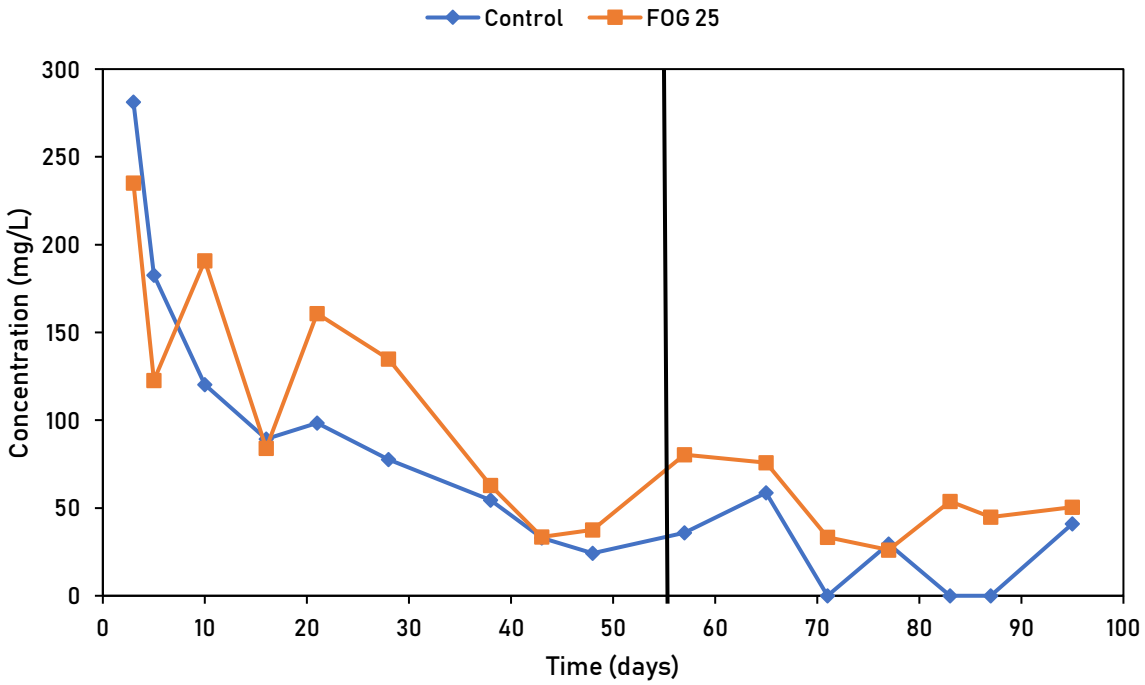

Figure 4.11 Stearate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

4.4.2.2 Palmitate

Palmitate was found to be the most abundant LCFA in the digesters during the early phases of operations. Addition of FOG increased the palmitate concentration almost two folds – 330.1 mg/L in control and 600.5 mg/L in FOG 25. The two primary sources of palmitate are feed sludge and byproduct formation of oleate degradation. As mentioned earlier, the first sample for LCFA analysis was taken at day 3 and most of the oleate is believed to have converted to palmitate since it has been found that conversion of unsaturated LCFA to (n-2) saturated LCFA is fast, non-limiting and independent of methanogenesis (Cavaleiro *et al.*, 2016). The concentration variation trends for FOG 25 are very similar to stearate – accumulation occurring around days 10 and 21, when concentration increased to

430.7 mg/L and 319.9 mg/L from 298.0mg/L and 163.0 mg/L, respectively. This shows that palmitate along with stearate at high concentrations inhibit methanogens and their activity. When the cumulative methane production plot is studied along with the palmitate profile, it can be seen that during the first 28 days of operation when high concentrations of palmitate had accumulated in FOG 25, 1541.36 mL of methane had been produced. On the other hand, in the following 27 days when the concentrations dropped, it produced 2819.35 mL of methane – a rise of 82.9%. After switching to semi-continuous mode, it was found that the difference in concentrations was not significant – average concentration was found to be around 38.2 mg/L and 77.7 mg/L for control and FOG 25, respectively which reflects in the methane production plot as well. The profiles have been shown in Figure 4.12.

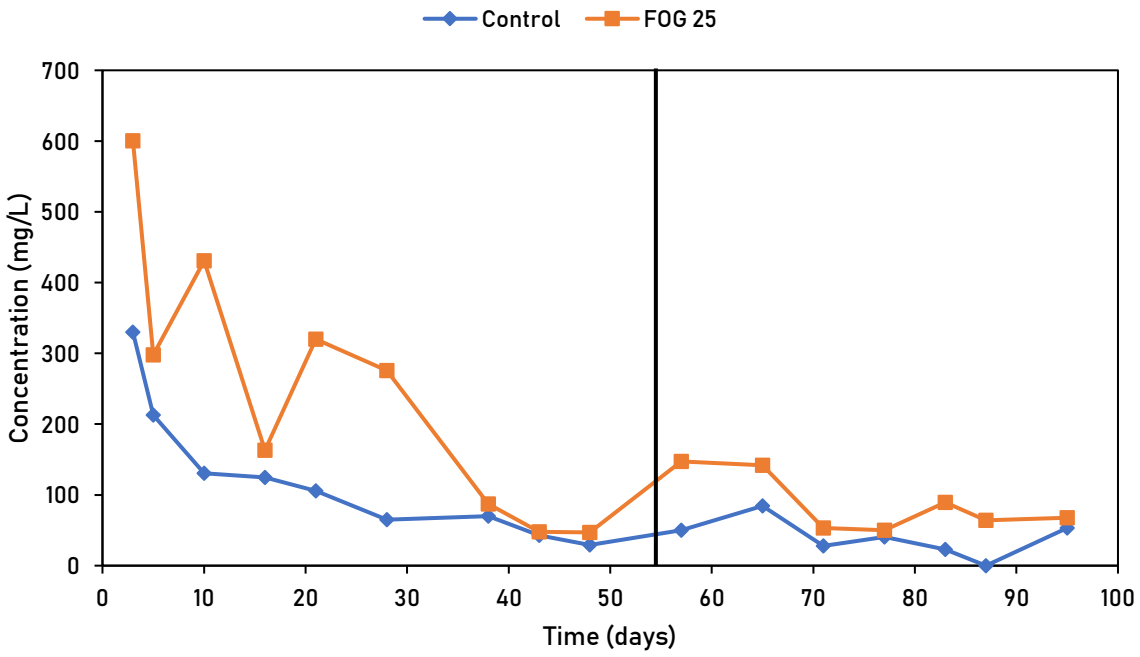

Figure 4.12 Palmitate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

4.4.2.3 Myristate

Myristate, which is a byproduct of palmitate and palmitoleate decomposition, was found to be present during the initial stages at concentrations of 68.9 mg/L (0.30 mM) and 25.6 mg/L (0.11 mM) in control and FOG 25, respectively and made up only 9.2% and 2.4% of the total LCFAs present in those digesters. Myristate is reported to have a minimal inhibitory concentration (MIC) of 2.6 mM and MIC_{50} of 4.8 mM which tends to become more toxic in presence of laurate (Koster and Cramer, 1987; Soliva *et al.*, 2003).

Later in our study, the concentration of myristate increased steadily with time (58.0 mg/L on day 57) in FOG 25. This steady rise can be attributed to the decomposition of either oleate, linoleate, palmitate or palmitoleate (David and Lalman, 2000; Cavaleiro *et al.*, 2016). During batch operation of the control, concentrations dropped from 110.2 mg/L to 44.2 mg/L from day 28 to day 57 which could be because of higher rate of degradation than generation. The increasing presence was also seen when the % compositions were studied. Myristate composition increased to 41.1% in control and 9.2% in FOG 25 by the end of batch operation. During semi-continuous operation, it contributed an average of 49.9% and 33.0% of the total LCFA present in control and FOG 25. The concentration profile of myristate has been shown in Figure 4.13. It was intriguing to find that none of the LCFAs with carbon length less than n(C) =14 were detected during the analysis which suggests that C14 LCFAs might be degrading directly to form volatile fatty acid of carbon lengths ranging from n(C) = 2 to 6. Apart from these three major LCFAs, noticeable quantities of myristoleate were also detected. This unsaturated C14:1 LCFA is believed to be entering the system from the feed since it was detected in trace amounts as shown earlier.

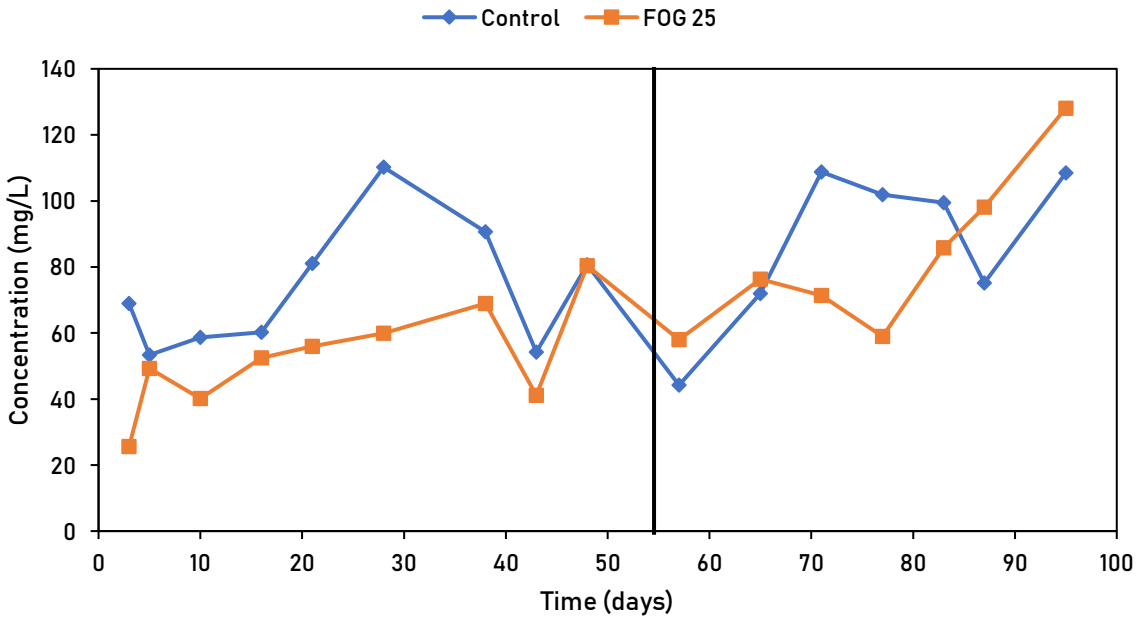

Figure 4.13 Myristate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

4.4.3 Volatile Fatty Acids Analysis

In order to develop a better understanding regarding the lag phase caused as a result of inhibition, it was deemed beneficial to identify and quantify different volatile fatty acids (VFAs) being formed during the breakdown of long-chain fatty acids in the two digesters. This in turn facilitates a direct evaluation of the effect of FOG addition to the digester. Six different VFAs – acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate were detected and thereafter, tracked during the batch operation.

During the start-up phase, 41.61 mM and 35.82 mM acetate was detected in the control and FOG 25 digesters on day 2, respectively. In both the reactors, acetate accumulated for the first few days and then, was consumed by the aceticlastic methanogens to form methane. The acetate present in the reactor during the initial phase is either already present in the feed or is formed as a result of fermentation and/or β-oxidation reactions. When accumulation of acetate was compared with daily biogas

production, we observed that acetate consumption and rise in biogas production coincided. This accumulation of acetate can be attributed to the stringent effect of oleate and palmitate on aceticlastic as well as hydrogenotrophic methanogens (Sousa *et al.*, 2013). It is evident in Figure 4.14 that acetate concentration starts decreasing from day 7, which is when the first lag phase ended in FOG 25. On day 10, control and FOG 25 produced 450 mL and 325 mL biogas, respectively and on the same day, concentration of acetic dropped to 13.8 mM and 24.72 mM, respectively. However, on day 18 acetate concentration was found to be 17.24 mM and 6.45 mM in control and FOG 25, which gradually started dropping corresponding to the end of second lag phase. For batch operation, no more accumulation of acetate was noticed since the acetate that was produced in the reactor was immediately being consumed by the microbial community that had adopted to lipid-rich substrate. Another rise in acetate concentration can be noticed beyond day 55 when reactors were switched to operate in semi-continuous mode. Equal volumes of samples were withdrawn and added every two days to maintain a retention time of 30 days which translated to feeding acetate-rich samples.

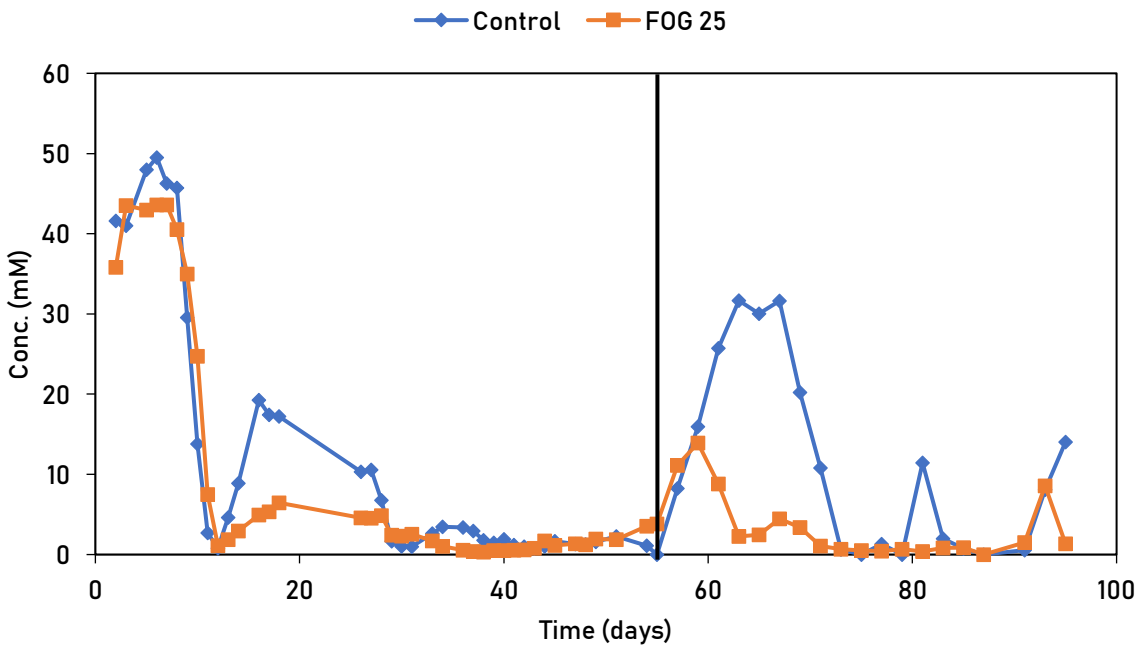

Figure 4.14 Acetate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

Propionate, on the other hand, was found to accumulate over time and achieved steady state value after 35 days of operations. Accumulation of propionate in the initial phases is typical of digesters and have been reported in several studies (Vavilin and Lokshina, 1996; Wang *et al.*, 2009). It has also been found that accumulation of propionate along with a drop in pH is indicative of digester upset (Nielsen, Uellendahl and Ahring, 2007; Franke-Whittle *et al.*, 2014). Propionate concentration during the start-up phase was found to be 9.35 mM and 9.50 mM in control and FOG 25, respectively, during which corresponding pH values 6.35 and 6.15 were noted. After day 35, an average concentration of 25.94±2.39 mM and 22.60±1.45 mM along with pH values in the range of 6.78 - 8.72 and 6.84 - 8.47 were maintained in control and FOG 25, respectively. The pH values remained around neutral which was indicative that the accumulation of propionate was not deteriorating the digester performance. Concentration profile for propionate and the pH variations during the operation of the digesters have been shown in Figure 4.15 and Figure 4.16 below. From the results obtained, it can be hypothesized that either propionate does not undergo degradation, or, that the rate of formation and consumption are balanced providing a steady value.

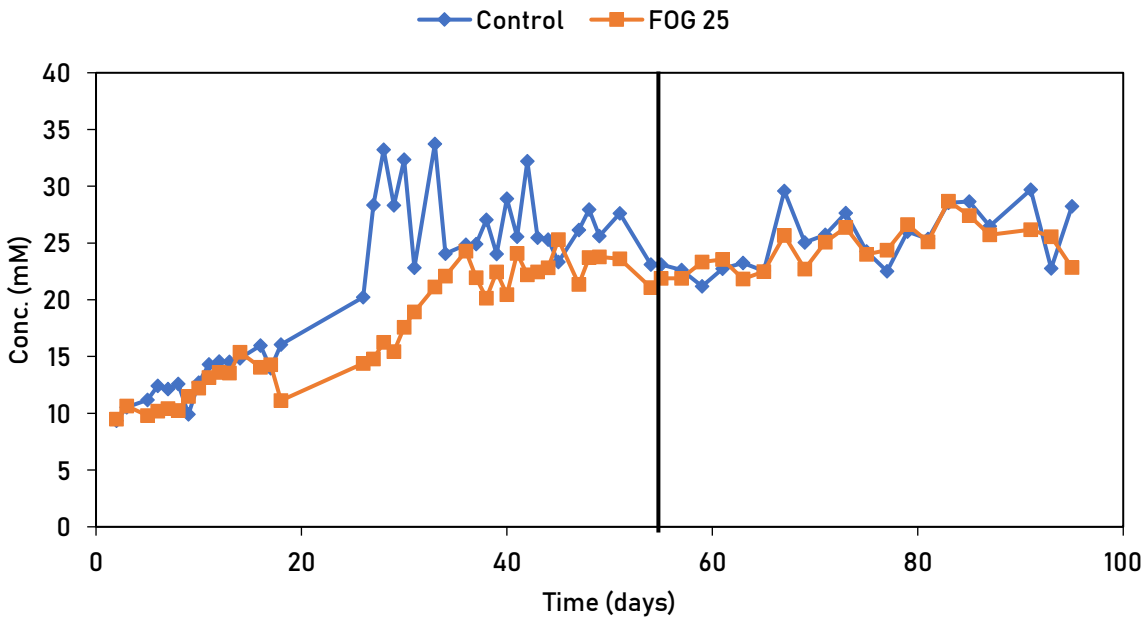

Figure 4.15 Propionate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

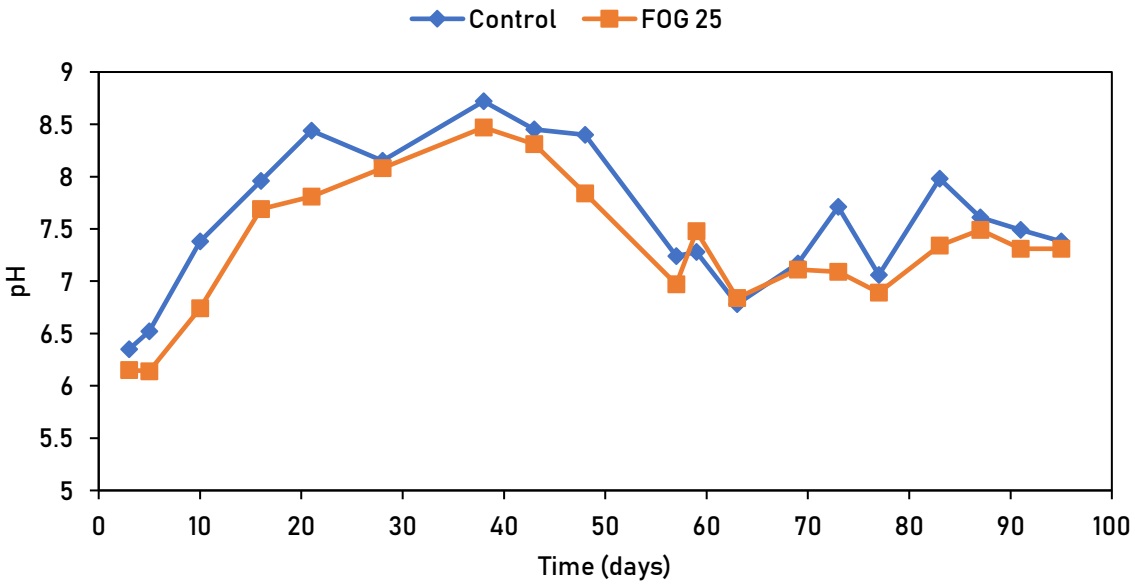

Figure 4.16 pH variations during the operation of bench-scale digesters

Among the different intermediates studied, iso-butyrate has provided some very interesting insights in understanding the effects of FOG loading on the ability of microbes to degrade certain intermediates. Hill et al. in the operation of a digester found that concentrations of 0.06-0.15 mM of iso-butyric and iso-valerate to have deteriorating effect on the functioning (Hill and Bolte, 1989). However, in our study, the initial concentration of iso-butyrate was found to be 3.04 mM and 2.55 mM in control and FOG 25 respectively. In the control, it was found that the concentration increased to 5.06 mM on day 26 and dropped to 0.87 mM on day 34. On the other hand, for FOG 25, the concentration increased to 4.54 mM and then dropped to 2.66 mM on day 33 but increased again to 4.12 mM on day 34. Beyond this point, no significant drop is observed. There could be two possible reasons behind this behavior. First, the microorganisms present in the control reactor responsible for degrading iso-butyrate have been inhibited by long-chain fatty acids present in FOG 25. Second, once the lag phase ends which coincides with the drop in iso-butyrate concentration, the consumption of existing iso-butyrate is compensated by production of iso-butyrate as a result of degradation of longer chain fatty acids (which may not be present or if they do, be in very low concentrations in the digester with no FOG loading), thereby maintaining a steady concentration. Although the difference between the concentrations in the

two reactors is not large, it helps in understanding the role of intermediates when co-digesting lipid-rich substrate. Iso-butyrate, like acetate, could already be present in the feed or form as a result of degradation reactions which can explain the accumulation after day 55, when digesters were switched to operate in semi-continuous mode as shown in Figure 4.17 below.

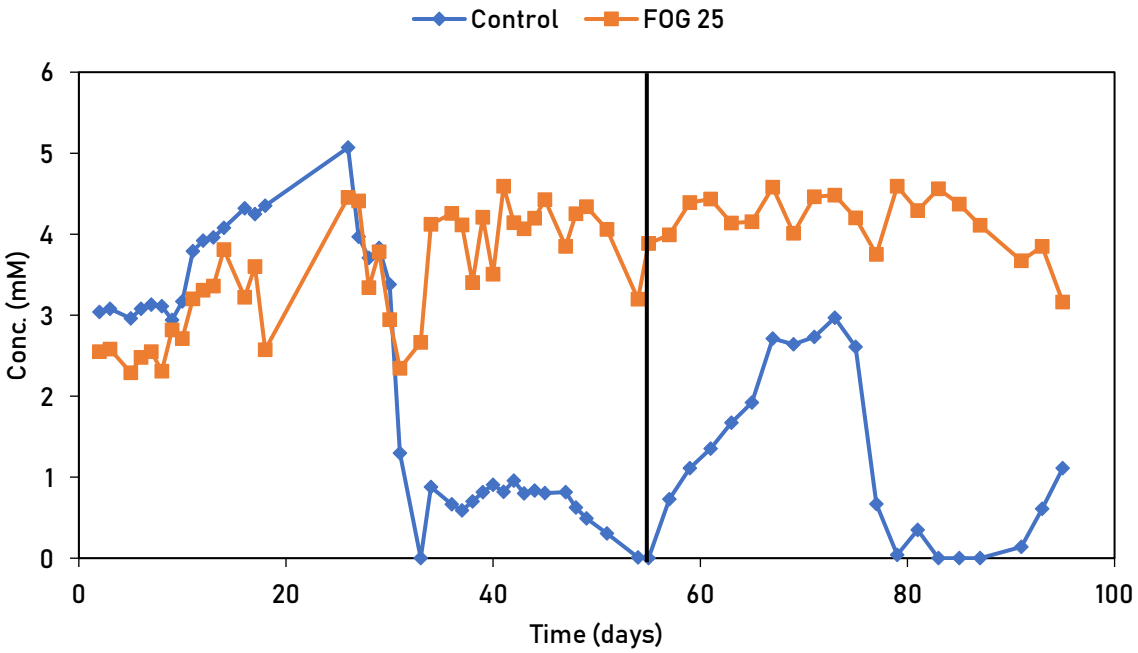

Figure 4.17 Iso-butyrate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

Another commonly found volatile fatty acid during the anaerobic degradation of complex substrates is butyrate plotted in Figure 4.18. Following butyrate and iso-butyrate concentration can be beneficial as it has been shown that they are reliable indicators to detect stress in digesters (Ahring, Sandberg and Angelidaki, 1995). During the initial phase, concentration of 7.86 mM and 10.13 mM were found in the control and FOG 25 digesters, respectively. The concentrations were found to be gradually decreasing until day 27 when no butyrate was detected in the control and was 4.49 mM in FOG 25. However, between days 28 and 33, no clear peak for butyrate peak was noticed and therefore, it was assumed to be zero in both the digesters. However, a very noticeable difference was observed in butyrate degradation in FOG 25 when compared to the control. In FOG 25, butyrate took 28 d to

decompose completely, whereas it took only 16 d in the case of control. This is because of the high concentration of LCFAs during the initial phases of operation resulting in the inhibition of butyrate degrading microbes. Inhibition of butyrate degradation has been reported earlier where they found that 100 mg/L of linoleate, 100 mg/L of total LCFA and 300 mg/L of total LCFA resulted in less than 5% removal in 10 days (Lalman and Bagley, 2002).

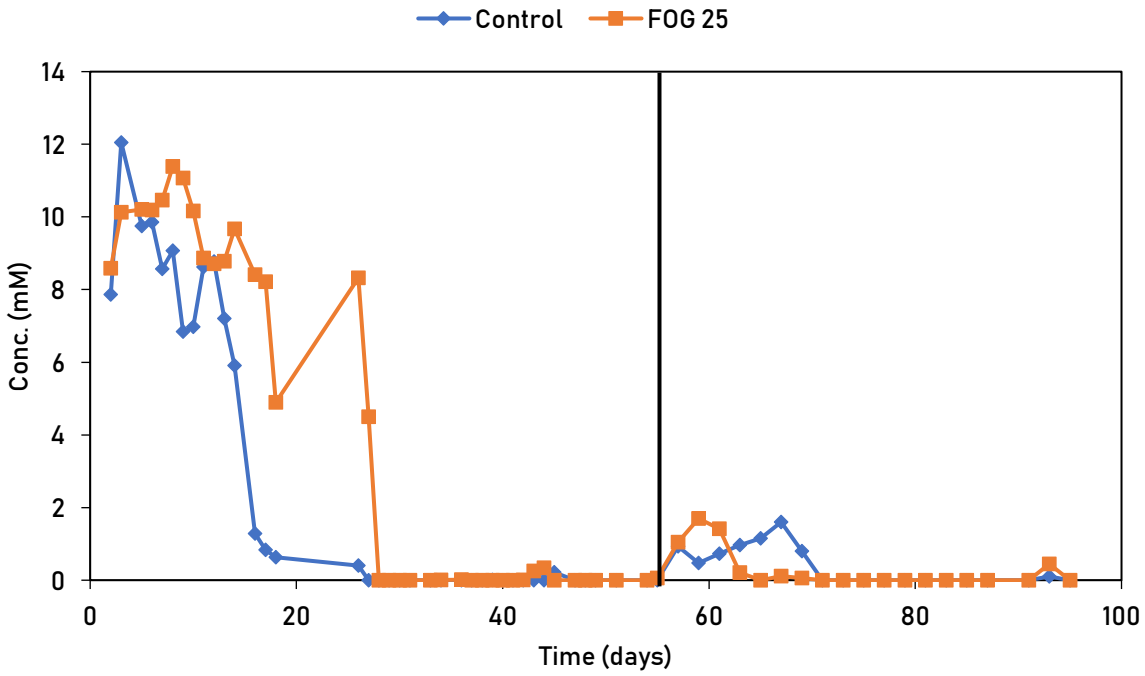

Figure 4.18 Butyrate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

Iso-valerate was another volatile fatty acid that was detected while conducting the HPLC analysis. Over the period of batch operation, no significant differences were observed in the concentrations in either of the digesters. Average concentrations of iso-valerate were maintained at 7.53 mM and 6.65 mM in control and FOG 25, respectively. The concentration of iso-valerate remains steady either due to the equal rates of formation and consumption of iso-valerate, or inability of the microorganisms to degrade. The concentration profile of iso-valerate can be seen in Figure 4.19.

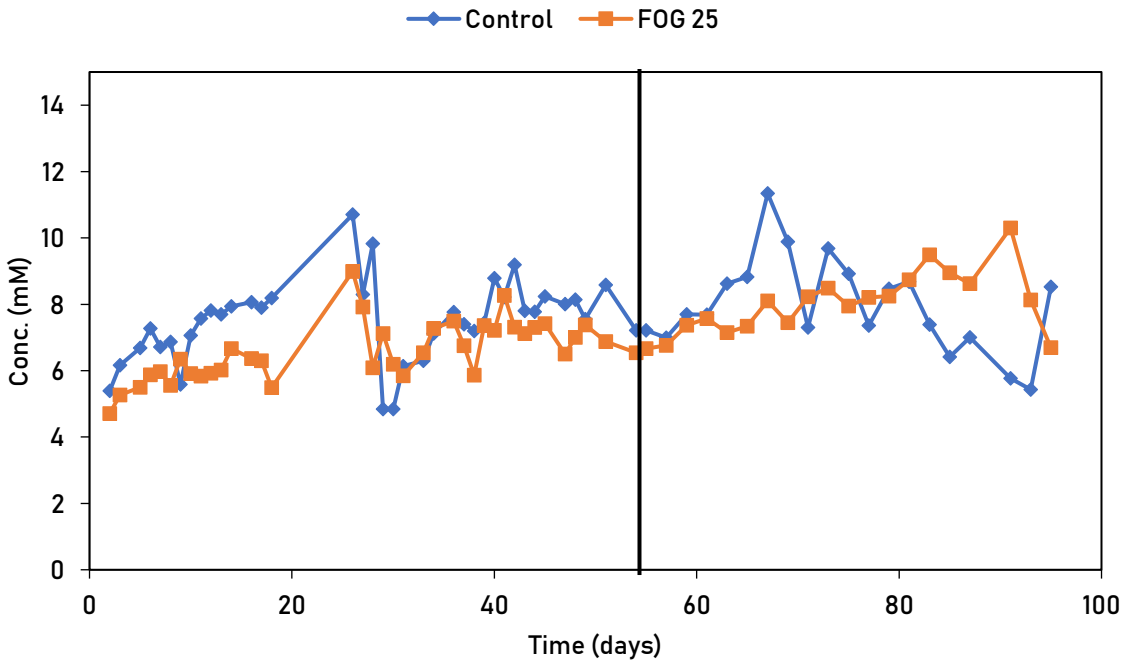

Figure 4.19 Iso-valerate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

The final volatile fatty acid of those detected during the analysis is valerate. The concentration of valerate were similar to those observed for iso-valerate with initial values of 3.02 mM and 4.30 mM for control and FOG 25, respectively. The concentrations did not change much until day 18 but was found to accumulate for a short period of time before it started dropping down again. In this case, it is interesting to observe that more valerate was accumulated in the reactor with FOG loading as compared to the control. Also, the degradation of valerate in FOG 25 was found to be slower. The accumulation discussed above occurs just before the inhibition is overcome in the second lag phase. Therefore, the accumulation and subsequent degradation of valerate may be have an impact on inhibitory effects of the intermediates that are formed during the process. The concentration profile for valerate has been shown in Figure 4.20.

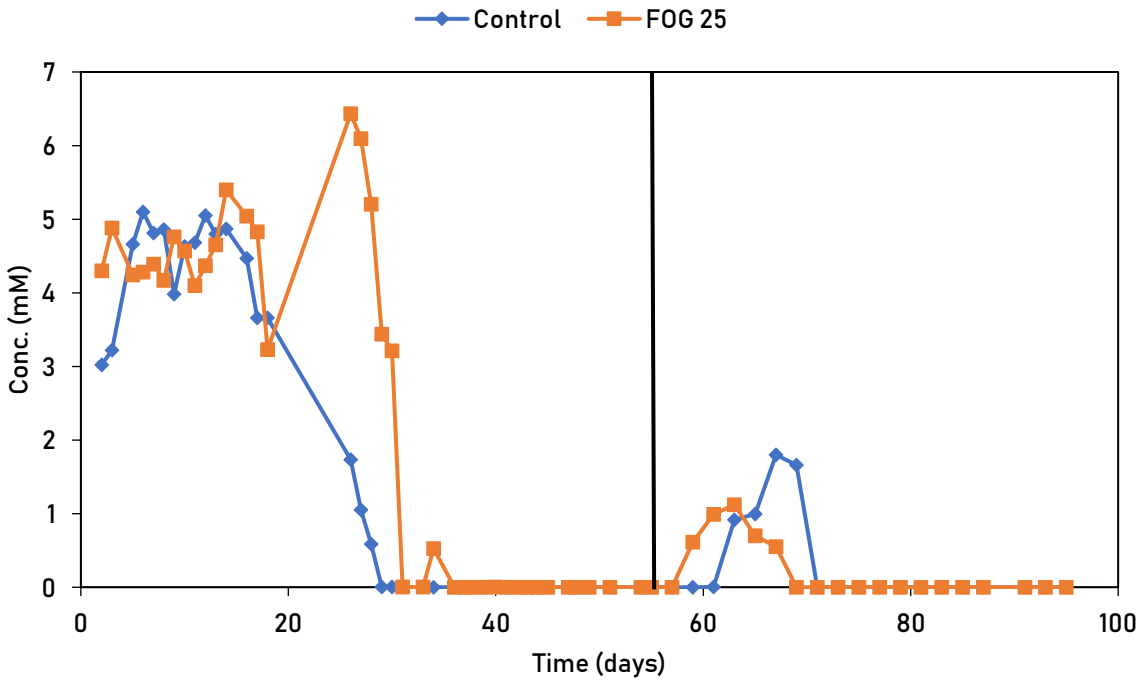

Figure 4.20 Valerate concentration profile in control and FOG 25 with black line showing transition from batch to semi-continuous mode.

The results obtained from all the experiments and analyses conducted show that LCFAs, especially stearate and palmitate can have a noticeable impact on the degradation process of different compounds thereby affecting biogas production via methanogenesis. Lag phases which were noticed during the operation in batch mode was found to coincide with accumulation of LCFAs, which in turn resulted in either accumulation or reduction in the rates of degradation of VFAs like propionate, butyrate, iso-butyrate, and valerate. It was also found through the results obtained from semi-continuous operation, that microbial culture acclimated to LCFAs can successfully metabolize at FOG loading of 25% on volume basis and that digester functioning at high FOG loadings can overcome inhibition.

5. CONCLUSIONS

This study to investigate the inhibition of anaerobic co-digestion of fats, oil and greases with municipal sludge has provided insights into the effects of FOG addition on the process of breakdown on numerous intermediates that are formed along with the obtaining the optimal ratio for maximizing biogas production. It has also enabled us to conclude the following:

(1) All batch reactors with FOG addition were affected due to high concentration of LCFAs and only FOG 25 recovered activity after 45 days and produced 771.5 mL biogas in the following 20 days, in contrast to 94.67 mL produced between days 20 and 45.

(2) During batch mode operation of the bench-scale digesters, cumulative methane production increase by 29.93% and corresponding % COD conversion values were 49.44% and 50.96% for control and FOG 25 which is in agreement with results obtained in other studies.

(3) Methane content was found to be higher in FOG 25 (61.57±4.28% during BMP and 64.34±3.17% during semi-continuous) than in control (59.38±3.53% and 62.41±4.80% during semi-continuous) after the acclimation phase which can be attributed to higher methane yields of lipids.

(4) During steady-state semi-continuous operation, addition of 25% FOG increased methane production rate by 7.5% and % COD conversion to methane increased by 30.34%.

(5) Accumulation of high concentrations of palmitate (>300 mg/L) and stearate (>150 mg/L) coincided with the lag phase periods which supports the notion that LCFAs at high concentrations are inhibitory. These concentrations are lower than those reported in the literature which shows that a combination of LCFAs have a synergistic effect and can be inhibitory at lower concentrations.

(6) Noticeable differences were observed between control and FOG 25 in terms of degradation of iso-butyrate, butyrate and valerate. Accumulation of iso-butyrate was observed in FOG 25 which had degraded after 30 days in control. In the cases of butyric and valerates, accumulation followed by delayed degradation was noticed which suggested that LCFA influenced not only methanogenesis but the acidogenesis as well.

The results obtained from these studies have shown that addition of FOG influence LCFA and VFA degradation processes and that there could possibly be LCFA degrading mechanisms other than β-oxidation during anaerobic co-digestion. Based on these outcomes, to better understand the factors responsible for the variations in processes, we recommend that future studies should focus on (a) effects of varying SRTs, (b) effects on microbial communities of FOG addition, and (c) kinetic modeling of biogas production and degradation of various intermediates

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APPENDIX A

Sample Characterization Table A1. BMP Test 1

Sample	TWAS	FOG	Inoculum
TS (g/L)	43.65	12.00	18.25
VS (g/L)	37.20	14.050	16.15
pH	7.36	4.47	7.82
Alkalinity (mg/L as $CaCO_3$)	304	154	862
tCOD (g/L as COD)	74.96	64.675	58.83

Table A2. BMP Test 2

Sample	PS	TWAS	FOG	Inoculum
TS (g/L)	31.00	44.50	62.00	20.50
VS (g/L)	26.50	34.00	59.50	15.00
tCOD (g/L as COD)	63.35	80.00	102.35	34.08
Ammonia (mg/L NH_3-N)	174	180	551	1535
Phosphorous (mg/L PO_4-P)	814	7625	1745	2990

Table A3. Batch Operation of Bench-scale Digesters

Sample	PS	TWAS	FOG	Inoculum
TS (g/L)	34.50	71.00	26.50	21.50
VS (g/L)	30.50	53.50	25.50	17.00
tCOD (g/L as COD)	47.95	70.15	64.85	25.29
Ammonia (mg/L NH_3-N)	244.5	509	384	1410
Phosphorous (mg/L PO_4-P)	687	9040	686	3475

Table A4. Semi-continuous Operation of Bench-scale Digesters

Sample	Raw FOG	Raw PS+TWAS	Control	FOG 25
TS (g/L)	27.53	37.24	25.28	21.45
VS (g/L)	23.86	26.80	16.64	14.69
tCOD (g/L as COD)	84.52	54.85	36.02	36.19
Ammonia (mg/L NH_3-N)	225	801.5	1692.5	1520.5
Phosphorous (mg/L PO_4-P)	558	3158	4562	3774

Table A5. Average Cumulative Biogas Produced During BMP Test 1 (per g VS added)

Day	FOG 0	FOG 25	FOG 33	FOG 50	FOG 100
	mL	*mL*	*mL*	*mL*	*mL*
0	0.0	0.0	0.0	0.0	0.0
1	18.5	16.2	14.8	13.5	12.1
2	35.6	26.3	23.3	20.2	14.0
3	49.2	35.1	31.7	26.8	15.0
4	62.7	43.3	38.3	32.2	16.0
5	75.9	51.3	45.1	37.4	17.4
6	85.3	60.1	52.9	42.8	18.6
7	93.8	70.1	61.5	50.3	19.9
8	102.2	80.4	72.3	59.5	20.8
9	111.2	90.0	83.1	71.0	21.6
10	120.4	97.5	90.7	79.7	22.3
11	129.2	103.4	94.5	84.5	22.8
13	144.2	115.2	102.1	84.7	23.8
14	154.6	125.3	110.3	87.9	24.0
15	162.6	136.9	120.0	93.1	24.9
16	170.9	148.4	130.4	93.6	25.7
17	177.0	159.3	144.6	102.6	26.4
18	181.1	171.1	155.8	111.1	28.8
20	189.5	192.9	172.7	130.1	48.0
21	196.0	208.5	188.1	146.1	63.0
22	202.9	221.2	202.9	159.8	78.0
23	203.3	229.5	218.4	175.2	83.1
24	214.6	234.8	231.8	189.9	86.5
25	221.4	238.9	242.3	204.6	87.9
27	231.9	246.1	250.5	225.0	89.2
28	238.4	252.4	254.0	238.2	89.9
29	243.6	258.7	256.8	248.3	91.3
30	248.0	265.6	260.3	257.9	92.3
31	251.4	271.6	266.5	267.5	93.7
32	253.7	276.8	273.6	275.4	95.0
34	257.1	285.9	285.0	286.9	96.4
35	259.3	292.4	292.2	295.2	97.4
36	261.3	298.4	299.7	303.8	98.1
37	263.1	304.1	307.4	313.4	98.8
38	264.9	308.3	315.6	321.5	99.5

Table A6. Average Cumulative Biogas Produced During BMP 2

Days	Blank	FOG 0	FOG 25	FOG 50	FOG 75	FOG 100
	mL	*mL*	*mL*	*mL*	*mL*	*mL*
0	0.0	0.0	0.0	0.0	0.0	0.0
1	21.3	67.0	36.0	23.3	18.8	9.7
2	32.7	124.3	51.0	28.0	18.8	9.7
3	41.7	179.0	62.7	40.3	29.2	13.7
4	52.3	233.3	69.0	47.3	34.5	17.7
5	62.7	296.7	75.7	53.0	39.2	19.7
6	70.3	356.3	78.0	56.0	40.8	19.7
7	78.0	419.7	82.8	59.8	44.5	21.3
9	87.0	515.7	98.0	67.2	49.7	25.8
10	90.3	541.7	110.2	71.5	50.0	26.7
11	90.7	556.5	126.0	78.0	51.0	27.2
13	95.7	576.7	168.8	105.8	54.8	29.7
16	103.8	601.3	181.3	153.0	64.8	42.2
17	107.0	617.0	187.0	171.8	77.2	58.8
18	109.3	627.2	190.3	180.2	94.3	80.2
19	109.3	635.7	191.7	181.8	122.0	110.8
20	112.0	643.2	193.7	185.5	152.3	136.2
21	114.8	651.8	194.8	188.7	177.8	156.2
23	117.8	667.5	196.8	192.0	196.5	170.0
25	120.3	681.0	199.5	196.7	210.2	181.8
28	123.3	694.8	203.5	200.5	218.0	191.5
30	124.5	703.8	208.5	204.8	224.0	199.3
32	127.7	711.8	215.3	209.0	229.7	205.7
35	128.7	721.0	227.5	216.3	235.3	211.3
38	131.7	732.0	244.8	227.3	241.3	217.2
41	135.8	740.3	262.5	233.3	246.2	221.8
43	136.2	745.7	275.0	236.0	248.5	224.8
45	136.7	750.8	288.3	238.2	250.2	227.5
48	138.3	756.5	313.5	239.0	252.3	231.2
51	139.3	762.2	360.7	239.8	254.0	234.0
55	140.8	769.7	482.0	242.8	256.8	238.0
62	148.0	783.7	792.0	249.2	262.5	244.7
65	148.0	787.7	892.3	250.7	264.5	246.0
68	148.0	791.8	965.7	251.5	266.3	247.7
72	148.8	797.8	1020.3	254.8	268.7	250.2
76	150.0	799.5	1059.8	256.7	269.8	251.7
79	152.0	805.5	1090.7	259.2	272.2	253.3
84	154.2	811.0	1133.3	262.0	274.2	254.7

Table A7. Cumulative Biogas Production During Batch Mode Operation of Bench-scale Digesters

Days	Control	FOG 25
	mL	*mL*
0	0.0	0.0
1	200.0	139.0
2	410.0	267.0
3	526.0	315.0
5	644.0	393.0
6	712.0	411.0
7	806.0	441.0
8	978.0	514.0
9	1191.0	680.0
10	1641.0	1005.0
11	2035.0	1302.0
12	2160.0	1552.0
13	2201.0	1560.0
14	2297.0	1577.0
16	2455.0	1613.0
17	2713.0	1615.0
18	3069.0	1616.0
20	3449.0	1625.0
22	3607.0	1700.0
23	4016.0	1737.0
25	4368.0	1845.0
26	4665.0	1954.0

Days	Control	FOG 25
	mL	*mL*
28	5272.0	2322.0
29	5544.0	2502.0
30	5807.0	2703.0
31	5940.0	2948.0
33	6142.0	3683.0
34	6201.0	4044.0
36	6266.0	4746.0
37	6289.0	4993.0
38	6342.0	5216.0
39	6353.0	5346.0
40	6385.0	5481.0
41	6405.0	5596.0
42	6412.0	5681.0
43	6414.0	5761.0
44	6420.0	5843.0
45	6441.0	5932.0
47	6499.0	6113.0
48	6509.0	6139.0
49	6524.0	6227.0
51	6559.0	6397.0
54	6625.0	6555.0
55	6625.0	6595.0

Table A8. Daily Biogas Production Semi-Continuous Mode Operation of Bench-scale Digesters

Day	Control	FOG 25
	mL	*mL*
56	229.0	276.0
57	37.0	56.0
58	186.0	170.0
59	98.0	131.0
61	304.0	454.0
62	208.0	334.0
63	152.0	277.0
64	206.0	360.0
65	232.0	339.0
67	535.0	730.0
68	317.0	415.0
69	395.0	570.0
70	471.0	638.0
71	348.0	429.0
72	504.0	599.0
73	468.0	452.0
75	728.0	768.0
76	461.0	509.0
77	320.0	311.0
78	529.0	511.0
79	272.0	336.0
81	627.0	925.0
82	342.0	467.0
83	292.0	444.0
84	398.0	482.0
85	318.0	373.0
86	439.0	620.0
87	242.0	371.0
91	795.0	1171.0
92	268.0	346.0
93	229.0	370.0
95	728.0	967.0

Table A9. Cumulative Methane Production During BMP 2

Days	Control	FOG 25	FOG 50	FOG 75	FOG 100
	mL	*mL*	*mL*	*mL*	*mL*
0	0.0	0.0	0.0	0.0	0.0
1	50.0	11.4	3.6	1.3	0.0
2	93.1	15.2	6.3	1.6	0.0
3	124.9	23.6	10.7	5.4	0.2
4	157.4	23.8	12.6	6.9	0.2
5	187.9	27.6	12.6	7.7	0.3
6	224.7	28.8	12.7	7.7	0.7
8	338.5	36.3	18.6	12.9	3.4
9	387.4	39.0	19.3	12.9	4.2
10	416.5	45.8	23.4	13.3	6.6
11	431.1	64.6	28.9	17.9	6.6
13	448.2	103.3	54.6	17.9	10.5
16	464.8	109.3	90.0	27.1	16.1
17	471.8	111.5	98.6	35.2	21.5
18	474.9	112.5	98.6	47.6	28.9
20	486.1	112.5	98.6	81.4	66.9
21	494.6	115.4	104.9	134.6	95.4
23	506.6	115.4	105.7	134.6	106.8
24	513.8	117.4	107.9	140.9	112.3
25	517.6	118.6	108.8	140.9	114.7
28	530.8	120.8	117.6	150.5	119.2
30	542.8	126.3	129.7	166.6	146.1
36	545.9	132.6	138.0	169.4	146.3
38	557.0	143.0	144.4	170.5	152.5
41	557.1	144.8	149.8	176.2	152.5
43	559.6	152.1	150.5	176.2	154.3
45	563.1	160.8	152.0	177.1	155.7
48	566.2	176.3	152.0	178.5	156.5
51	567.2	203.3	152.0	178.5	157.8
55	573.0	290.4	155.2	179.5	160.7
58	577.6	382.1	157.2	187.5	161.0
62	580.3	478.6	161.1	187.5	161.0
65	583.7	537.5	161.5	187.5	161.0
68	583.7	600.8	168.3	197.1	171.0
72	592.5	628.8	168.3	197.1	171.0
76	594.1	661.5	172.1	198.4	172.1
79	598.1	687.2	172.1	198.7	172.2
84	604.5	727.8	178.8	202.3	173.4

Table A10. Cumulative Methane Production During Batch Mode Operation of Bench-scale Digesters

Days	Control	FOG 25
	mL	*mL*
0	0.0	0.0
1	64.1	14.2
2	157.1	34.2
3	230.4	54.7
5	300.5	107.4
6	341.8	125.7
7	382.5	149.3
8	502.0	158.8
9	645.8	309.2
10	944.2	536.5
11	1284.3	802.9
12	1351.2	946.7
13	1353.7	946.7
14	1415.4	958.7
16	1501.7	978.3
17	1661.9	983.1
18	1912.6	1019.6
20	2192.4	1019.6
22	2294.5	1077.3
23	2584.2	1108.8
25	2837.7	1194.7
26	3071.5	1260.6

Days	Control	FOG 25
	mL	*mL*
28	3583.4	1541.4
29	3834.1	1708.1
30	4021.7	1820.3
31	4150.8	1981.6
33	4340.1	2575.9
34	4340.1	2772.3
36	4361.5	3199.9
37	4394.7	3341.7
38	4436.5	3491.5
39	4459.6	3537.4
40	4472.3	3674.6
41	4494.1	3724.4
42	4494.1	3815.1
43	4528.4	3879.7
44	4528.4	3955.3
45	4559.5	3982.9
47	4559.5	4114.5
48	4563.0	4132.2
49	4592.6	4174.4
51	4618.4	4274.1
54	4660.0	4346.6
55	4660.0	4360.7

Table A11. Daily Methane Production During Semi-Continuous Operation of Bench-scale Digesters

Days	Control	FOG 25
	mL	*mL*
56	54.1	132.2
57	*27.4*	*32.8*
58	57.0	56.9
59	55.1	70.7
61	149.4	265.1
62	56.8	179.4
63	123.7	147.8
64	39.5	208.8
65	161.5	219.1
67	319.8	434.3
68	190.8	252.7
69	284.6	368.8
70	285.8	441.3
71	251.1	293.1
72	342.8	391.3
73	297.0	312.8
75	523.6	489.3
76	296.0	354.6
77	234.5	176.8
78	223.8	319.7
79	252.7	254.5
81	379.0	543.5
82	203.4	309.3
83	153.6	272.1
84	262.3	260.4
85	220.3	296.0
86	240.2	306.6
87	154.0	279.8
91	510.7	810.3
92	81.8	175.3
93	148.4	245.7
95	421.9	661.5

Table A12. % COD Destroyed During BMP Test 2

Days	Control	FOG 25	FOG 50	FOG 75	FOG 100
0	0.0	0.0	0.0	0.0	0.0
1	2.4	0.5	0.2	0.1	0.0
2	4.4	0.7	0.3	0.1	0.0
3	5.9	1.1	0.5	0.3	0.0
4	7.5	1.1	0.6	0.3	0.0
5	8.9	1.3	0.6	0.4	0.0
6	10.7	1.4	0.6	0.4	0.0
8	16.1	1.7	0.9	0.6	0.2
9	18.4	1.9	0.9	0.6	0.2
10	19.8	2.2	1.1	0.6	0.3
11	20.5	3.1	1.4	0.9	0.3
13	21.3	4.9	2.6	0.9	0.5
16	22.1	5.2	4.3	1.3	0.8
17	22.5	5.3	4.7	1.7	1.0
18	22.6	5.4	4.7	2.3	1.4
20	23.1	5.4	4.7	3.9	3.2
21	23.5	5.5	5.0	6.4	4.5
23	24.1	5.5	5.0	6.4	5.1
24	24.5	5.6	5.1	6.7	5.3
25	24.6	5.6	5.2	6.7	5.5
28	25.3	5.8	5.6	7.2	5.7
30	25.8	6.0	6.2	7.9	7.0
32	25.9	6.0	6.2	7.9	7.0
36	26.0	6.3	6.6	8.1	7.0
38	26.5	6.8	6.9	8.1	7.3
41	26.5	6.9	7.1	8.4	7.3
43	26.6	7.2	7.2	8.4	7.3
45	26.8	7.7	7.2	8.4	7.4
48	27.0	8.4	7.2	8.5	7.4
51	27.0	9.7	7.2	8.5	7.5
55	27.3	13.8	7.4	8.5	7.7
58	27.5	18.2	7.5	8.9	7.7
62	27.6	22.8	7.7	8.9	7.7
65	27.8	25.6	7.7	8.9	7.7
68	27.8	28.6	8.0	9.4	8.1
72	28.2	29.9	8.0	9.4	8.1
76	28.3	31.5	8.2	9.4	8.2
79	28.5	32.7	8.2	9.5	8.2
84	28.8	34.7	8.5	9.6	8.3

Table A13. % COD Destroyed During Batch Mode Operation of Bench-scale Digesters

Days	Control	FOG 25
0	0.00	0.00
1	0.48	0.10
2	1.18	0.25
3	1.74	0.40
5	2.27	0.79
6	2.58	0.92
7	2.88	1.10
8	3.78	1.17
9	4.86	2.27
10	7.14	3.94
11	9.67	5.90
12	10.17	6.96
13	10.19	6.96
14	10.67	7.04
16	11.35	7.19
17	12.49	7.22
18	14.37	7.49
20	16.48	7.49
22	17.36	7.91
23	19.42	8.15
25	21.33	8.78
26	23.08	9.26

Days	Control	FOG 25
28	27.13	11.32
29	28.82	12.55
30	30.22	13.37
31	31.20	14.56
33	32.86	18.93
34	32.62	20.37
36	32.78	23.51
37	33.03	24.55
38	33.58	25.65
39	33.52	25.99
40	33.61	27.00
41	33.77	27.36
42	33.78	28.03
43	34.24	28.50
44	34.04	29.06
45	34.27	29.26
47	34.27	30.23
48	34.50	30.36
49	34.53	30.67
51	34.72	31.40
54	35.03	31.93
55	35.03	32.04

Table A14. Daily Methane Production Rate During Semi-Continuous Operation of Bench-scale Digesters

Day	Control	FOG 25
	*L CH_4/(g VS added*d)*	*L CH_4/(g VS added*d)*
56	0.26	0.26
57	0.05	0.05
58	0.11	0.09
59	0.10	0.11
61	0.14	0.21
62	0.11	0.28
63	0.23	0.23
64	0.07	0.32
65	0.30	0.34
67	0.30	0.34
68	0.36	0.39
69	0.55	0.57
70	0.54	0.68
71	0.47	0.45
72	0.65	0.60
73	0.56	0.48
75	0.49	0.38
76	0.56	0.55
77	0.44	0.27
78	0.45	0.49
79	0.48	0.39
81	0.36	0.42
82	0.38	0.48
83	0.29	0.42
84	0.49	0.40
85	0.42	0.46
86	0.45	0.47
91	0.24	0.32
92	0.15	0.27
93	0.28	0.38
94	0.40	0.51
95	0.40	0.51
Avg.	**0.40**	**0.43**

Table A15. pH Variations During Operation of Bench-scale Digesters

Day	pH Value	
	Control	FOG 25
3	6.4	6.2
5	6.5	6.1
10	7.4	6.7
16	8.0	7.7
21	8.4	7.8
28	8.2	8.1
38	8.7	8.5
43	8.5	8.3
48	8.4	7.8
57	7.2	7.0
59	7.3	7.5
63	6.8	6.8
69	7.2	7.1
73	7.7	7.1
77	7.1	6.9
83	8.0	7.3
87	7.6	7.5
91	7.5	7.3
95	7.4	7.3

Table A16. HPLC Results for Control Bench-scale Digester

Day	Acetate	Propionate	Iso Butyrate	Butyrate	Iso Valerate	Valerate
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
2	41.6	9.4	3.0	7.9	5.4	3.0
3	41.0	10.5	3.1	12.1	6.2	3.2
5	48.0	11.2	3.0	9.8	6.7	4.7
6	49.5	12.4	3.1	9.9	7.3	5.1
7	46.3	12.2	3.1	8.6	6.7	4.8
8	45.7	12.6	3.1	9.1	6.9	4.9
9	29.6	9.9	2.9	6.8	5.6	4.0
10	13.8	12.7	3.2	7.0	7.1	4.6
11	2.7	14.3	3.8	8.6	7.6	4.7
12	0.8	14.6	3.9	8.8	7.8	5.1
13	4.6	14.5	4.0	7.2	7.7	4.8
14	8.9	14.8	4.1	5.9	7.9	4.9
16	19.3	16.0	4.3	1.3	8.1	4.5
17	17.4	14.0	4.3	0.8	7.9	3.7
18	17.24	16.07	4.35	0.63	8.19	3.66
26	10.32	20.23	5.07	0.41	10.70	1.73
27	10.55	28.36	3.97	0.00	8.29	1.05
28	6.79	33.23	3.71	0.00	9.83	0.59
29	1.68	28.33	3.83	0.00	4.84	0.00
30	1.04	32.36	3.38	0.00	4.84	0.00
31	0.98	22.82	1.30	0.00	6.13	0.00
33	2.63	33.75	0.00	0.00	6.30	0.00
34	3.45	24.06	0.88	0.00	7.12	0.00
36	3.36	24.85	0.67	0.00	7.77	0.00
37	2.94	24.92	0.59	0.00	7.40	0.00
38	1.77	27.04	0.70	0.00	7.19	0.00
39	1.45	24.04	0.82	0.00	7.43	0.00
40	1.92	28.90	0.91	0.00	8.79	0.00
41	1.20	25.56	0.82	0.00	8.22	0.00
42	0.99	32.21	0.96	0.00	9.20	0.00
43	0.74	25.48	0.80	0.00	7.81	0.00
44	1.02	25.29	0.84	0.00	7.78	0.00
45	1.67	23.33	0.80	0.22	8.24	0.00
47	1.31	26.15	0.82	0.00	8.00	0.00
48	1.26	27.94	0.63	0.00	8.14	0.00
49	1.59	25.60	0.49	0.00	7.56	0.00
51	2.25	27.60	0.31	0.00	8.58	0.00
54	1.11	23.11	0.01	0.00	7.21	0.00

Day	Acetate	Propionate	Iso Butyrate	Butyrate	Iso Valerate	Valerate
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
55	0.00	23.11	0.00	0.00	7.21	0.00
57	8.24	22.64	0.73	0.93	7.00	0.00
59	15.94	21.18	1.11	0.47	7.70	0.00
61	25.71	22.73	1.35	0.73	7.69	0.00
63	31.67	23.24	1.67	0.97	8.63	0.92
65	30.02	22.53	1.92	1.15	8.82	1.00
67	31.64	29.58	2.71	1.60	11.34	1.80
69	20.20	25.06	2.64	0.80	9.89	1.66
71	10.79	25.72	2.73	0.00	7.30	0.00
73	0.61	27.64	2.97	0.00	9.68	0.00
75	0.00	24.27	2.61	0.00	8.92	0.00
77	1.31	22.50	0.67	0.00	7.36	0.00
79	0.00	26.02	0.04	0.00	8.47	0.00
81	11.46	25.37	0.35	0.00	8.68	0.00
83	2.00	28.55	0.00	0.00	7.39	0.00
85	0.73	28.65	0.00	0.00	6.41	0.00
87	0.00	26.48	0.00	0.00	7.00	0.00
91	0.53	29.71	0.14	0.00	5.77	0.00
93	8.10	22.77	0.61	0.10	5.43	0.00
95	14.04	28.24	1.11	0.00	8.53	0.00

Table A17. HPLC Results for FOG 25 Bench-scale Digester

Day	Acetate	Propionate	Iso Butyrate	Butyrate	Iso Valerate	Valerate
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
2	35.82	9.50	2.55	8.59	4.71	4.30
3	43.50	10.63	2.58	10.13	5.27	4.88
5	42.94	9.80	2.29	10.21	5.50	4.24
6	43.61	10.18	2.48	10.19	5.87	4.28
7	43.59	10.42	2.55	10.46	5.97	4.39
8	40.54	10.26	2.31	11.39	5.56	4.17
9	34.96	11.50	2.82	11.07	6.35	4.76
10	24.72	12.22	2.71	10.16	5.91	4.57
11	7.49	13.15	3.20	8.87	5.84	4.10
12	1.11	13.61	3.31	8.71	5.92	4.37
13	1.86	13.54	3.36	8.78	6.02	4.65
14	2.93	15.37	3.81	9.67	6.66	5.40
16	4.94	14.05	3.22	8.41	6.37	5.04
17	5.32	14.27	3.60	8.22	6.30	4.83
18	6.45	11.12	2.57	4.90	5.49	3.23
26	4.58	14.39	4.45	8.32	8.99	6.43
27	4.55	14.78	4.41	4.50	7.92	6.09
28	4.88	16.26	3.34	0.00	6.09	5.21
29	2.44	15.43	3.78	0.00	7.12	3.44
30	2.28	17.59	2.94	0.00	6.19	3.21
31	2.53	18.95	2.34	0.00	5.84	0.00
33	1.73	21.14	2.66	0.00	6.54	0.00
34	1.01	22.10	4.12	0.00	7.27	0.53
36	0.56	24.30	4.26	0.01	7.50	0.00
37	0.38	21.96	4.11	0.00	6.75	0.00
38	0.30	20.16	3.40	0.00	5.87	0.00
39	0.52	22.47	4.21	0.00	7.36	0.00
40	0.52	20.45	3.51	0.00	7.21	0.00
41	0.55	24.10	4.59	0.00	8.27	0.00
42	0.58	22.21	4.14	0.00	7.31	0.00
43	0.80	22.46	4.07	0.25	7.12	0.00
44	1.70	22.83	4.20	0.34	7.30	0.00
45	1.16	25.31	4.43	0.00	7.42	0.00
47	1.33	21.35	3.85	0.00	6.50	0.00
48	1.25	23.73	4.25	0.00	7.00	0.00
49	1.95	23.79	4.34	0.00	7.38	0.00
51	1.88	23.61	4.06	0.00	6.88	0.00
54	3.53	21.08	3.20	0.00	6.54	0.00

Day	Acetate	Propionate	Iso Butyrate	Butyrate	Iso Valerate	Valerate
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
55	3.82	21.88	3.89	0.06	6.66	0.00
57	11.14	21.89	3.99	1.05	6.76	0.00
59	13.93	23.34	4.39	1.69	7.37	0.61
61	8.80	23.56	4.43	1.41	7.57	0.99
63	2.26	21.83	4.14	0.20	7.14	1.12
65	2.45	22.49	4.15	0.00	7.34	0.70
67	4.47	25.68	4.58	0.11	8.10	0.55
69	3.37	22.72	4.01	0.06	7.45	0.00
71	1.07	25.07	4.46	0.00	8.23	0.00
73	0.67	26.38	4.48	0.00	8.49	0.00
75	0.50	24.01	4.20	0.00	7.95	0.00
77	0.41	24.37	3.75	0.00	8.21	0.00
79	0.67	26.63	4.59	0.00	8.25	0.00
81	0.37	25.10	4.29	0.00	8.74	0.00
83	0.84	28.69	4.56	0.00	9.49	0.00
85	0.88	27.42	4.37	0.00	8.95	0.00
87	0.00	25.73	4.11	0.00	8.62	0.00
91	1.50	26.17	3.67	0.00	10.30	0.00
93	8.57	25.55	3.85	0.45	8.13	0.00
95	1.34	22.86	3.16	0.00	6.69	0.00

Table A18. Long Chain Fatty Acid Results for Raw Samples

		Raw FOG Sample		Raw PS+TWAS Sample	
	Fatty Acid	**Concentration**	**Composition**	**Concentration**	**Composition**
		µg/mL	*%*	*µg/mL*	*%*
C12:0	Lauric	25.31	0.63	0.00	0.00
C13:0	Tridecanoic	0.00	0.00	0.00	0.00
C14:0	Myristic	165.84	4.16	44.36	5.79
C14:1	Myristoleic	14.13	0.35	34.55	4.51
C15:0	Pentadecanoic	0.00	0.00	0.00	0.00
C16:0	Palmitic	1635.75	41.03	389.40	50.86
C16:1	Palmitoleic	34.56	0.87	0.00	0.00
C17:0	Heptadecanoic	24.03	0.60	0.00	0.00
C18:0	Stearic	441.69	11.08	0.00	0.00
C18:1n9t	Elaidic	259.93	6.52	0.00	0.00
C18:1n9c	Oleic	698.10	17.51	269.74	35.23
C18:2n6t	Linolelaidic	47.72	1.20	0.00	0.00
C18:2n6c	Linoleic	256.40	6.43	0.00	0.00
C20:0	Arachidic	19.78	0.50	0.00	0.00
C18:3n6	r-Linolenic	0.00	0.00	0.00	0.00
C20:1	cis-11-Eicosenoic	17.14	0.43	0.00	0.00
C18:3n3	Linolenic	0.00	0.00	0.00	0.00
C21:0	Heneicosanoic	15.87	0.40	0.00	0.00
C20:2	cis-11,14-Eicosadienoic	62.05	1.56	0.00	0.00
C22:0	Behenic	20.33	0.51	0.00	0.00

Table A19. Three Long Chain Fatty Acid Concentration Profiles

	Stearate		Palmitate		Myristate	
Day	**Control**	**F25**	**Control**	**F25**	**Control**	**F25**
	µg/mL	*µg/mL*	*µg/mL*	*µg/mL*	*µg/mL*	*µg/mL*
3	281.2	235.1	330.1	600.5	68.9	25.6
5	182.6	122.7	212.8	298.0	53.3	49.3
10	120.2	190.8	130.7	430.7	58.7	40.2
16	89.3	84.1	124.6	163.0	60.2	52.5
21	98.4	160.7	105.5	319.9	81.1	56.0
28	77.6	134.9	65.1	275.7	110.2	59.9
38	54.5	62.8	70.0	87.2	90.6	68.9
43	33.0	33.5	43.0	47.8	54.2	41.1
48	24.3	37.6	29.2	47.0	80.7	80.4
57	35.9	80.3	50.1	147.4	44.2	58.0
65	58.7	75.8	84.5	141.9	71.9	76.3
71	0.0	33.4	28.0	53.3	108.8	71.4
77	29.6	26.1	40.5	50.0	101.9	59.0
83	0.0	53.7	22.9	89.4	99.5	85.8
87	0.0	44.9	0.0	63.9	75.1	98.1
95	41.0	50.5	53.0	67.8	108.5	128.1
Feed	269.7	441.7	60.8	1635.8	6.9	165.8
Average =	21.6	47.4	38.2	77.7	94.3	86.5

Table A20. Three Long Chain Fatty Acid Composition Profiles

	Stearate		Palmitate		Myristate	
Day	**Control**	**F25**	**Control**	**F25**	**Control**	**F25**
	%	**%**	**%**	**%**	**%**	**%**
3	37.4	22.3	43.9	57.0	9.2	2.4
5	34.2	9.5	39.8	23.0	10.0	3.8
10	31.1	22.2	33.8	50.1	15.2	4.7
16	24.1	18.5	33.7	36.0	16.3	11.6
21	27.0	21.5	29.0	42.8	22.3	4.7
28	25.2	21.9	21.1	44.8	35.7	9.7
38	15.9	15.3	20.4	21.3	26.5	16.8
43	13.7	12.9	17.9	18.5	22.6	15.9
48	12.4	4.3	14.9	5.4	41.1	9.2
57	14.6	18.1	20.4	33.2	18.0	13.1
65	18.3	17.6	26.3	33.0	22.4	17.7
71	0.0	14.8	14.8	23.5	57.4	31.6
77	15.7	16.0	21.4	30.7	53.8	36.2
83	0.0	18.7	13.6	31.2	58.7	29.9
87	0.0	17.4	0.0	24.8	63.3	38.1
95	16.6	17.6	21.5	23.6	44.0	44.6
Feed	35.2	11.1	50.9	41.0	5.8	4.2
Average =	8.4	17.0	16.3	27.8	49.9	33.0

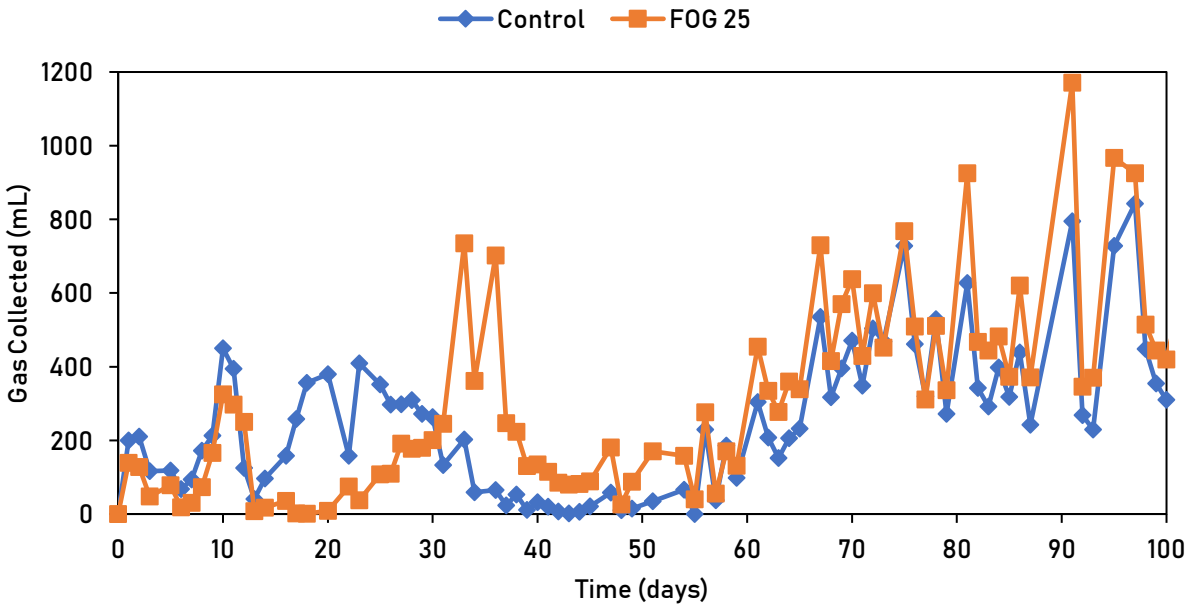

Figure A1. Daily biogas production during bench-scale digester operation

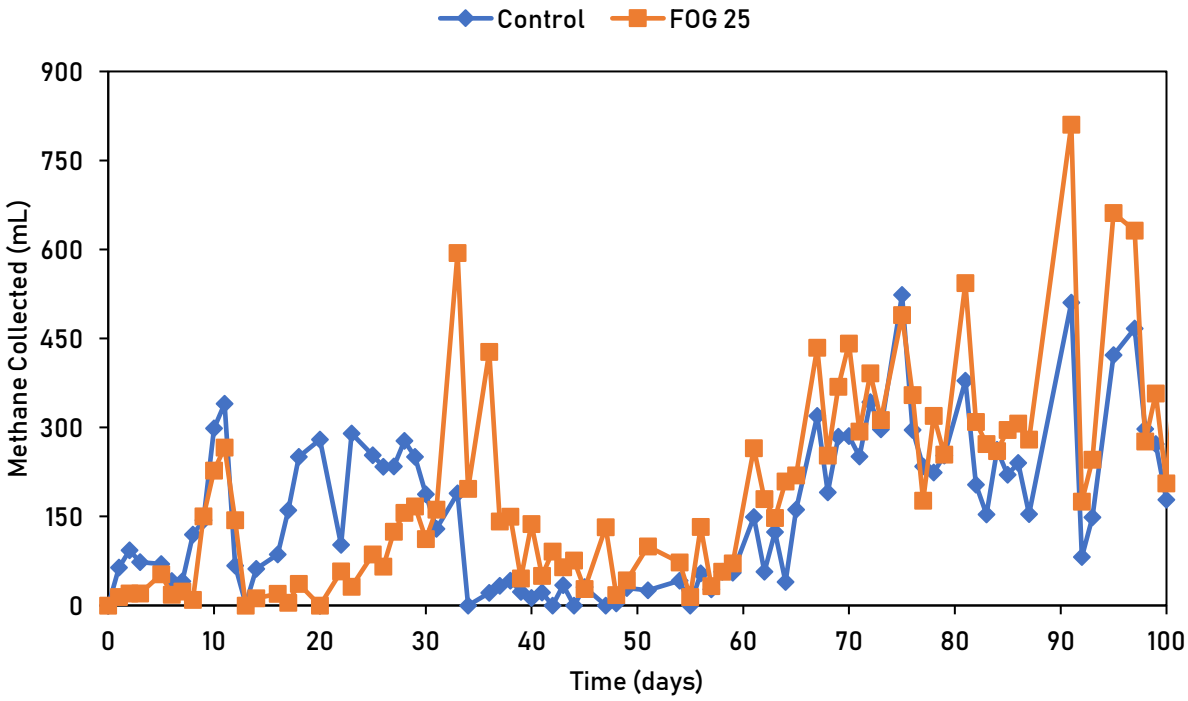

Figure A2. Daily methane production during bench-scale digester operation

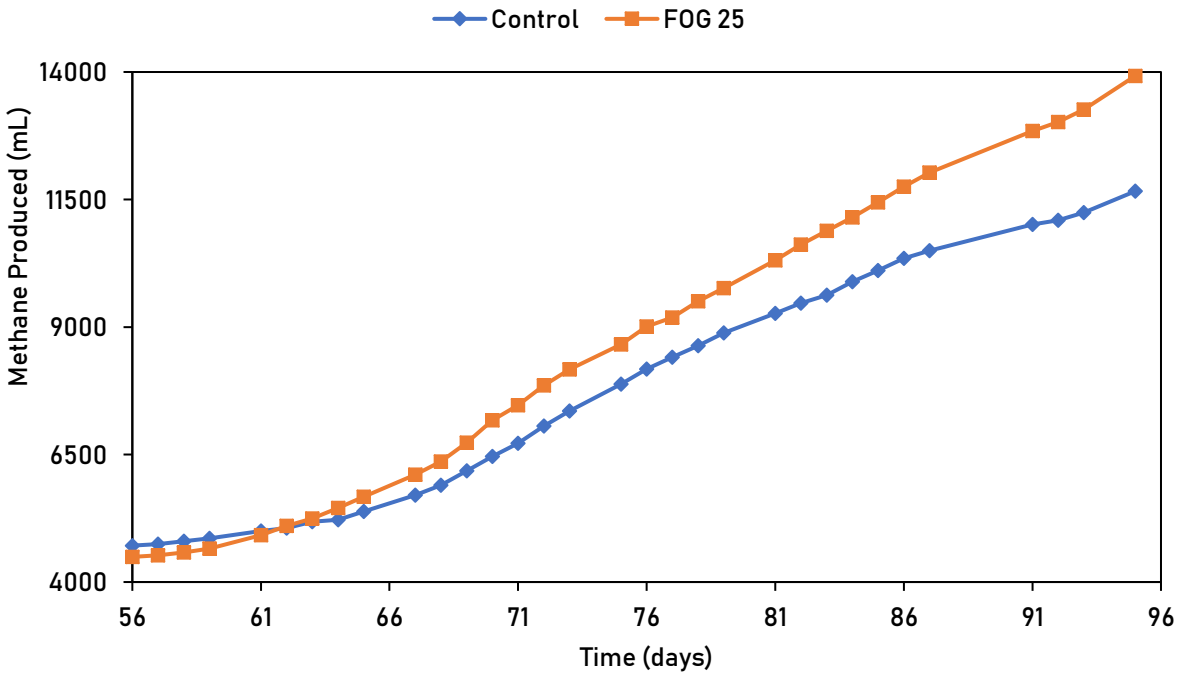

Figure A3. Cumulative methane production during semi-continuous bench-scale digester operation

Figure A4. Serum bottles used during BMP tests with aluminum caps and rubber septa

Figure A5. BMP test setup in shaker incubator maintained at 35℃ and 130 rpm

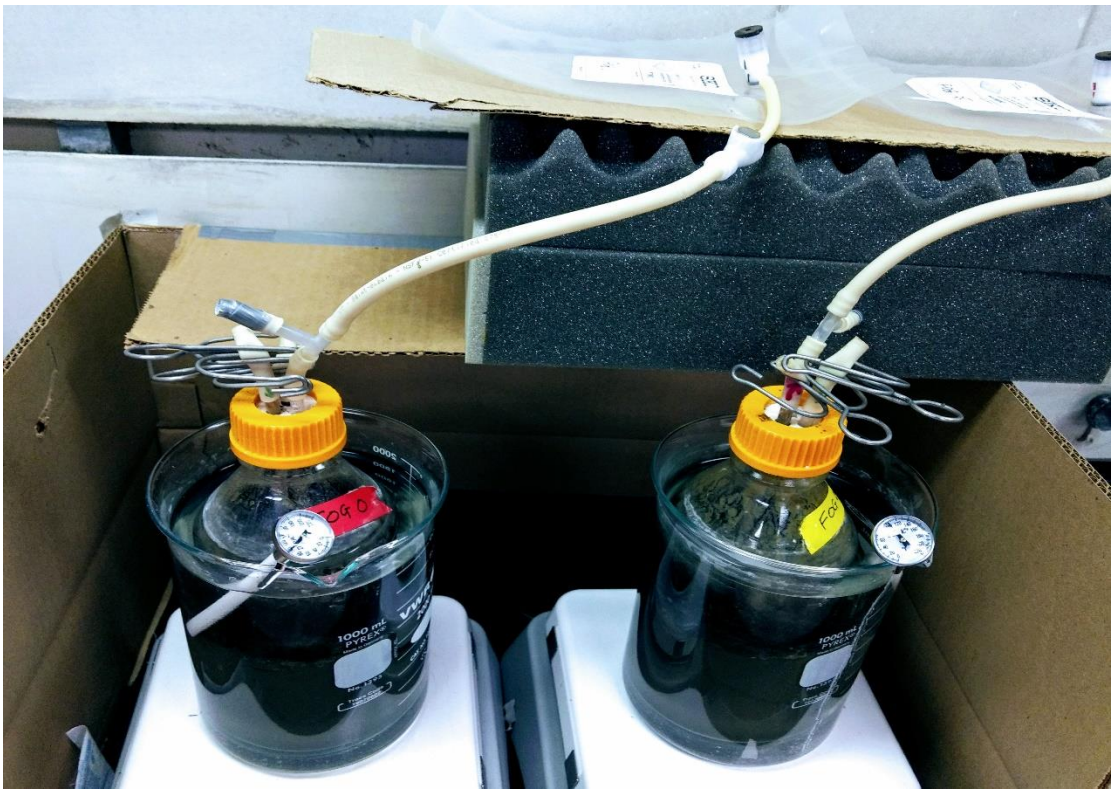

Figure A6. Bench-scale digesters placed in water bath heater at 35℃ using hot plate

Figure A7. Bench-scale digesters – biogas collection in Tedlar bags

Figure A8. Biogas measurement from a Tedlar bags using 50 mL disposable syringe

Figure A9. Methane content measurement using GC-TCD

Figure A10. VFA analysis using HPLC

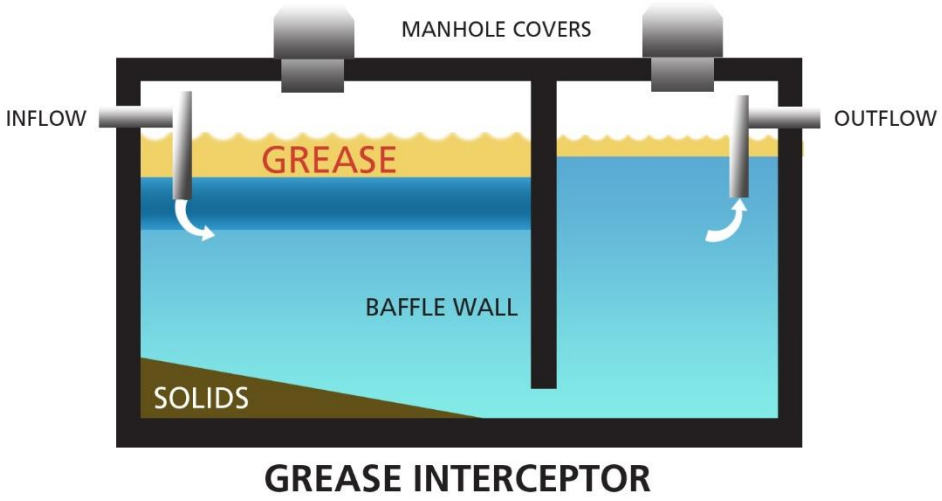

Figure A11. Grease interceptor design for separating FOG waste from wastewater (https://www.mix96.co.uk/news/local/2202044/fatberg-causes-sewer-blockage-in-bierton)

Figure A12. Example of clogged sewer lines a.k.a. fatbergs due to FOG deposition (https://baystatesewage.com/grease-trap-pumping-grease-interceptor-pumping)