Anaerobic co-digestion of municipal waste sludge with fat, oil and grease: Effectiveness of process configurations, and point of failure

by

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Abstract

Fat, oil and grease (FOG) collected from restaurant grease trap is a well-known co-substrate for anaerobic digestion (AD) process to improve the process efficiency, since FOG has theoretically high biogas production potential. FOG hydrolysis leads to fast long chain fatty acid (LCFA) formation; however, LCFA accumulation can occur at a high FOG loading rate, which can lead to AD process failure. This study investigated the effect of FOG addition to the AD process utilizing municipal sludge and identified the optimum operational condition to reach the highest stable biogas production.

Initially, two semi-continuous flow single-stage anaerobic digesters were operated under mesophilic condition (35°C) as a control and a co-digester. FOG addition to the co-digester was increased in a stepwise manner, this corresponded to organic loading rates (OLR) of 1.84 and 3.15 g volatile solids (VS)/L/d for control and co-digester, respectively. Results indicated that increasing FOG/sludge ratio up to 40% VS led to a stable digester performance and at 50% VS FOG, digester failure was observed. As a result of the addition of FOG at 40%, up to 99, 28 and 13% improvements in methane yield, VS removal, and chemical oxygen demand (COD) removal was achieved, respectively. Moreover, Class B biosolids were produced from the digesters for potential land application as fertilizer.

In a reattempt to achieve a stable AD process at 50% VS FOG addition, two sets of temperature--phased AD (TPAD) systems were run according to the following scenarios: TPAD-1 with acid and phase temperatures of 55 and 38°C, respectively, and TPAD-2 with acid and methane phase temperatures of 70 and 38°C, respectively. They were run at 50%VS FOG addition and methane phase digester failure was observed in TPAD-1 and TPAD-2 after 35 and 49 days of operation, respectively. Hence, no improvement over the single-stage co-digester was achieved.

Finally, batch experiments were conducted with three different biochar dosages and it was concluded that biochar has the potential to remove inhibitory LCFAs from AD. However, further studies are needed for biochar addition to continuous-flow AD process to identify the optimal biochar dose and AD configuration for an effective LCFA inhibition mitigation.

Lay summary

Treating wastewater sludge, a by-product of a wastewater treatment, through AD is a favorable method to reduce pathogens and odor potential. Moreover, methane-rich biogas is produced through this process and converted to heat and electricity that is a great source of energy for the wastewater treatment plant (WWTP). The process efficiency can be improved by adding other substrates to the main substrate. In this research, FOG was collected from restaurants grease traps and added to the AD process to improve the process efficiency in terms of methane production and solids removal. Moreover, the optimum operational condition to reach the highest biogas production was investigated. The effect of other digester configuration, named TPAD system, on this process was studied since TPAD has advantages on methane production compared to conventional AD. In addition, the effect of adsorbent addition to the process in order to mitigate the process limitation was assessed.

Preface

The research presented in this thesis included the design of the experiment, the design and operation of bioreactors, data analysis and writing the thesis. This work was conducted by the author with the assistance of Dr. Cigdem Eskicioglu at the Bioreactor Technology Group in the School of Engineering, University of British Columbia. David Krisa, who was an undergraduate research assistant (URA) at Bioreactor Technology Group at the time, helped with digester set-up and performance monitoring.

A portion of the results of this research was presented at the 10th International Water Association (IWA) Symposium on Waste Management Problems in Agro-Industries which was held in Greece in June 19-21, 2019. Furthermore, a journal paper is being prepared for submission to *Renewable Energy*.

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List of Acronyms and Abbreviations

AP	Acid phase
AD	Anaerobic digestion
ANOVA	Analysis of variance
BNR	Biological nutrient removal
BOD	Biochemical oxygen demand
CST	Capillary suction timer
COD	Chemical oxygen demand
CFU	Coliform forming unit
FOG	Fat, oil and grease
FPS	Fermented primary sludge
GC	Gas chromatography
HRT	Hydraulic retention time
LCFA	Long chain fatty acids
MP	Methane phase
MPN	Most probable number
OLR	Organic loading rate
OMRR	Organic matter recycling regulations

PS	Primary sludge
RO	Reverse osmosis
SRT	Sludge retention time
STP	Standard temperature and pressure
TPAD	Temperature-phased anaerobic digestion
TS	Total solids
TWAS	Thickened waste activated sludge
VFA	Volatile fatty acids
VS	Volatile solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

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At last but not least gratitude goes to my amazing husband for his endless love, patients and encouragement. He supported me every step of the way, as he always does. This would have been impossible without him.

To my parents, sister and my husband

Chapter 1 . Introduction

Over the last few years, there has been an increase in FOG production from restaurants, food industries, and residences due to population growth and changes in people's eating habits. The increase in FOG production has raised worldwide environmental concerns, as it has become a major source of municipal organic waste. Direct disposal into sewer systems can lead to accumulation in pipes and sanitary sewer blockage and overflow. Moreover, cleaning, repairing, and replacing the pipes can cost millions of dollars every year. It can also lead to the release of high concentration of pathogens, nutrients, and solids in water bodies because of unwanted bacterial growth (Wallace *et al.* 2017).

If FOG is discharged to the sewer system and reaches to a WWTP, it can stick to the pipes and cause pipe blockages and overflows. Moreover, FOG can also decrease the efficiency of settlement and clarification processes in WWTPs (Wallace *et al.* 2017).

A method that restaurants use to prevent FOG entry into pipelines is to collect it at its point of origin using grease traps. The disposal of collected FOG in landfills is detrimental for public health and the environment, as it reduces the hydroconductivity of soil and generates greenhouse gas emissions; thus, this practice is no longer permitted (Razaviarani *et al.* 2013; Husain *et al.* 2014). Other disposal alternatives include using FOG as a feedstock for biodiesel (Park *et al.* 2010), biomaterials (i.e. biodegradable plastics, polyhydroxyalkanoates) (Wallace *et al.* 2017) and AD. Of these, AD has received much attention to improve biomethane production in WWTPs (Salama *et al.* 2019).

Compared to conventional waste disposal methods such as incineration, and landfilling, AD is a more effective biological process for sludge treatment due to its environmental and economic advantages (Davidsson *et al.* 2008). In conventional AD, one substrate is mainly used; however,

in co-digestion a secondary or other organic substrates (co-substrate) (i.e. organic municipal solid waste, grease and waste oils, fruits and vegetable wastes, glycerin) can be added to the process to improve its efficiency.

More recently, studies have shown that using FOG as a co-substrate in AD can enhance biomethane production (Li, C. *et al.* 2015; Alqaralleh *et al.* 2018). This enhancement is due to its theoretically higher biogas production potential with a higher methane content than biogas yield from proteins and carbohydrates. However, this process is faced with many challenges that can lead to process failure, such as long chain fatty acids (LCFAs) accumulation, scum foaming, and AD overflowing. Under the AD process, FOG is degraded to LCFA and glycerol, which are then further degraded to acetate and hydrogen to yield methane. In this process, LCFA degradation is the rate limiting process that can lead to LCFA accumulation in AD. The accumulated LCFA can be absorbed on cell walls, hinder mass transfer to substrates, and limit product release from cells. This can ultimately lead to volatile fatty acids (VFA) accumulation and process failure. Moreover, LCFAs accumulation reduces surface tension and causes sludge floatation and washout. Hence, the addition of FOG as a co-substrate to AD requires a careful feeding strategy and process design to prevent digester failure.

1.1 Motivation of research

Numerous studies have investigated the effectiveness of FOG addition as a co-substrate in anaerobic co-digestion. These studies focused more on the effectiveness of different parameters such as temperature, OLR, and FOG characteristics on the process efficiency in batch mode and lab scale. Moreover, some studies focused on different pre-treatment effectiveness on FOG, prior to biological process, to overcome its limitations in AD and improve the process efficiency. Process failure has been documented as a result of LCFA accumulation from a high FOG

concentration. However, there is still lack of information on the optimum condition required to maximize biogas (methane) production without process inhibition. To fill this gap, FOG should be added in a stepwise manner to acclimatize the microbial cultures to FOG and it's by-products (i.e. LCFAs, glycerol) and find the optimum OLR to achieve the highest biogas production. Furthermore, the inhibition parameters such as LCFA and VFA concentrations should be monitored during the bench-scale testing to find their threshold. In addition, different digester configurations can be operated to investigate the possible differences on anaerobic co-digestion performance. The literature has not focused on some of the digestate (digester effluent) characteristics (fecal coliform content, dewaterability, and heavy metal content) as a result of FOG addition to co-digester; thus, it will be beneficial to identify possible effects of FOG on biosolids quality for further usage as fertilizer.

1.2 Research objectives

In this study, anaerobic co-digestion of municipal waste sludge with restaurant grease trap was conducted and the main objectives were as follows:

I. Exploration of various anaerobic digester configurations to maximize bioenergy recovery from FOG and municipal sludge
II. Assessment of the quality of digested biosolids in comparison to Organic Matter Recycling Regulations (OMRR) in the province of British Columbia.
III. Investigation of the potential of biochar addition to remove LCFAs from digesters to reduce inhibition to methane producing cultures.

1.3 Thesis outline

In this thesis, a brief introduction about the main topic is provided in chapter 1, as well different steps of the project to achieve the main goal of this research are defined. In chapter 2, detailed

discussion on literature results provides a better understanding of FOG's impact on anaerobic co-digestion, and the influence of different parameters on the process efficiency. Chapter 3 presents the experimental design, materials and analytical procedures used in this research. The fourth chapter provides the obtained results of the study and compares them with previous findings in literature. The last chapter provides a summary of major findings of this research and some suggestions for future work.

Chapter 2 . Literature review

This chapter provides general information about municipal wastewater and WWTP. As well as information about FOG: it's sources of generation and composition, status of FOG management, difficulties of dealing with it, benefits and barriers when using this energy source to produce higher biomethane in WWTP. In this regard, the literature review is most focused on anaerobic co-digestion, the current status of using FOG as a co-substrate in AD, and the importance of different parameters in this process. In addition, different digester configurations and different pre-treatment methods, which are studied to enhance biomethane production in AD, are discussed in detail.

2.1 Background

Untreated wastewater sludge has numerous pathogenic microorganisms and nutrients which can be a threat to human health and the environment if discharged without treatment. For these reasons, wastewater sludge needs treatment to reduce nutrients, trace chemicals, and heavy metals to an acceptable level before discharging back into the environment.

Among the different treatment methods, AD is one of the most effective process for pathogen removal and sludge stabilization. It is also a favorable method to produce biomethane which can be converted to electricity/heat and supply energy to a WWTP or nearby communities (Mao *et al.* 2015).

2.2 Wastewater treatment

The main objectives of wastewater treatment are (1) to remove suspended and floatable materials, (2) to treat dissolved biodegradable organic matter and convert it to methane-rich biogas (the removal of these materials reduces the disposal volume and thus significantly decreases the cost of disposal), (3) to eliminate pathogenic microorganisms. As a side note, the elimination of organics and microorganisms also helps to reduce unpleasant odors. The most common parameters

used to define organic matter concentration in wastewater are COD and biological oxygen demand (BOD). BOD is the amount of oxygen consumed by microorganisms when they are oxidizing organic matter in wastewater. Moreover, BOD only measures the concentration of biodegradable organic matter and requires pre-treatment if the wastewater consists toxic compounds. On the other hand, COD is equivalent to the amount of oxygen required for the degradation of both biodegradable and non-biodegradable organic matter. Moreover, it takes about 2.5 hours to complete, while BOD assays can take days. It is reported that the typical BOD/COD ratio for municipal wastewater, before treatment, is between 0.3 to 0.8 (Metcalf & Eddy 2014).

2.2.1 Wastewater treatment plant

Wastewater consists of high amount of organic and inorganic matter and their composition is highly depended on their source of generation (domestic, industrial, and agricultural). Untreated wastewater enters the WWTP and is disposed to the environment after going through different unit processes (physical, chemical, and biological) (Sonune & Ghate 2004). All WWTPs require a combination of unit processes, as it is not possible to remove unwanted constituents using only one unit process. Some WWTPs also have some additional units for further treatments based on the discharge requirements of the treated wastewater. There are strict regulations for the discharge of treated wastewater streams into the environment. The regulations vary regionally; however, the ultimate purpose is to make the liquid stream safe to discharge back to the environment. In general, there are numerous ways for a WWTP to reduce the negative effects of pollutants in wastewater. The typical process flow diagram of a WWTP that is treating municipal wastewater is shown in Figure 2-1. As shown in Figure 2-1, there are three main sections for the treatment of wastewater. Primary treatment is used to remove suspended organic matter, secondary treatment is implemented to remove colloidal and dissolved organics, and tertiary treatment (physical,

chemical, and biological) is used to remove nutrients, residual particulates, pathogens and chemical contaminations. These sections are described in detail in the following sections.

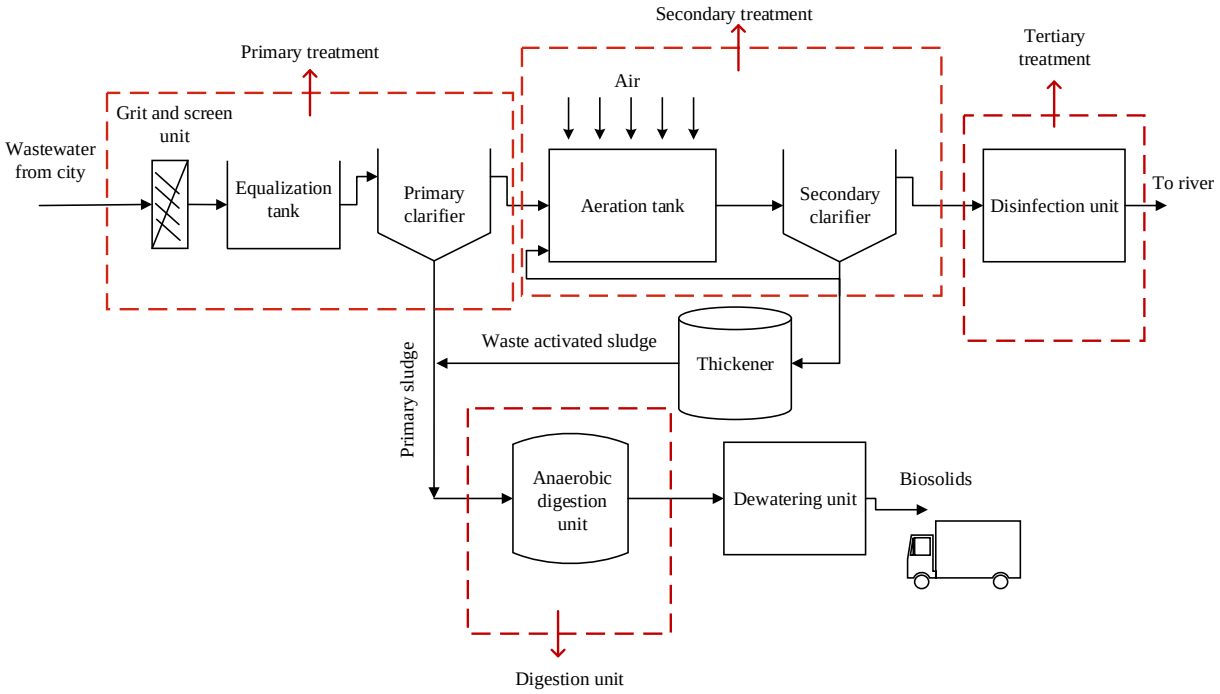

Figure 2-1: Wastewater and wastewater sludge processing flow diagram

2.2.2 Primary treatment

Primary treatment mostly consists of physical treatment units; however, chemical and biological processes can be added to increase the efficiency of organic matter removal and VFAs production, respectively (Chanona *et al.* 2006; Johnson *et al.* 2008). The first unit process in primary treatment is a screening unit, which removes large solid materials from the wastewater stream. Removing the large solid materials eliminates their negative effects on the equipment in the following treatment sections. After screening, the wastewater stream goes through a grit chamber and stays there long enough for the dense materials to settle down to the bottom of the tank. The dense materials are then collected and disposed separately. The organic concentration in the influent of WWTP varies throughout the day and in early morning and early evening, the peak

flows typically occur. To prevent the shock loading to the unit processes at WWTP during peak flows, the grit chamber is followed by an equalization tank. The equalization tank improves the performance of the WWTP by providing an almost constant flowrate to the rest of the unit processes. The last unit in this section is the primary clarifier. It removes a significant portion (55-70%) of the suspended solids by settling them through the use of gravity (Metcalf & Eddy 2014). The settled solids, known as primary sludge (PS), are watery and have a high concentration of pathogenic microorganisms. Hence, they are collected and thickened for further treatment.

2.2.3 Secondary treatment

In general, secondary treatment refers to physical and biological treatments used to remove dissolved biodegradable organic substances (Sonune & Ghate 2004). This is accomplished by converting the organic substances to biological cell tissue that can be settled. For municipal wastewater treatment, the most common biological method used is the activated sludge process. This treatment technique is a two-step process in which an aeration basin is followed by a secondary clarifier (Muralikrishna & Manickam 2017). In the aeration basin, oxygen is provided to microorganisms to activate them and allow them to form carbon dioxide, new microbial cells, and water from organic pollutants. The suspended wastewater with microbial cells is called mixed liquor suspended solids that is used to refer to the population of the microorganisms in the aeration basin. The effluent of the aeration basin flows through a secondary clarifier, which uses gravitational settling to separate the microbial cells and other suspended materials from the liquid portion. A recycle stream collects sludge from the bottom of the secondary clarifier and transports a portion of it back to the aeration tank. This helps to keep the microorganism population constant in the aeration tank. The remaining sludge, referred to as waste activate sludge (WAS), is collected for further treatment.

2.2.4 Tertiary treatment

The treated wastewater still has some residual suspended solids, pathogenic microorganisms and significant amount of nutrients (phosphorus and nitrogen). Failure to remove these nutrients can lead to the eutrophication of water bodies. Thus, further treatment is required to make the wastewater safe to discharge back to the environment. One of the most effective techniques to remove nutrients during advanced treatment in a WWTP is a biological nutrient removal (BNR) system. The BNR system involves a combination of sequencing aerobic, anoxic, and anaerobic processes on the effluent from secondary treatment. Ammonia can be removed by simultaneous nitrification and denitrification processes that ultimately convert nitrogenous compounds to nitrogen gas. Phosphorus compounds can be removed by using a group of bacteria called phosphorus accumulating organisms or by precipitation via adding metal salts (Metcalf & Eddy 2014). Some WWTPs also apply filtration and disinfection via chlorine or ultraviolet irradiation (Das 2001; Anthony *et al.* 2007) to treated wastewater to reduce turbidity and pathogens, respectively, before discharge.

2.3 Wastewater sludge characteristics

There are many different treatment techniques in a WWTP to remove pollutants and pathogenic microorganisms before disposal to the environment. During these physical, chemical, and biological treatment processes, by-products, called wastewater sludge streams or simply sludge, are formed. Environmental concerns have caused biological methods (i.e. composting, anaerobic and aerobic digestion) to replace conventional sludge disposal methods (i.e. incineration, landfilling, and ocean dumping). Among the sludge treatment processes, aerobic digestion and AD are commonly used methods; however, AD is more favorable as it requires less energy and produces bioenergy. AD is a well known process with advantages such as sludge stabilization,

volume reduction, pathogen removal, and biogas production. However, it also has some challenges, such as requiring a long retention time to digest (Appels *et al.* 2008). Thus, a large digester volume is required. Although, the process can be speeded up by using pre-treatment methods applied to digester feed (substrate) prior to biological process. These pre-treatment methods accelerate the hydrolysis (rate limiting) step in AD (Carrère *et al.* 2010).

Wastewater sludge characteristics depend on the source of the wastewater (municipal, agricultural or industrial) and the treatment techniques applied to the wastewater. Sludge requires costly and energy demanding treatment as it is potentially hazardous (Anjum *et al.* 2016). Thus, a good understanding of the sludge characteristics is needed to choose the optimum sludge treatment technique.

The PS, containing settleable organic and inorganic matter (from primary sedimentation), and the WAS, containing microbial cells and extracellular polymeric substances, from secondary sedimentation) are the main sludge streams in a WWTP. These sludge streams are produced in high quantities and are often mixed prior to further treatment (i.e. aerobic digestion or AD). The typical characteristics of PS and WAS are provided in Table 2-1.

Primary sludge has a high concentration of readily biodegradable substances, which makes it an appropriate feedstock for AD. However, WAS has complex structural compounds (i.e. extracellular polymeric substances) which are not digested easily and thus need longer retention time to digest. According to Table 2-1, in comparison to WAS, PS produces more energy due to higher organic content and therefore COD concentration (Metcalf & Eddy 2014).

Table 2-1: Characteristics of untreated wastewater sludge [a]

Item	Primary sludge (PS)	Waste activated sludge (WAS)
Total solid (TS) (% w/w)	1-6	0.4-1.2
Volatile solid (VS) (% of TS)	60-85	60-85
Grease and fats (% of TS)	5-8	5-12
pH	5-8	6.5-8
Specific gravity (-)	1.02	1.05
Alkalinity (mg $CaCO_3$/l)	500-1500	580-1100
Total chemical oxygen demand (g/g VS)	2.0	1.4
Energy content (kJ/kg VS)	23,000-29,000	19,000-23,000

[a] The data is adopted from (Metcalf & Eddy 2014)

The end product of sludge stabilization is referred to as biosolids. Biosolids are nutrient-rich compounds which can be used for land application if they meet criteria set by regulators. In the province of British Columbia, based on fecal coliform density and heavy metals concentration, biosolids are categorized as class A or class B biosolids according to OMRR (OMRR 2008).

2.4 FOG

Fat, oil, and, grease are lipids that are most commonly found in used cooking oil. It can be also found in salad dressing, sauces, butter, cheese, ice cream etc. Sources of FOG generation include restaurants, domestic properties, and food processing sites (i.e. meat plants). (Husain *et al.* 2014; Wallace *et al.* 2017).

Over the last few decades, there has been an increase in the consumption of fast food and prepared foods. Moreover, the population growth in developed countries has led to an increase in FOG generation from food services. The FOG production per capita is estimated to be 50 kg/annum in developed countries versus less than 20 kg/annum in less developed countries. More recently, in 2015, a European organization estimated the production of used cooking oil as 8 L per capita in Europe (Andrea Salimbeni & Valeria Magnolfi 2015; Wallace *et al.* 2017). This large amount of FOG generation can cause many problems for environment and public health (discussed in the next section) and it needs to be managed in a proper way to minimize its adverse effects.

2.4.1 FOG problems/challenges

Fat, oil and grease is present in wastewater and can have detrimental effects on sewer systems. Studies on FOG deposits in sewer systems showed that it has high concentration of fatty acids and calcium. The FOG deposits are calcium salts of fatty acids, which are formed from saponification reaction, conversion of fat or oil in presence of an alkali agent to soap, between fatty acids with calcium ions. Calcium ions are present in wastewater naturally and there is evidence that concrete corrosion is also a source of calcium in sewer systems. The saponified solids adhere to the interior wall of pipe and act as a fixed core that un-reacted free fatty acids can accumulate around. Based on van der Waals attraction, un-reacted free fatty acids attract calcium and other cations to the fixed core. This leads to the build up of more saponified solids. Studies show that the FOG deposit quality (i.e. hardness and adhesion) depends on pH, temperature, water hardness, and type of free fatty acids. At a lower pH, more calcium is released and in the presence of unsaturated free fatty acids, there is a build up of more viscous solids than in the presence of saturated free fatty acids (He *et al.* 2011; He *et al.* 2013). Because of adhesion character of hardened FOG, debris in wastewater also accumulate in the pipes and it gradually decreases the capacity of the pipe.

Ultimately, FOG accumulation leads to complete blockage of the pipes and possibly overflow of the sanitary sewer system. Overflow of the system is a serious risk for human health and the environment as it releases a high concentration of pathogenic compounds and nutrients. Therefore, pipes need cleaning by high pressure water flush to remove the accumulated FOG. Moreover, municipalities spend millions of dollars every year to repair and replace the blocked pipes of the sewer systems (Long *et al.* 2012).

Skimming tanks can be used to remove FOG from wastewater stream in WWTPs. Other techniques for FOG separation include dissolved air floatation, filtration, and centrifugation. The FOG can cause serious problems, such as sticking to the pipes, pipe blockages, and overloading, if it is not removed in the skimming tank. Additionally, it has adverse effects on steps later on in the treatment process, such as a decrease in efficiency of settling tanks (Wallace *et al.* 2017).

Recently, municipalities have mandated the collection of FOG at its source of generation. Grease traps, also called grease abatement, are installed at the waste effluent stream of restaurants to separate FOG by gravitational force. A grease trap is usually a tank smaller than 55 gallons that has different sections, where FOG is collected at the top and solids are collected at the bottom (Wallace *et al.* 2017). Moreover, grease traps need regular cleaning. Some countries have regulations about the cleaning of grease traps, as their efficiency is highly dependant on the frequency of cleaning (Husain *et al.* 2014).

In Kelowna, it costs more than $60,000 annually to clear FOG from the sewer system (City of Kelowna 2017a). Regional District of Central Okanagan requests residences to not to pour the leftover household cooking oil and kitchen grease down the drain. Residences can collect the cooking oil and congealed grease and dispose them at the Westside Residential Disposal and Recycling Centre in West Kelowna, or at the Glenmore Landfill in Kelowna (RDCO 2017).

Using grease traps devices to collect used cooking oil in commercial food and restaurants in Kelowna is mandated and grease traps should be cleaned when grease or oil level reached to 25% of the liquid level in a trap (City of Kelowna 2017b).

2.4.2 FOG management

In the past, collected FOG was disposed in landfills; however, this practice is now banned in many developed countries. This is due to its release of carbon dioxide and methane into the atmosphere and consequently adverse effects on public health and the environment (Husain *et al.* 2014).

Potential alternatives to treat collected FOG from grease traps in restaurants and WWTPs are AD, biodiesel, and biopolymer production (Wallace *et al.* 2017). Recently, biodiesel production from used frying oil, grease traps, and other waste oils has been a favorable option compared to conventional fuels due to its environmental advantages and lower biodiesel production costs. However, using restaurants grease traps (40% to 100% free fatty acids) as a feedstock to produce biodiesel is challenging since biodiesel production requires the feedstock to have the lowest possible free fatty acids concentration. This is due to the adverse effect of a high free fatty acids concentration on the biodiesel yield and glycerol production. There are studies to find the optimal condition to use an acid catalyst to treat free fatty acids in a grease trap, prior to the transesterification step for biodiesel production (Park *et al.* 2010). Although, for operation on a larger scale, proper disposal of the acidic stream at the end should be considered. The acid catalyst can be removed through some separation units, to make the management of final stream easier (Pastore *et al.* 2014). It is estimated that 31.5% of biodiesel production of U.S. can be fulfilled by recovering and using grease trap waste (Wallace *et al.* 2017).

Since FOG has great potential for methane production, using it as a co-substrate in AD is a viable alternative for its treatment. The methane produced from co-digestion of FOG with wastewater sludge can be used as a renewable source of energy for a WWTP. However, there are some challenges in this process such as sludge floatation, scum foaming, and LCFA accumulation, which causes process failure. Hence, it is essential to have a good understanding of the process and its inhibition parameters, to find the best operating condition to prevent process failure and produce methane as discussed in the subsequent sections.

2.4.3 FOG characteristics

Generally, FOG is semi solid at room temperature, its density is lower than water, and it is not soluble in water. However, it is soluble in organic solvents such as hexane and ether (Husain *et al.* 2014). Physico-chemical properties of FOG are highly dependant on its source of generation (Table 2-2). Moreover, FOG from the same source collected at different times, can also have different characteristics based on waste collection frequency (Long *et al.* 2012). Although, almost all the collected FOG wastes have an acidic pH due to the presence of free fatty acids that are mainly formed through hydrolysis and oxidation of oils (Husain *et al.* 2014). FOG also has a high solid content (total solids (TS) and VS). Its high VS causes an increase in its COD. Previous studies have reported that FOG is mainly composed of saturated fats (i.e. palmitic acid and stearic acid), monounsaturated fats (i.e. oleic acid), and polyunsaturated fats (i.e. linoleic acid) (Salama *et al.* 2019). FOG characteristics in Table 2-2 provide a better understanding of its potential as an organic feedstock to improve biogas production in AD.

Table 2-2: FOG characteristics from different sources

Source	Dairy farm in BC (Srinivasan *et al.* 2018)		Polymer-dewatered FOG (Kabouris *et al.* 2008)	Organic Resource Management Inc. in Ottawa (Alqaralleh *et al.* 2018)	Primary skimmer of a plant in Spain (Martín-González *et al.* 2011)	Thickened grease trap sludge (Davidsson *et al.* 2008)	FOG receiving facility in Columbus (Wan *et al.* 2011)	Meat processing plant (Luostarinen *et al.* 2009)
pH	4.5	2.9	4.03	4.2	NM[a]	4.38	4.2	5.1
TS	2.4%	90.9%	42.4%	29.1%	11.6%	17%	3%	25%
VS	NM	NM	40.9%	28.2%	10.4%	17%	3%	25%
(VS/TS)*100	NM	NM	96%	97%	89%	98%	93.9%	99%
VFA	1.6 g/l	4.3 g/l	3,473 mg COD/l	NM	NM	NM	857.4 mg/l	NM
TCOD	49 g/l	534 g/l	1,211 g/kg	261.2 g/kg	NM	NM	NM	NM
SCOD	17 g/l	286 g/l	13.7 g/l	NM	NM	NM	NM	NM
Ammonia	NM	NM	356 mg/l	NM	NM	136 mg/l	NM	NM
Alkalinity	NM	NM	NM	NM	NM	NM	NM	NM
TKN	NM	NM	5.1 g/kg	NM	2.4%	NM	NM	NM
TOC	NM	NM	NM	NM	55%	NM	NM	NM
Total phosphorus	NM	NM	670 mg/kg	NM	NM	NM	NM	NM

TS: total solids; VS: volatile solids; VFA: volatile fatty acids; TCOD: total chemical oxygen demand; SCOD: soluble chemical oxygen demand; TKN: total kjeldahl nitrogen; TOC: total organic carbon; [a]Not measured

2.5 Sludge stabilization

Sludge stabilization involves the removal of volatile or organic compounds, to make the growth of microorganisms, in the organic fraction of sludge, unsuitable. Failure to control the volatile compounds leads to pathogens survival, the release of offensive odors, and putrefaction. Sludge stabilization can be achieved by one of the following methods: alkaline stabilization, AD, aerobic digestion, or composting. Benefits of sludge stabilization also include an improvement in dewaterability, volume reduction, and methane-rich biogas production (Metcalf & Eddy 2014).

2.5.1 Alkaline stabilization

One of the most common methods of sludge stabilization is the addition of a chemical (usually lime). Enough lime should be added to maintain the sludge pH at or above 12. However, lime is also consumed in other reactions, which can lead to a decrease in pH. Thus, excess amounts of lime should be used to keep the pH at the specified range required for adequate stabilization (Metcalf & Eddy 2014).

Alkaline stabilization is a method that requires simple technology to produce class A and B biosolids (based on the criteria defined by OMRR) (OMRR 2008). Its operation is also flexible. However, compared to other methods, this method has a higher volume of biosolids for final disposal and has the potential to produce odorous material (Metcalf & Eddy 2014).

2.5.2 Anaerobic digestion

In AD, organic matter is decomposed in absence of oxygen and converted to methane-rich biogas. This method can also produce biosolids suitable for land applications depending on digester temperature and specific SRT used. In order to obtain the desirable biogas production and achieve beneficial biosolids production, a suitable process design with a good understanding of

the principles of the process is needed (Metcalf & Eddy 2014). A detailed discussion on AD process is provided in section 2.6.

2.5.3 Aerobic digestion

Aerobic digestion usually takes place in an open-top tank with a continuous air (or oxygen) supply. Once microorganisms have consumed the available substrate in sludge, they enter a phase of endogenous respiration and consume their own protoplasm to survive. This leads to a reduction in sludge volume. In this process class B biosolids can be produced at specific SRT and temperatures. This process is also simpler and easier to operate than AD. However, this process requires a higher capital cost to provide the high amount of energy needed for aeration and continuous mixing. Moreover, there is no energy recovery in this process (Metcalf & Eddy 2014). Therefore, it is preferred by small- to medium-size WWTPs.

2.5.4 Composting

Composting involves the degradation of the organic fraction of sludge through biological reactions in an enclosed reactor, windrows, or piles. A suitable environment for biological activity is provided by addition of a bulking agent. Organic decomposition in the sludge leads to an increase in temperature, which activates more bacteria and reduces pathogens. Moreover, unstable nitrogen forms are converted to stable organic forms and volume of waste is reduced in this process (Sunar *et al.* 2014). One of the most significant drawbacks of this process is the production of odours. One way to minimize odours is to combine composting with aerobic digestion. This also helps elevate temperature and thus increase pathogen removal (Metcalf & Eddy 2014).

2.6 Fundamentals of anaerobic digestion

A favorable technique for sludge stabilization is AD, due to its potential for organic matter decomposition, methane-rich biogas production, and odor and pathogen reduction. Moreover, the

final biosolids are classified based on heavy metal and pathogenic microorganism concentrations and can be used for land application as fertilizer if they meet the criteria or composted for further stabilization.

Anaerobic digestion is a biochemical process which consists of four main steps. As it is shown in Figure 2-2, these steps are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each step involves a different diversity of microorganisms. The hydrolysis step achieves the conversion of insoluble matter to soluble substances (Parkin & Owen 1986; Adekunle & Okolie 2015). The higher molecular weight compounds are also broken down to lower molecular weight compounds, mainly by hydrolytic fermentative bacteria. The hydrolysis step is followed by the acidogenesis step, in which hydrolysed compounds are further degraded to form VFA, carbon dioxide, ammonia and hydrogen sulfide. These compounds are utilized in the acetogenesis step to mainly produce acetic acid (Batstone *et al.* 2002). In the last step, two groups of methanogens are involved; a group of them (i.e. aceticlastic methanogens) converts acetate into methane and carbon dioxide. The other group of methanogens (i.e. hydrogenotrophic methanogens) use hydrogen and carbon dioxide as an electron donor and acceptor respectively, to produce methane (Appels *et al.* 2008).

Performance of AD depends on different operating parameters including temperature, pH, alkalinity, VFA, SRT, OLR, substrate characteristics, and inhibitory compounds. Temperature is one the most important parameters which affects the process in terms of microbial ecology in the digester and biological activity. By increasing the temperature, chemical and biological reactions are accelerated, and solubility is improved. Moreover, pathogenic microorganisms are reduced and class A biosolids are produced. However, elevating temperature may lead to a decrease in system's tolerance to inhibitory substances due to reduced microbial diversity and require more energy input

for heating. As a result, temperature increase requires a good strategy to avoid process failure (Appels *et al.* 2008).

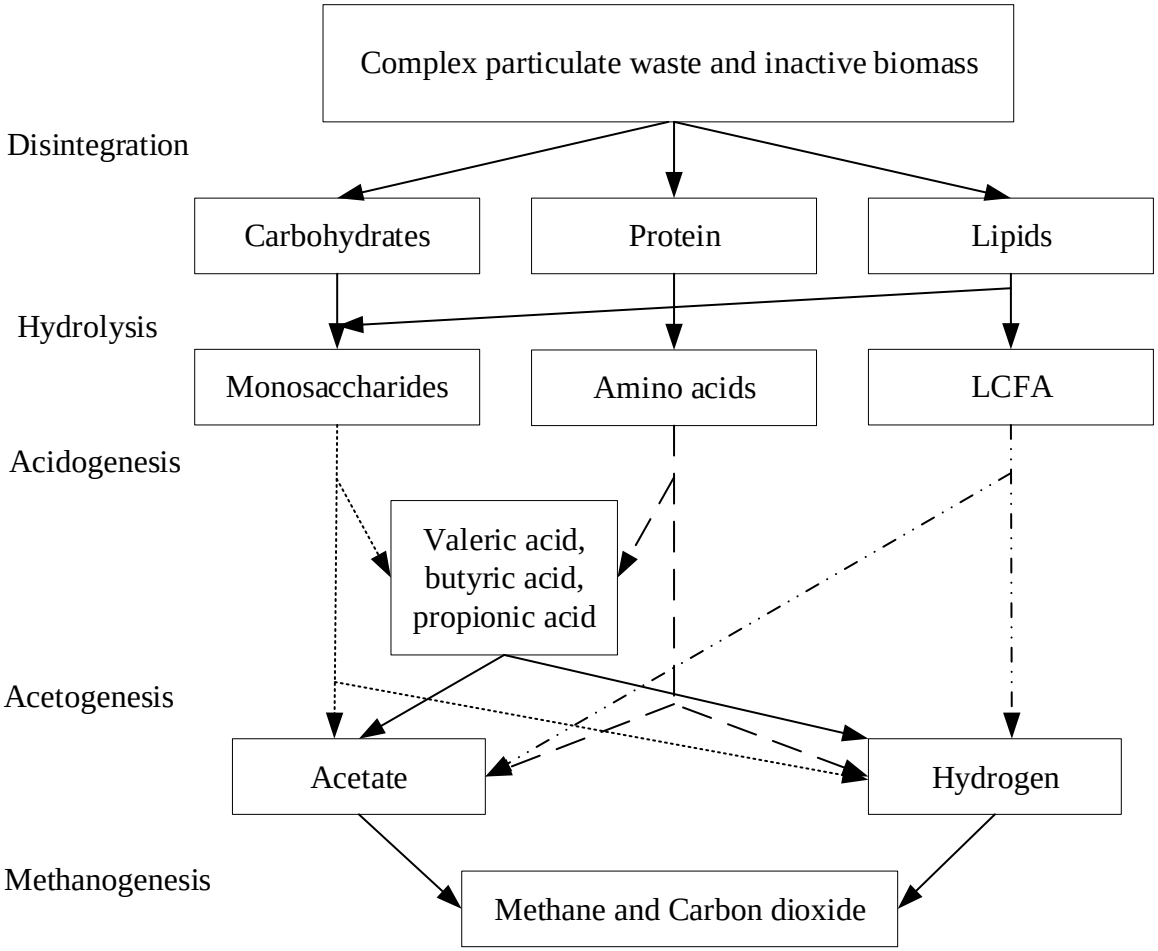

Figure 2-2: Main stages of sludge anaerobic digestion process (adapted from (Batstone *et al.* 2002))

Another key parameter is pH which is influenced by VFA and alkalinity in the digester. Methanogens are the most sensitive groups of microorganisms to pH. The pH preference of methanogens and fermentative organisms is between 6.5 to 7.2 and 4 to 8, respectively. The overall optimal pH is between 6.6 and 7.4 for a single-stage AD where acid and methane formation occurs simultaneously (Boe & Angelidaki 2006). VFA production normally results in a pH drop that would interrupt the activity of methanogens; however, this is prevented by alkalinity production (carbon dioxide, ammonia, and bicarbonate) during methanogenesis. It is noted that to achieve

stable digester performance the molar ratio of bicarbonate to VFA should be maintained as at least 1.4:1. The addition of bicarbonate externally is a common practice to increase digester pH if VFA accumulation occurs (Appels *et al.* 2008).

The average time that solids are held in the digester is defined as SRT and it is equal to hydraulic retention time (HRT), the average time that liquid is held in the digester, for a continuously stirred tank reactor without recycle. The SRT significantly affects the AD performance and process efficiency. There should be a balance between the microorganisms which are withdrawn from the digester and the newly grown cells that are produced by microorganism growth and reproduction. The rate of microbial production is not the same for different groups of microorganisms, for example, acidogenesis needs a shorter SRT than methanogenesis. At a SRT of 5-8 days, VFA accumulation and incomplete compound breakdown is reported. Therefore, it is noted that a minimum 10 days of SRT is required for the reduction of all sludge compounds and stable AD performance (Appels *et al.* 2008). In literature it is reported that at a mesophilic condition (35°C) approximately 15-20 days is required to treat municipal wastewater under AD process (Ho *et al.* 2014). However, at a thermophilic condition (55°C) the growth rate of methanogenesis is higher and the required SRT may be reduced to 5-8 days (Chen *et al.* 2018).

Organic matter is introduced to the digester on a daily constant rate (i.e. OLR) and expressed as a daily input VS per volume of the digester (g VS/l/day). The typical OLR for an anaerobic digester running under a mesophilic condition is ranged between 1.6 to 4.8 g VS/l/day (Metcalf & Eddy 2014). More organic matter is provided for acidogenesis to produce VFA at a higher OLR. Methanogens utilize the produced VFA and subsequently more methane is produced by increasing the OLR. However, there should be a balance between VFA production and their conversion to methane, due to the slower growth rate of methanogens compared to acidogens. If the OLR was

suddenly increased, the VFA would start to accumulate and cause a pH drop. In the case of insufficient alkalinity and buffering capacity, process failure can occur as described earlier. Foaming is another drawback of shock loading that can result in partial sludge degradation and the threshold organic loading of 2.5 g VS/l/d was reported in literature to prevent foam initiation (Ganidi *et al.* 2011). The optimum OLR of 2.9 g VS/l/day was reported to achieve the highest solids reduction in AD of municipal solid waste and domestic sewage (Elango *et al.* 2007).

2.6.1 Anaerobic co-digestion

In general, conventional AD utilizes a single substrate fed to the digester. However, based on the digester capacity, there may be an opportunity to increase OLR by adding one or more secondary substrates. Simultaneous digestion of two or more organic substrates, called co-digestion. In this process, the main substrate is the primary substrate (i.e. wastewater sludge) and it is mixed with one or more secondary substrates that result in organics (cellulose and hemicellulose) biodegradation improvement (Hagos *et al.* 2017) and subsequently enhance biogas production. Beside advantages on energy recovery, co-digestion improves buffering capacity of the mixture which can protect the digestion process from possible failures due to accumulation of inhibitory compounds (i.e. VFA) (Cuetos *et al.* 2011). In addition, production of a mixture with optimal C/N ratio and dilution of inhibitory compounds are reported in literature as a result of co-digestion process (Esposito *et al.* 2012). Beside these process benefits, economical and environmental advantages have made this process more favorable than conventional AD (Metcalf & Eddy 2014).

In literature, better process performances as a results of the addition of different co-substrates such as typical food waste and straw (Yong *et al.* 2015), household organic wastes (Zupančič *et*

al. 2008), rice straw and chicken manure (Mei *et al.* 2016), wine/fruit juice production waste or landfill leachate (Barrantes Leiva *et al.* 2014) to the main substrate are reported.

Fat, oil, and grease are one of the most favorable organic substrates compared to other high strength liquid organic wastes such as whey from cheese production or residual glycerin from biodiesel production (Metcalf & Eddy 2014). Moreover, studies showed higher theoretical methane production from lipids (0.990 l/g) in comparison to theoretical methane production from proteins (0.633 l/g) and carbohydrates (0.415 l/g) (Alves *et al.* 2009). The high energy value of lipids makes them an ideal co-substrate for AD. Lipid addition to the AD process, even a fraction in the feed, can improve the net energy production (Alves *et al.* 2009).

Fat, oil, and grease are lipids consisting of glycerol linked by ester or ether to LCFAs and alcohols. Fats and oil are categorized as a subgroup of lipids that are mainly in form of triglycerides in which all three hydroxyl functional groups of glycerides are esterified by LCFAs (Figure 2-32-3). Fat structures consist of saturated LCFAs (i.e. palmitic acid ($C_{16}H_{32}O_2$)) (Figure 2-4(a)) and oil structures consist of unsaturated LCFAs (i.e. oleic acid ($C_{18}H_{34}O_2$)) (Figure 2-4(b)) (Alves *et al.* 2009).

By feeding FOG to AD, fats and oil undergo hydrolysis and break down to their major components: glycerol and LCFA. The glycerol is mainly degraded to acetate and hydrogen to form methane. The LCFA undergoes β-oxidation via a syntrophic relationship between acetogenic bacteria and methanogenic archaea, to form acetate, hydrogen, and carbon dioxide. These biochemical reactions eventually produce methane. Studies showed that all bacteria that β-oxidize LCFA, belong to one of two families: *Syntrophomonadaceae* within the class of *Clostridia* or *Syntrophaceae* within the subclass of *Deltaproteobacteria*. It has been proven that LCFA degradation is the rate-limiting step in lipid degradation during AD and can inhibit the process at

a high concentration (Ziels *et al.* 2015; Ziels *et al.* 2016). Process failure was reported at OLRs of 3.4 g VS/l/day, 4.1 g VS/l/day, and 7.39 g VS/l/day as a result of co-digestion of FOG with thickened waste activated sludge (TWAS), PS and TWAS and primary raw sludge in different studies (Wan *et al.* 2011; Noutsopoulos *et al.* 2013; Li, C. *et al.* 2013).

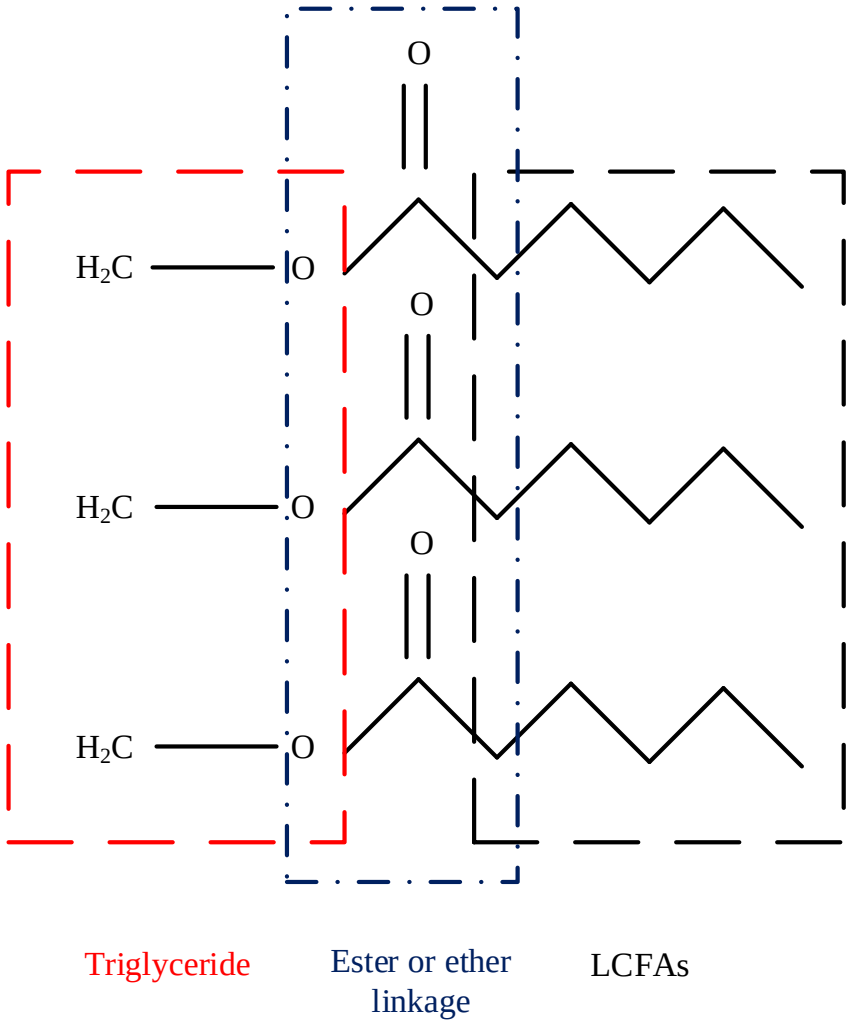

Figure 2-3: FOG structure

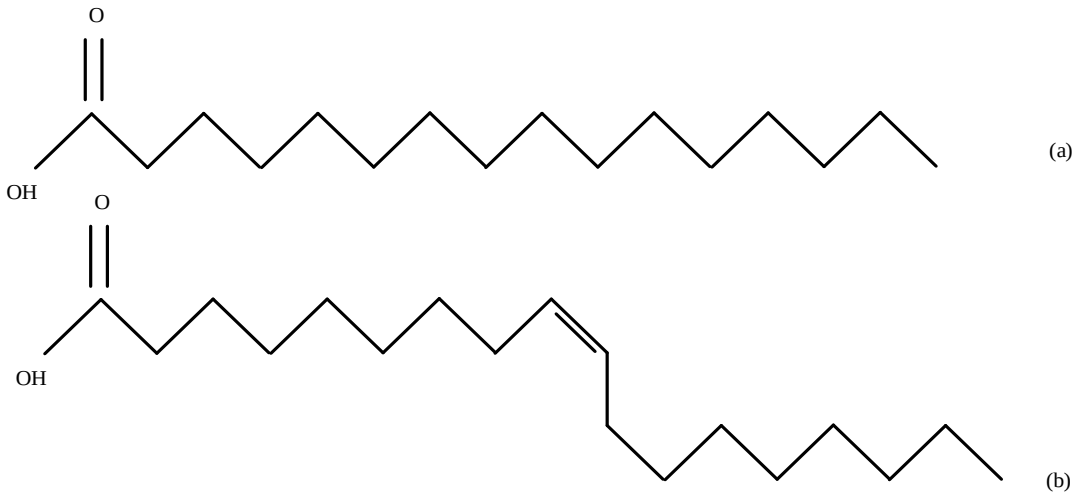

Figure 2-4: LCFA structure (a) palmitic acid (saturated LCFA) (b) oleic acid (unsaturated LCFA)

The challenges of adding FOG to AD are categorized into two main groups: (1) LCFA accumulation in digester which can cause sludge floatation and biomass washout, and (2) LCFA absorption to cell walls and their toxic effect on acetogenic bacteria and methanogenic archaea (Alves *et al.* 2009).

Long chain fatty acids have an amphiphilic structure. This means that they have a hydrophobic aliphatic tail and a hydrophilic carboxylic head (Figure 2-4). At neutral pH, they act as a surfactant that decreases the surface tension. As a result of the lowered surface tension, adhesion properties of acetogens that are characterized as hydrophobic, decrease. Therefore, sludge floatation and sludge washout are expected as a result of LCFA accumulation (Alves *et al.* 2009).

The accumulated LCFAs can also absorb to the biomass cell surfaces and hinder the mass transfer to the substrate. Moreover, LCFAs hinder biogas release (Pereira *et al.* 2005). The rate of LCFA absorption is closely related to their concentration. Their accumulation can also significantly decrease digester performance. Moreover, the adverse effects of the mixture of LCFAs are more severe in comparison with single LCFA compounds (Salama *et al.* 2019).

Permanent toxic effect of LCFAs on acidogenic bacteria and methanogenic archaea was reported in previous studies (Angelidaki & Ahring 1992; Rinzema *et al.* 1994). However, more recent research has shown that this toxic effect is reversible (Pereira *et al.* 2005).

As it is described above, adding FOG to AD process as a co-substrate without a proper feeding strategy can be risky and lead to process failure. Therefore, a number of studies have been conducted to investigate the effectiveness of different parameters on digester performance. Table 2-3 summarizes the results of different studies on anaerobic co-digestion with FOG, with different sources of generation (restaurant grease trap, meat processing plant, WWTP, etc.). As it can be concluded from Table 2-3, the process performance is highly dependant on the source of FOG and its characteristics, along with digester OLR, temperature, and reactor configuration.

Davidsson et al. conducted laboratory batch tests with two pure LCFAs (solid stearic acid and liquid oleic acid), as well as with a mixture of sewage sludge from a WWTP and restaurant grease, at different ratios (VS-basis). These tests investigated the methane yield and compared the results to theoretical methane yields at a mesophilic temperature (38°C). The results showed that pure substrates had the closest methane potential to the theoretical value (1014 Nml CH_4/g VS). Furthermore, different concentrations of restaurant grease as a single substrate (10, 25, and 60% VS-basis) without sewage sludge showed a similar methane production trend, as they all reached to 845-925 Nml CH_4/g VS. This indicated that restaurant grease is mostly consisted of fats. Based on results from mixtures of sewage sludge and restaurant grease at different ratios (restaurant grease:sewage sludge of 10:90, 25:75, 60:40, and 0:100 VS-basis), the methane production was the same in all reactors during the first 80 h. However, after 80 h, the methane production increased by increasing restaurant grease:sewage sludge ratio. Moreover, in the second test round of this study, continuous-flow reactors' performance was investigated with different restaurant

grease:sewage sludge ratios (100:0, 90:10, 70:30, and 0:100 VS-basis). Digesters with restaurant grease as a single substrate failed to stabilize. However, the addition of restaurant grease to sewage sludge (10 and 30% of total VS) showed improvement of 9-27% in methane yield. The continuous-flow ADs utilizing restaurant grease and sewage sludge at 10 and 30% of total VS also reached to 90% and 75% of calculated methane yield from the batch experiment, respectively (Davidsson *et al.* 2008).

Ziels et al. investigated the effectiveness of FOG addition to the main substrate (fermented primary sludge (FPS) and TWAS). In this study, FOG ratio was increased to 52% of total VS over 94 days. The experiment was conducted by operating two semi-continuous flow ADs at a mesophilic temperature (37°C) with 20 days SRT. At first, digesters were fed with FPS+TWAS to reach to steady-state level. Then, FOG was added to one of the digesters at 13% of total VS and the ratio was increased to 52% in a stepwise manner. Co-digester performance was observed to be enhanced by a 31% improvement in specific methane yield over control (AD fed with FPS + TWAS only): 420 and 320 ml CH_4/g VS, respectively. Moreover, an improvement in VS reduction was achieved, as it reached to 63% in the co-digester in comparison with 51% in the control. Based on this result, VS reduction of FOG was estimated to be 74%. Furthermore, LCFA concentration (summation of palmitic acid, stearic acid, and oleic acid) was measured by gas chromatography (GC) during the experiment and at 52% VS FOG addition to FPS+TWAS (OLR = 2.9 g VS/l/day), it reached the highest value of 104 mg LCFA/g TS without any process failure (Ziels *et al.* 2016).

Other studies were performed to investigate the effectiveness of different digester temperatures on anaerobic co-digestion of FOG. A lab-scale study was performed in batch and continuous-flow reactor feeding mode, to investigate the impact of FOG addition (from a WWTP) as a co-substrate on anaerobic co-digestion performance. The experiment was carried out at a mesophilic

temperature (37°C) and the optimum ratio of FOG to the main substrate was estimated to be 1:6 (VS: VS) (Martín-González, L. *et al.* 2010). The authors continued their study with another set of experiments to investigate the effectiveness of a higher digester temperature on the efficiency of the process. The optimum FOG/feed ratio was applied to the anaerobic co-digestion process at a thermophilic temperature (55°C), and the process performance was compared with obtained results at a mesophilic temperature. It was reported that at 55°C, 25% and 40% improvements in biogas and methane yields in comparison with the mesophilic condition were observed, respectively (Martín-González, Lucia *et al.* 2011).

In another study, the effectiveness of different operation parameters including SRT (12 and 24 days) and OLR (1.19 - 8.97 g VS/l/day) as well as process temperatures (37°C and 55°C) of anaerobic co-digestion on FOG degradation were investigated. At SRT of 24 days and OLR of 2.43 g VS/l/day the best digester performance was achieved. At a thermophilic temperature, 32.8% and 7.10% higher improvements in biogas production and methane content were observed, respectively, compared to a mesophilic condition at the optimum operational condition (SRT = 24 days and OLR = 2.43 g VS/l/day) and the results confirmed the effectiveness of a higher temperature on process performance. However, for different OLRs, the biogas production pattern was the same at mesophilic and thermophilic temperatures (Li, C. *et al.* 2013). The results, summarized in Table 2-32-3, illustrated the positive impact of a higher temperature on anaerobic co-digestion efficiency with FOG. However, financial consideration should be considered, since higher energy input is required for elevating the process temperature from mesophilic to thermophilic.

Adding collected FOG from restaurant grease interceptors, to the AD process was implemented in a full-scale wastewater sludge digester in City of Riverside, California. It was reported that,

FOG was added to the digester in a 15% volumetric ratio to the mixed sludge and 58 to 133% improvement in daily biogas production was observed over the control during a year. Moreover, improvement in methane percentage from 60% to 68% in the produced biogas and about 16% improvement in reduction of biosolids production were achieved during this process (Bailey 2007).

Table 2-3: Studies on anaerobic co-digestion of FOG*

Author, year	FOG type/ Co-substrate type	Digester conditions/ Digester type	Main results
(Davidsson *et al.* 2008)	Grease trap (17% VS, 17.3% TS VS/TS = 98%) Mixed sludge (PS:WAS 50:50%) (4.1% TS, 3.1% VS VS/TS = 75%)	Temperature: 38°C Lab-scale batch and continuous-flow anaerobic digestion SRT = 10-13 days	Up to 27% improvement in methane yield in continuous-flow anaerobic digestion achieved by increasing FOG up to 30% of total VS (OLR = 2.4 g VS/l/day)
(Ziels *et al.* 2016)	Waste cooking oil (VS/TS ≈ 99%) Waste primary sludge + WAS	Temperature: 37°C Semi-continuous flow anaerobic digestion SRT =20 days	Up to 31% improvement in specific methane yield achieved in co-digester over control at 52% VS FOG in feed (OLR = 2.9 g VS/l/day). Furthermore, 23% improvement in VS reduction improvement was observed over the control.
(Luostarinen *et al.* 2009)	Grease trap from a meat processing plant (25.4% TS, 25.2% VS VS/TS = 99%) Municipal sludge (3.2% TS, 1.8% VS, VS/TS = 67%)	Temperature: 35°C Batch and semi-continuous flow anaerobic digestion SRT = 20, 18 and 16 days	Grease trap sludge was increased up to 46% of total VS and no inhibition sign was observed (SRT of 16 days, OLR = 3.46 g VS/l/day). Methane yield at 46% VS in co-digester reached to 463 ml CH_4/g VS in comparison with 278 ml CH_4/g VS in control. At 55-71% of total VS grease trap, process did not reach to steady state level.

Table 2-3: Studies on anaerobic co-digestion of FOG (cont'd)

Author, year	FOG type/ Co-substrate	Digester conditions/ Digester type	Main results
(Noutsopoulos *et al.* 2013)	Surface skimming of primary settling tank of a WWTP (71% TS, 64% VS VS/TS = 90%) Mixed sludge (PS:TWAS 74:26% VS basis)	Temperature: 35°C Semi-continuous flow anaerobic digestion SRT = 15 days	Co-digestion of mixed sludge with grease sludge under OLR up to 3.5 g VS/l/day increased biogas yield up to 55% over control. Grease sludge OLR could be increased up to 2.4 g VS/l without process inhibition.
(Wan *et al.* 2011)	FOG receiving facility (VS/TS = 93.9%) TWAS (VS/TS = 84%)	Temperature: 37°C Semi-continuous flow anaerobic digestion SRT = 15 days	Co-digestion of 64% VS FOG with TWAS resulted in 137% improvement in methane production. Adding synthetic micronutrient to the mixture of FOG and TWAS resulted in no significant improvement in biogas production and process stability.
(Martín-González, L. *et al.* 2010)	Primary skimmer of the sewage treatment plant (TS = 120.2 g/kg VS = 99.4 g/kg VS/TS = 82%) Organic fraction of municipal solid waste (TS = 370 g/kg, VS = 275 g/kg, VS/TS = 74%)	Temperature: 37°C Batch and semi-continuous flow anaerobic digestion SRT = 14.5 days	15% FOG addition was chosen for semi-continuous flow anaerobic co-digestion based on batch experiments with different FOG ratios (5%, 15%, 35% and 100%, VS basis). In semi-continuous flow experiment, OLR increased to 4.5 g VS/l/day after FOG addition and 72% and 46% higher biogas production and methane yield were observed over the control.

Table 2-3: Studies on anaerobic co-digestion of FOG (cont'd)

Author, year	FOG type/ Co-substrate	Digester conditions/ Digester type	Main results
(Martín-González, Lucia *et al.* 2011)	Primary skimmer of the sewage treatment plant (TS = 116.6 g/kg VS = 103.9 g/kg VS/TS = 89%) Organic fraction of municipal solid wastes (TS = 373 g/kg, VS = 338 g/kg, VS/TS = 90%)	Temperature: 55°C Semi-continuous flow anaerobic digestion SRT = 16 days	FOG was added to the main substrate (municipal solid waste) in 1:6 ratio (VS basis) and improved the biogas production by 52% and methane yield by 36%.
(Li, C. *et al.* 2013)	Restaurant's waste oil (TS = 1119 g/l VS = 951 g/l VS/TS = 85%) Primary raw sludge (TS = 41.9 g/l, VS = 31.6 g/l, VS/TS = 75%)	Temperature: 55°C and 37°C Semi-continuous flow anaerobic digestion SRT = 12 and 24 days	At mesophilic temperature (SRT = 12 days) with OLR of 4.22 g VS/l/day, biogas production increased during the first days and gradually decreased to the same level as control's biogas production. Higher OLRs (7.39 and 8.97 g VS/l/day) led to process inhibition. Thermophilic digesters performance had the same patterns as mesophilic digesters with higher biogas production and higher methane yield. The highest biogas production was achieved at OLR of 2.5 g VS/l/day and SRT of 24 days. Biogas production and methane content at the thermophilic temperature were 32.8% and 7.10% higher than the identical condition at the mesophilic temperature.

Table 2-3: Studies on anaerobic co-digestion of FOG (cont'd)

Author, year	FOG type/ Co-substrate	Digester conditions/ Digester type	Main results
(Alqaralleh *et al.* 2016)	Organic Resources Management Inc. (TS = 290.1 g/kg VS = 282.8 g/kg VS/TS = 97%) TWAS (VS/TS = 72%)	Temperature: 55°C and 70°C Batch tests	Batch tests were run by using mixture of FOG and TWAS at different VS ratios (0, 20, 40, 60 and 80% FOG) at a thermophilic condition and hyper-thermophilic/thermophilic conditions (after 2 days, assays were transferred from 70 to 55°C). Hyper-thermophilic (70°C) digestion of TWAS with FOG has shown significant improvement over TWAS digestion under a thermophilic temperature (55°C) (112.7% improvement in methane production). At 80% FOG, the lowest biogas production was obtained at both temperatures, even less than control.

*FOG: Fat, oil and grease; TS: total solid; VS: volatile solid; PS: primary sludge; WAS: waste activated sludge; TWAS: thickened waste activated sludge; SRT: solid retention time; OLR: organic loading rate

2.7 Advanced anaerobic digestion

Advanced AD can produce class A biosolids for land application and removes volatile solids better than conventional single-stage mesophilic AD. Thermophilic digestion (50-57°C) is an option for advanced AD. Its acceleration of biochemical reactions allows it to reduce residual sludge volume and pathogens more than mesophilic digestion. However, the process has a higher energy requirement and a more difficult heat recovery system (Metcalf & Eddy 2014).

Staged AD and sludge pre-treatment are other commonly used options for advanced AD. These processes are further discussed in the following sections.

2.7.1 Staged anaerobic digestion

The staged AD system involves two or more digesters in series. Staged thermophilic digestion has a larger reactor as a first stage and one or more smaller subsequent digesters at thermophilic temperatures. This sequence of digesters is used to achieve class A biosolids. Staged mesophilic digestion also has two reactors in series, at mesophilic temperatures, to enhance digestate dewaterability and produce less odorous and more stable biosolids (Metcalf & Eddy 2014).

Temperature-phased anaerobic digestion (TPAD) is another well-known alternative of the staged AD system. TPAD consists of a thermophilic (55°C) or hyper thermophilic (60-70°C) digester as a first stage (acid phase) with a shorter SRT. In the acid phase, the first two steps of anaerobic digestion (hydrolysis and acidogenesis) take place and VFAs are formed. The acid phase is followed by a mesophilic digester (methane phase) with a longer SRT, to produce methane. The mesophilic digester in this system enhances sludge stabilization and the thermophilic digester improves the digestion rate. The combination of these two digesters make this system more efficient in terms of VS removal and sludge stabilization than single stage mesophilic or

thermophilic digesters (Metcalf & Eddy 2014). Moreover, the energy requirement is much smaller than a staged thermophilic digester. This process also produces class A biosolids.

There are few studies on dual stage anaerobic co-digestion with FOG. As it is mentioned in the previous section, researchers (Li, C. *et al.* 2013) concluded that single stage anaerobic co-digestion with FOG has better performance at thermophilic temperatures than at mesophilic temperatures. In another study, researchers investigated the effectiveness of the feed pre-treatment on a thermophilic two stage digestion system. Two sets of dual stage thermophilic anaerobic co-digestion were operated under a thermophilic temperature (55°C) with 24 days SRT. Raw primary sludge was used as a main substrate and FOG, collected from the garbage waste oil of a graduate club restaurant, was used as a secondary substrate. Digester configurations, operational conditions, and feed mixture were identical for both systems (except pre-treated feed for one of the systems). The digester performance was enhanced using thermo-chemical pre-treatment. The pre-treatment involved adjusting the pH of substrate mixture (FOG + raw primary sludge) to around 10, using sodium hydroxide, and incubating in a batch reactor at 55°C for one day. The pre-treated substrate was fed to first stage digester and first stage digester effluent was fed to the second stage digester to ensure sufficient digestion. It was concluded that pre-treatment significantly improves biogas production and COD and soluble COD removal for a two stage digestion system (Li, C. *et al.* 2015).

As mentioned in Table **Error! Reference source not found.**, other researchers (Alqaralleh *et a l.* 2016) conducted some biochemical methane potential tests which confirmed that elevating process temperature to hyper-thermophilic condition can improve the process efficiency and methane production. More recently, the authors studied co-digestion of FOG with TWAS in a two stage semi-continuous flow reactor system. The authors concluded that there were advantages of

applying hyper-thermophilic condition to the system. The first stage digester was operated at a hyper-thermophilic (70°C) temperature and the second stage digester was operated at a thermophilic (55°C) temperature. The performance of digesters was compared to a single stage co-digester and a control digester operated at a thermophilic temperature (55°C). The single stage co-digester was fed with the same TWAS+FOG mixture while the control digester was only fed with TWAS. In this study, the co-digester performance at different SRTs (9, 12 and 15 days) was evaluated. Moreover, the FOG addition to the feed mixture was increased to find the maximum possible FOG % (VS basis). The best digester performance was achieved at 15 days SRT with 70% VS FOG addition. This combination improved methane yield of the dual stage co-digestion system, up to 148.2%, over the control. The single stage thermophilic co-digestion performance was enhanced by FOG addition up to 65% in the feed and the methane yield was 88.3% more than the control. Moreover, the total coliforms were measured at 40%VS FOG at different SRTs (9, 12 and 15 days). It was also shown that the hyper-thermophilic/thermophilic dual stage system was able to produce class A biosolids even at a short SRT (9 days). However, the single stage co-digester and control required a longer SRT (at least 12 days) to produce class A biosolids (Alqaralleh *et al.* 2018).

2.7.2 Sludge pre-treatment for enhanced anaerobic digestion

Aerobic and anaerobic digestions are common methods for sludge stabilization; however, presence of complex organics in feed decreases the process efficiency. Sludge pre-treatment methods are employed to alter the structure of these rate limiting compounds, by disrupting the sludge structure before AD. TWAS is the main concern in sludge pre-treatment due to its lower biodegradability characteristics in comparison with PS. Pre-treatment methods (i.e. thermal, chemical, mechanical methods, and their combinations) have different sludge disintegration

potentials under different conditions. However, all the pre-treatment methods are employed to increase hydrolysis (rate limiting step of the process) by applying a form of energy to the sludge. Furthermore, pre-treatment is beneficial to reduce the required SRT in digesters, by accelerating biological treatment (Metcalf & Eddy 2014; Anjum *et al.* 2016). In the following sections, some forms of sludge pre-treatment processes are described in more detail and literature results on their application for FOG pre-treatment are provided.

2.7.2.1 Thermal hydrolysis pre-treatment

Thermal hydrolysis improves the hydrolysis step in anaerobic digestion by providing a relatively high temperature condition in the range of 150 to 200°C. This is highly effective in reducing organic matter and digestion volume. Through this process, long chain organic matter is converted to shorter chains and subsequently faster and in some cases, higher biogas production is achieved. It is noted that this process is also beneficial in class A biosolids production with enhanced dewaterability characteristics. This process also produces less odorous products (Metcalf & Eddy 2014).

There are several techniques, which can be employed for thermal hydrolysis, including conventional heating, microwave, and radio frequency. In conventional heating, heat provided from an external supplier, to the surface of the medium, is diffused to the cooler parts of the load through thermal conductivity (energy transferred from more energetic particles to less energetic ones). It is best to avoid a thermal gradient inside the load; however, it is challenging to provide uniform heating all over the medium (Tyagi & Lo 2013). However, uniform heat can be obtained through the medium, by decreasing the heating rate. This results in more energy loss and increases reaction time.

In the microwave heating technique, media is exposed to microwave irradiation and some energy is absorbed by the molecules. Molecular kinetic energy is also increased which results in a temperature increase inside and on the surface of the material. This method provides more rapid and selective heating than conventional heating. Moreover, this method can be also more efficient and cost effective than conventional heating depending on the material heated, due to its ability to be instantly controlled by an on/off switch (Tyagi & Lo 2013; Anjum *et al.* 2016). However, currently, for municipal sludge heating, the recent studies indicated that the net energy production is negative due to commercial microwave systems not designed/optimized for sludge pre-treatment for enhanced AD (Kor-Bicakci *et al.* 2019).

2.7.2.2 Mechanical pre-treatment

There are different methods for mechanical pre-treatment namely, sonication, lysis- centrifuge, liquid shear (collision plate and high pressure homogenization) and grinding. Among these mechanical pre-treatment techniques, sonication and high pressure homogenization are the most commonly used.

The sonication process includes two cyclic phases (compression and rarefaction) in which cavitation bubbles formation and their collapse occurs. This contributes to local temperature and pressure increase. In compression cycle, positive energy is applied to the media to squeeze the molecules. In the rarefaction cycle, negative energy is applied to the media to pull apart the molecules. These cycles are repeated until micro bubbles form, grow, and collapse. The sonication method uses different frequencies ranged from 20 kHz to 500 MHz (Pilli *et al.* 2011). It is reported that at a high frequency sonication or a high sonication time, conversion of solubilized matter to methane decreases. Therefore, an energy threshold for cell disintegration and sludge solubilization is reported in most cases (Carrere *et al.* 2016).

For mechanical pre-treatment, high pressure homogenization can be implemented prior to the biological treatment process for sludge disintegration. This technique includes a narrow orifice in which extremely high pressure is applied (up to 900 bar) to the sludge (Carrère *et al.* 2010). High pressure causes shear force on cell membrane and disrupts the cell membrane. The sludge is then rapidly depressurized, and its contents are released into the liquid. In this method no chemical changes occur. It is an interesting alternative to sludge pre-treatment due to its low investment requirement and its ease of operation (Zhang *et al.* 2012). However, it is reported that it can have a negative impact on sludge dewaterability (Carrere *et al.* 2016).

2.7.2.3 Chemical pre-treatment

In chemical pre-treatment, an external chemical compound is added to the sample, to disrupt cell walls and membranes. Consequently, organic matter bioavailability, for enzymatic attacks, is enhanced. Strong reagents for chemical pre-treatment are used, of which acids, alkali and oxidants are the most common (Zhen *et al.* 2017).

Acid and alkali pre-treatments are low cost methods, compared to energy intensive methods (i.e. microwave high pressure homogenization, sonication) described above, and easy to operate, as well as efficient in improving methane production. The most common acids which are employed for acid-treatment are HCl, H_2SO_4, H_3PO_4 and HNO_3. This method improves enzymatic digestibility through hydrolysis of high-molecular weigh compounds, such as hemicellulose. However, it suffers from toxic by-products formation which can inhibit microbial activities. Another drawback is the corrosivity characteristic of pretreated substrate that results in equipment corrosion due to low pH. Therefore, acid-thermal pre-treatment with a low dose chemical agent at a moderate temperature (120-130°C), lower than sole thermal pre-treatment, is a proposed method to overcome the problems mentioned above (Zhen *et al.* 2017).

In the alkaline hydrolysis method, an alkali agent is employed to improve hydrolysis of substrate before AD. The alkali agent breaks down the lignin and other slowly biodegradable compounds and enhances the accessibility of substances to enzymes. Moreover, the agent provides additional alkalinity which enhances the buffer capacity of the sample and improves process stability. Alkali agents that are employed commonly for chemical pre-treatment include NaOH, KOH, $Mg(OH)_2$, and $Ca(OH)_2$. Of these, NaOH is the most effective alkali agent. Some drawbacks of this method include the necessity of sludge re-neutralization after chemical treatment, increasing mineral content, and the formation of non-biodegradable compounds as a result of a high concentration of chemical agent (Zhen *et al.* 2017). Employing a combination of low concentration alkaline hydrolysis and thermal pre-treatment can also prevent overdose of the chemical compound (Carrère *et al.* 2010).

A well-known peroxidation process among oxidation methods is ozonation. It is effective in sludge solubilization and reduction. Sludge solubilization is dependent on ozone dosage and at higher ozone dosages, solubilization can decrease due to oxidation of some of the solubilized compounds. Therefore, the optimum ozone dosage is between 0.05-0.5 g O_3/g TS. This method requires high energy in terms of ozone production and its transfer to the sample, as well as liquid oxygen production. The microbubble ozonation process is a proposed method in order to speed up hydroxyl radical formation and decrease the capital cost (Zhen *et al.* 2017).

Another alternative of oxidation technology is Fenton oxidation. In this method, H_2O_2 is used as a strong oxidant and highly active hydroxyl radicals are formed; moreover, an iron ion is used as a catalyst. This method enhances sludge solubilization and dewaterability due to the release of intracellular materials and bound water, in the sample. In addition, it improves biogas production and reduces the final sludge volume to be disposed of. The process effectiveness is affected by

different parameters including temperature, pH, treatment duration, and hydrogen peroxide and catalyst ratio (Zhen *et al.* 2017).

2.7.2.4 FOG pre-treatment

According to the literature, numerous studies have investigated the increase in effectiveness of pre-treatment on wastes with high lipid contents (oily wastewaters, food wastes, dairy products, vegetable oils etc.). These studies have proven the potential of pre-treatment on enhancing the anaerobic co-digestion performance. However, there is not much research on the impact of pre-treatment on FOG waste, prior to anaerobic co-digestion. FOG is semi-solid at room temperature and non-soluble in water. As a result, advanced studies are needed to apply different pre-treatment techniques on FOG prior to the digestion process, to assess their impact on FOG solubility and overcome its limitations, as well as identify their potential for operation on a larger scale. The results from previous studies on FOG pre-treatment are summarized in Table 2-4.

A study was conducted to investigate the effectiveness of thermal pre-treatment through microwave irradiation combined with chemical treatment of FOG (Srinivasan *et al.* 2018). Along with microwave irradiation, H_2O_2 was chosen as a powerful oxidant to enhance disintegration of the FOG structure. The combination of these two pre-treatments was called the microwave-enhanced advanced oxidation process. This experiment was done in two levels of temperature (90 and 100°C) and seven hydrogen peroxide dosages (ranged 0-4% H_2O_2/%TS) to treat FOG with different solid contents (90 and 2.5% TS by weight). Analysis of total COD and soluble COD revealed that increasing hydrogen peroxide dosage and temperature improved final solubilisation of FOG (quantified by soluble COD concentration) with lower TS content (2.5% TS). However, total COD analysis for FOG with higher TS content (90%) indicated that by adding more hydrogen peroxide (2.5% H_2O_2/ TS or more), much higher total COD in treated sample was detected than in

the initial sample. The reason was the presence of residual hydrogen peroxide in the treated solution, which affected COD determination. Furthermore, it was shown that FOG consists of LCFAs such as palmitic acid and oleic acid, as well as shorter fatty acids such as myristic acid, lauric acid and capric acid. However, treated FOG consists of shorter chain fatty acids and by increasing the hydrogen peroxide dosage up to 4%, no LCFA was detected for both temperature levels. The result has proven that microwave-enhanced advanced oxidation process under suitable conditions has the potential to degrade FOG components to lower molecular weight substrates. Although this study did not conduct anaerobic biodegradability testing for the treated samples with microwave-enhanced advanced oxidation process, the authors have postulated that this would improve hydrolysis in anaerobic co-digestion with FOG and mitigate inhibitory substrates (Srinivasan *et al.* 2018).

In another study, the authors (Li, Chenxi *et al.* 2013) studied the effect of ultrasonic and thermo-chemical pre-treatment on methane production from a mixture of FOG and WAS, as a mixture substrate. The study was conducted by running two sets of biochemical methane potential tests. After ultrasonic pre-treatment of substrate mixture (WAS + FOG) at different conditions (5300-36000 kJ/kg TS power input, 5-40 minutes treatment), no significant improvement was reported in methane production. However, thermo-chemical pre-treatment on the same mixture of FOG + WAS at different pH (8, 10 and 12) showed methane production enhancement, compared to the same mixture with no pre-treatment. The authors reported 9.9% improvement in methane yield when sodium hydroxide was used as an alkaline agent. In this treatment, the pH of the mixture was adjusted to 10 and the mixture was stirred at 55°C for 1.5 h (Li, Chenxi *et al.* 2013).

Slaughterhouse wastes are similar to FOG in terms of high solids concentration and high lipid contents. A study was conducted to investigate chemical pre-treatment effectiveness on the

biodegradation of slaughterhouse wastes (aeroflotation waste and carcass waste) in anaerobic digestion. In this study, chemical pre-treatment was done with an alkali agent (NaOH), which is also called saponification. The process was performed at different temperatures (60, 120 and 150°C) for 3 h to investigate the best performance condition. Improvement was observed for pre-treated waste in comparison with raw waste and the best performance was achieved for saponified carcass waste under 120°C. However, the LCFA concentration did not change significantly under saponification and the authors reported that improvement was observed due to bio-availability improvement of fatty acids under the process (Battimelli *et al.* 2010).

In a study carried out by Mouneimne *et al*. (2003), effectiveness of saponification pre-treatment on biodegradation of solid fatty residues collected from a WWTP in France, was assessed. The waste was a mixture of lipidic residues and materials which were separated by air flotation at the preliminary treatment section of the plant. In this study, lipidic fraction of the waste before and after the treatment was analyzed by hexane extractible matter method, to quantify the achieved degradation. Fatty residues were degraded and partially converted to VFA. To calculate the acidification ratio, VFA concentration was measured by GC and the ratio of VFA production to lipidic compounds degradation was called the acidification ratio. It was reported that two alkali agents were employed for the saponification (KOH and NaOH). The acidification ratio was lower for treated waste with NaOH due to toxic substrates formation that inhibited VFA production. Moreover, pre-treatment process was performed at two different pH (6.5 and 8.5) and the best performance was observed at pH of 8.5 with KOH (Mouneimne *et al.* 2003).

Table 2-4: Studies on pre-treatment of FOG prior to AD*

Author, year	Pre-treatment method used	FOG type/ Co-substrate	Pre-treatment conditions/ digester type	Main results
(Srinivasan *et al.* 2018)	Microwave-enhanced advanced oxidation process	Local dairy farm in BC, Canada Two different samples at two different time periods (TS = 25 and 909 g/l) Source separated organics (TS 30-89 g/l)	BMP tests Two different temperature levels: 90 and 100°C Different hydrogen peroxide dosages (0-4 %H_2O_2/ %TS)	FOG was mainly consisted of LCFAs, which were broken down to lower molecular weight substrates under applied treatment condition. Organic matter solubility was enhanced by increasing hydrogen peroxide dosage and temperature.
(Li, Chenxi *et al.* 2013)	Ultrasonic and thermo-chemical	Restaurant in Ontario, Canada (VS = 940 g/l) WAS (VS = 9.9 and 13.5 g/l)	BMP tests at 37°C Alkali agent: NaOH pH: 8, 10 and 12 Treatment temperature: 55°C	No significant improvement was observed under ultrasonic pre-treatment of FOG + WAS. Thermo-chemical pre-treatment at pH=10 and 55°C improved methane yields up to 9.9%.
(Battimelli *et al.* 2010)	Thermo-chemical	Slaughterhouse wastes: aeroflotation waste (TS; VS: 155; 138 g/kg), carcass waste (TS; VS: 935; 934 g/kg)	BMP tests Alkali agent: NaOH Treatment temperatures: 60, 120 and 150°C	Waste biodegradation was improved by pre-treatment and the best performance was observed for treated carcass waste at 120°C without significant change of LCFAs composition.

Table 2-4 Studies on pre-treatment of FOG prior to AD (cont'd)

Author, year	Pre-treatment method used	FOG type/ characteristics	Pre-treatment conditions/ digester type	Main results
(Mouneimne *et al.* 2003)	Thermo-chemical	Solid fat residues (TS = 60%, VS = 59%)	Lab-scale continuously stirred tank reactor at 35°C Alkali agents: NaOH and KOH pH: 6.5 and 8.5 Treatment temperature: 80°C	Saponification improved bioavailability of fatty residues with both alkali agents. However, less VFA production was achieved under pre-treatment with NaOH due to biotoxic matter generation during the process. Pre-treatment at pH 8.5 resulted in higher fatty matter degradation in comparison with pH 6.5.

*FOG: Fat, oil and grease; TS: total solid; VS: volatile solid; LCFA: long chain fatty acid; BMP: biochemical methane potential; TWAS: thickened waste activate sludge; WAS: thickened waste activated sludge; VFA: volatile fatty acid

2.8 Biochar

Using carbonaceous adsorbents in the AD process is a relatively new practice to remove inhibitors and improve the process efficiency. Improvements in the removal of contaminations are observed as a result of the addition of the adsorbents such as zeolites and activated carbon to the AD process (Fagbohungbe *et al.* 2017). Adsorbents have porous structure with adhesion properties that cause biofilm formation by accumulation of atoms, ions or molecules on their surface (Mumme *et al.* 2014; Fagbohungbe *et al.* 2017). Biochar is another alternative as an adsorbent which is prepared by pyrolysis of the residues of biomass in zero/low oxygen condition at a high temperature (180-950°C) (Codignole Luz *et al.* 2018). Biochar is a more favorable adsorbent than activated carbon and zeolite as it is relatively cheaper; however, activated carbon has a larger surface area compared to biochar. Adsorption is occurring through different stages: adsorbate is settled on the surface of the particles of the adsorbent, a layer is formed on the surface and the pores are filled until an equilibrium state is achieved, adsorbent is almost saturated (Fagbohungbe *et al.* 2017). The performance of the adsorbent is depending on operational conditions: contact time, temperature, adsorbent and adsorbate dosages; as well adsorbent characteristics: pore distribution, particle size, surface properties and pH (Fagbohungbe *et al.* 2017).

Adding biochar during the AD process is effective in adsorbing the inhibitory compounds, immobilizing the bacterial cells by providing a condition for bacterial growth. Moreover, the addition of biochar to the AD improves the buffering capacity of the system by accelerating the formation of dissolved substances from macromolecules transformation (Pan *et al.* 2019). To date, it is proven that biochar has potential to mitigate inhibitory ammonia/VFAs and enhance biomethane production (Mumme *et al.* 2014; Cai *et al.* 2016; Pan *et al.* 2019) but it has not been

tested for an anaerobic co-digestion system utilizing FOG with municipal sludge to mitigate inhibitory effects of LCFA accumulation.

2.9 Summary

Food industry FOG is mainly produced through cooking in restaurants. The grease traps are employed to collect FOG at the source of generation. It is essential to eliminate FOG in the waste stream to minimize its negative impacts on environment and public health. The collected FOG can be treated in different ways, of which AD is a favorable method. Theoretically, FOG has a good methane production potential and is a desirable organic waste to be added to the main substrate in AD to improve biomethane production. However, this process is challenging due to some limitations. FOG is degraded to LCFA and glycerol during fermentation and LCFA degradation to acetate is the rate limiting process which can lead to process failure. As a result, a good feeding strategy for the addition of FOG is essential to prevent process failure. It is necessary to find the optimal substrate feeding and digester operational condition to achieve the highest biomethane production, while monitoring inhibitory parameters to find their thresholds.

Furthermore, pre-treatment methods can enhance FOG biodegradability and increase its solubility. However, further research is needed to validate the effectiveness of different pre-treatment methods and compare their efficiencies on this process. Moreover, different digester configurations for co-digestion of FOG needs to be evaluated to explore their impacts on biomethane production and inhibition parameters. Another potential practice to overcome FOG limitations is the addition of an adsorbent to the process to mitigate LCFA accumulation. The following chapter will implement some of these strategies to maximize biomethane yield by optimizing FOG addition to municipal sludge and determine the point of AD process failure.

Chapter 3 . Materials and methods

In this chapter, the equipment used to conduct the research are listed. The characteristics of feed (mixed sludge, FOG) and inoculum are summarized. Additionally, analytical test procedures are illustrated in detail.

3.1 Equipment

In Table 3-1 a list of equipment used in this research is provided.

Table 3-1: List of equipment

Equipment	Type and manufacture
Balance	XS204DR, Mettler Toledo
Capillary suction timer	440, Fann
Centrifuge	Sorvall Lengend XT, Thermo Scientific
pH probe	13-636-XL25, Fisher Scientific
Gas chromatograph-A	7890A, Agilent
Gas chromatograph-B	7820A, Agilent
Gas manometer	Custom Built
Incubator/shaker	Innova 44R, New Brunswick Scientific
Muffle furnace	W-13, Paragon Industries
Micro centrifuge	Sorvall Legend Micro 21, Thermo Scientific
Oven	Isotemp Oven, Fisher Scientific
Polytron homogenizer	PT 10-35 GT, Fisher Scientific
Spectrophotometer	Genesys 10, Thermo Electron Corporation
Thermotron	S-1.5-3200, Thermotron

3.2 Mixed sludge and FOG characteristics

Mixed sludge (FPS and TWAS) was provided by Westside Regional wastewater treatment plant operated by the Regional District of Central Okanagan. In Westside Regional wastewater treatment plant, after preliminary treatment section in which rags and large solids are removed, the wastewater goes through a primary clarifier to separate suspended solids. The sludge (PS) settled at the bottom of the primary clarifier, with a high concentration of organics and microorganisms, flows through a fermenter where organic compounds are degraded to form VFA. These VFA are collected from the surface of the fermenter and pumped to the BNR system (anaerobic zone) for phosphorus release. The remaining sludge is collected and sent to dewatering. The effluent of primary clarifier is also sent to the modified BNR system, with three sequential steps (anaerobic, anoxic and aerobic), to remove nitrogen and phosphorus compounds. The biomass from BNR system is separated in secondary sedimentation tanks and the excess sludge collected at the bottom of the secondary settling tanks (WAS) is sent to a dissolved air floatation tank where TWAS is obtained. Then, TWAS and FPS are mixed and sent to the centrifuge system where solids are separated from wastewater by centrifugal forces. The nutrient rich separated liquid stream is called centrate. At the final stage, the dewatered biosolids are transported to land application sites. For this research, FPS and TWAS were collected and transported to UBC's Bioreactor Technology Group's Laboratory, on a monthly basis. Mixed sludge was prepared by mixing FPS and TWAS in 33:67% volumetric ratio, which represents the volumetric ratio at Westside Regional wastewater treatment plant. To maintain the consistency among different batches of sampling from the plant for long-term bench-scale digester testing, VS content was adjusted by adding reverse osmosis (RO) water to each mixed sludge batch. The mixed sludge characteristics are summarized in Table 3-2.

Table 3-2: Mixed sludge characteristics

Parameter	Average value	(St. dev.; number of replicates)
Total solids (TS) (% w/w)	4.2	(0.3; 26)
Volatile solids (VS) (% w/w)	3.6	(0.3; 26)
Chemical oxygen demand (COD) (mg/l)	60,736	(6,152; 31)
Ammonia (mg/l)	452	(148; 20)
Alkalinity (mg/l as $CaCO_3$)	1,239	(174; 18)
Volatile fatty acids (VFA)[a] (mg/l)	2,461	(532; 22)
pH	5.8	(0.1; 23)

[a]Summation of acetic, propionic, and butyric acids

FOG was collected by D & L Environmental Services in Kelowna (BC) and provided to Bioreactor Technology Group. The grease wastes were collected from various food processing services grease traps (i.e. fast food restaurants, coffee shops, bakeries, meat/butter shops, sea food places etc.) and were stored in 1250 gallon hard plastic storage tanks which are located in ground. Then, the grease is disposed of in Kamloops when the tanks are full (approximately after 2-3 days). For this research, two batches of FOG were collected from the upper layers in the grease storage tanks with sampling dates of March 1, 2019 and Sep 4, 2019, and the characterization results are provided in Table 3-3.

Table 3-3: FOG characteristics

Parameter	Batch 1	Batch 2
Total solids (TS) (% w/w)	53.7 (3.3; 7)[a]	67.5 (1.6; 3)
Volatile solids (VS) (% w/w)	53.2 (3.2; 7)	66.4 (1.2; 3)
TS/VS	0.99	0.98
Chemical oxygen demand (COD) (mg/l)	742,448 (354,109; 9)	619,736 (339,336; 4)
pH	4 (0; 7)	4 (0; 7)
Long chain fatty acids (LCFA)[b] (mg/g TS)	396.5	527 (3; 109.7)

[a](St. dev.; number of replicates); [b]Summation of palmitic, stearic, and oleic acids

3.3 Experimental design

Three sets of digestion scenarios were conducted at bench-scale to achieve the main goals of this project. First, two semi-continuous flow single stage ADs (control and co-digester) were run simultaneously to compare their performances under the identical conditions and determine the FOG/sludge ratio with the highest biogas/biomethane production. The second scenario was conducted by running two TPAD systems with different acid phase temperatures, to compare the process efficiency with single stage AD and assess TPAD's potential in mitigating process inhibition. Finally, some batch adsorption tests were conducted to investigate the effectiveness of the addition of biochar to the digestate from AD-2 to remove LCFA.

3.3.1 Single stage anaerobic digestion

Two automated fermenters with mechanical mixing were set up to simulate single stage AD and anaerobic co-digestion to perform the first digestion scenario (Figure 3-1). Both digesters were connected to a controller. In order to provide constant heating, one of the vessels was equipped

with a heat blanket and the other one with a water jacket. In addition, their temperatures were continuously monitored by temperature probes. Each vessel had 5 L working (liquid) volume and was operated under a mesophilic condition (35°C) at SRT of 20 days. Initially, both digesters were fed manually (once/day, 7 days/week) with mixed sludge for 40 days, to reach the steady-state in terms of stable daily biogas production and parameters affecting digester performance (i.e. COD, VFA, pH, alkalinity). Then, 10% FOG was added to one of the vessels (AD-2) on VS basis and increased in 10% increments. The other vessel (AD-1), which was a control system, was continued to receive mixed sludge only. The FOG/sludge ratio was kept constant for AD-2 for a duration equal or longer than 3 x SRT (i.e. 60 days) to re-establish steady-state and then the FOG/sludge ratio was increased. Additionally, the AD-1 and AD-2 effluents were characterized continuously before changing the FOG/sludge ratio. AD-1 was run for 239 days and AD-2 was run for 304 days.

Figure 3-1: Automated fermenters simulating single stage anaerobic digesters

3.3.1.1 Anaerobic inoculum

The inoculum, to set up AD-1 and AD-2, were collected from a semi-continuous flow anaerobic digester that was fed with mixed sludge from Westside Regional wastewater treatment plant. The semi-continuous flow anaerobic digester was operated, in UBC's Bioreactor Technology Group Laboratory, under a mesophilic condition (35°C) for more than 2 years with an OLR of 2.54 g VS/l/day. The inoculum characteristics are summarized in Table 3-4.

Table 3-4: Characteristics of single stage anaerobic (AD-1 and AD-2) inoculum

Parameter	Value
Total solids (TS) (% w/w)	2.1
Volatile solids (VS) (% w/w)	1.7
Chemical oxygen demand (COD) (mg/l)	28,949
Ammonia (mg/l)	1,332
Alkalinity (mg/l as $CaCO_3$)	4,550
Volatile fatty acids (VFA)[a] (mg/l)	14
pH	7.6

[a]Summation of acetic, propionic, and butyric acids

3.3.1.2 Feeding strategy for anaerobic co-digester

The daily amount of FOG and mixed sludge for feeding was calculated based on mixed sludge VS, FOG VS, and the total volume of feed injection (calculations are summarized in Table 3-5 for different FOG/sludge ratios). The addition of FOG to AD-2 was based on VS in feed. The FOG to mixed sludge ratio was incremented based on VS in 10% steps. As mentioned earlier, AD-2 was run at SRT of 20 days and the digester liquid volume was 5 liters; therefore, once a day, 250 ml feed was injected into the digester after 250 ml reactor content was withdrawn.

Table 3-5: Experimental design for co-digester daily feeding at different FOG/sludge ratios

Mixed sludge VS (% w/w)	Mixed sludge mass (g)	FOG VS (% w/w)	FOG mass (g)	FOG VS/Feed VS (%, VS basis)
4.2	248	53.2[a]	2	$\frac{2 * 53.2}{((248 * 4.2) + (2 * 53.2))} * 100 = 10\%$
4.2	245.4	53.2	4.6	$\frac{4.6 * 53.2}{((245.4 * 4.2) + (4.6 * 53.2))} * 100 = 20\%$
4.2	242	53.2	8	$\frac{8 * 53.2}{((242 * 4.2) + (8 * 53.2))} * 100 = 30\%$
4.2	238	53.2	12	$\frac{12 * 53.2}{((238 * 4.2) + (12 * 53.2))} * 100 = 40\%$
4.2	235.5	66.4	14.5	$\frac{14.5 * 66.4}{((235.5 * 4.2) + (14.5 * 66.4))} * 100 = 50\%$

[a]First batch of FOG was used for digester feeding from 10 to 40% FOG/sludge and at 50% FOG/sludge the second batch was used

3.3.2 Temperature-phased anaerobic digestion (TPAD)

The first digestion scenario experienced process failure at 50% FOG/sludge (further discussed in the results section). Therefore, the second digestion scenario aimed to find a stable digestion configuration that will allow for increase of FOG addition to anaerobic co-digestion process. Based on literature review, the second scenario involved the operation of additional semi-continuous flow ADs, to simulate TPAD and to investigate it's effectiveness on methane production from FOG and mixed sludge. TPAD systems were comprised of thermophilic acid phase (AP) vessels followed by mesophilic methane phase (MP) vessels (Figure 3-3). Two digesters (AP-1 and AP-2, made out of 0.5 L side-armed Erlenmeyer flasks with 0.3 L liquid volume) were set up and kept at 55 and 70°C in separate temperature-controlled shakers, to simulate acid phases of TPAD systems. The MP digesters (MP-1 and MP-2) were made out of 2 L side-armed Erlenmeyer flasks with 1 L of liquid volume. They were kept at mesophilic conditions (38°C) in a temperature-controlled shaker, which provided uniform mixing and temperature. TPADs were fed in the same manner as AD-2

(co-digester of the first digestion scenario), with 40% FOG for 45 days. Then, the FOG ratio was changed to 50% (like AD-2). After AP-1 and AP-2, fed with 50% FOG, reached steady-state, their effluent was introduced to the mesophilic digesters. The AP-1 and AP-2 effluent were used as feed for the methane-phase-1 (MP-1) digester and the methane-phase-2 (MP-2) digester, respectively. The schematic for the experimental design of TPAD is shown in Figure 3-2. For consistency with the digesters at the first scenario, TPADs were run for an overall SRT of 20 days (i.e. AP-1 and AP-2 with SRT of 2 days, MP-1 and MP-2 at SRT of 18 days). Figure 3-3 shows the bench-scale anaerobic methane phase and acid phase digesters, used in this experiment. Each digester was sealed with a rubber stopper and silicone sealant. Two holes were placed through the stopper to insert the glass rods. The longer glass rod was used to withdraw the sludge every day and the shorter glass rod was used to collect biogas in a Tedlar bag. The digesters were fed every 24 hours (7 days/week), through the side arm of the flask. The feeding line and effluent line were sealed by clamps, when not in use, to prevent any oxygen entrance into the digester.

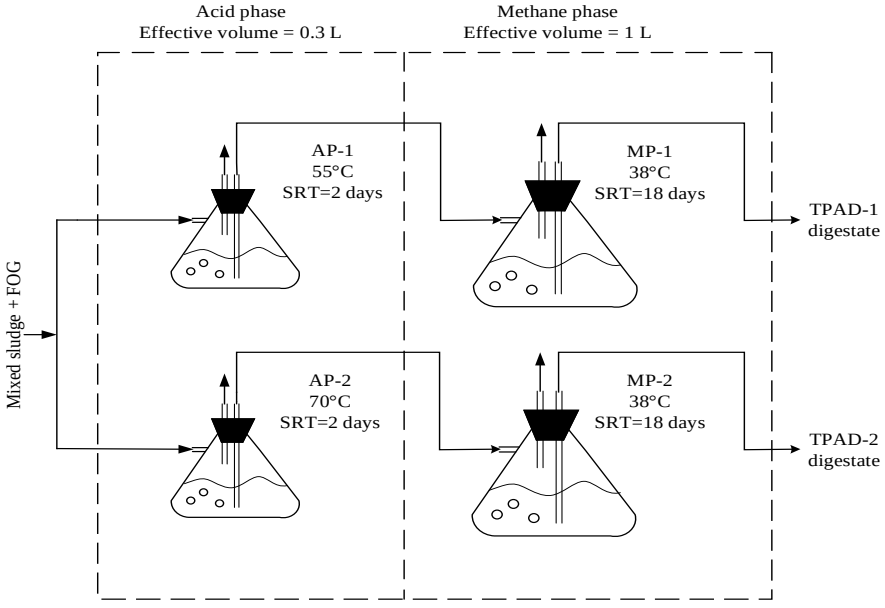

Figure 3-2: Experimental design of TPAD systems (AP: acid phase, MP: methane phase)

Figure 3-3: Image of bench-scale anaerobic digesters with TPAD configuration

3.3.2.1 TPAD inocula

The inoculum for the methane phases (MP-1 and MP-2) was collected from the first digestion scenario's co-digester (AD-2), while it was fed with 40% FOG. The inocula for AP-1 and AP-2 were collected from two semi-continuous flow acid phase digesters, operated at UBC's Bioreactor Technology Group's Laboratory at the same temperature as AP-1 (55°C) and AP-2 (70°C). They were fed with mixed sludge from Lulu Island WWTP (BC, Canada) and were run at a SRT of 2 days for more than 2 years. The acid phase inocula characteristics are summarized in Table 3-6.

Table 3-6: Characteristics of acid phase digesters (AP-1 and AP-2) inocula

Parameter	AP-1	AP-2
Total solids (TS) (% w/w)	47.2	37.3
Volatile solids (VS) (% w/w)	41.7	34.0
Chemical oxygen demand (COD) (mg/l)	64232	59961
Ammonia (mg/l)	963	759
Alkalinity (mg/l as $CaCO_3$)	2146	1436
Volatile fatty acids (VFA)[a] (mg/l)	4758	3742
pH	5.4	5.3

[a]Summation of acetic, propionic, and butyric acids

3.3.3 Biochar

In order to conduct batch adsorption test to investigate the impact of biochar on LCFA concentration, biochar was provided by SoilMatrix from Air Terra, located in Red Deer (Alberta). Biochar was sieved prior to the start of the experiment, and the particle sizes were greater than 850 micrometers (sieve #20) and less than 4.75 mm (sieve #4). Appendix C includes more detailed characterization data for the biochar tested.

Three sets of experiments were conducted with different biochar dosages. To initiate the adsorption assay, two 500 ml Wheaton bottles (duplicate each) were used. First, 100 ml of digestate from AD-2 (fed with 50% VS FOG and mixed sludge) was placed in to the bottles. Then, biochar was added to one of the bottles and the other one was used as a control without biochar. The digestate was purged with nitrogen to remove any residual oxygen and the bottles were sealed with rubber septa and plastic caps. Then, bottles were kept at 55°C in the temperature-controlled shaker for consistent heating and mixing. The pressure created by biogas production in bottles was released daily by puncturing the septa with needle. Every other day after mixing, 5 ml of digestate

was withdrawn from both bottles (with and without biochar addition) with needle and prepared for LCFA analysis (in duplicate).

To evaluate the influence of biochar dosage on LCFA removal by adsorption, biochar addition was increased from 0.17 g biochar/g TS digestate to 2 g biochar/g TS and 3 g biochar/g TS. The first set was run for 6 days and the other two sets were run for 10 days.

3.4 Analytical methods for sample characterization

Various analytical methods were used to compare the performance and process efficiency of the digesters. The list of analyzed parameters, and their frequency, is summarized in Table 3-7. In the following sections, each test procedure is explained in detail.

Table 3-7: Analyzed parameters during the experiment and their measurement frequency

Parameter	Frequency	Sample location
Alkalinity	Once a week	Influent/Effluent of each digester
Ammonia	Once a week	Influent/Effluent of each digester
Biogas composition	Once a week	Headspace of digester
Biogas volume	Daily	Headspace of digester
Chemical oxygen demand	Once a week	Influent/Effluent of each digester
Dewaterability	Minimum three time at each FOG ratio	Effluent of final stage digester
Fecal coliform	Minimum three time at each FOG ratio	Effluent of final stage digester
Heavy metals	Once sampling	Effluent of final stage digester
Long chain fatty acids	Once a week	Influent/Effluent of each digester
pH	Once a week	Influent/Effluent of each digester
Total solid/volatile solid	Once a week	Influent/Effluent of each digester
Volatile fatty acids	Once a week	Influent/Effluent of each digester

3.4.1 Total solids and volatile solids

The solid contents (TS and VS) of the digester streams (digestate and feed) were measured based on APHA 2540 B and 2540 E procedures (APHA 2005). Crucibles were prepared prior to the start of the analysis, by soaking in diluted sulfuric acid (20%) for about 2 hours. Then, they were washed, heated at 550°C (to remove any impurities and residues), and placed in a desiccator (to prevent the absorption of moisture). In this analysis, well-mixed samples were first weighed in the crucibles. Then, the crucibles were dried in the oven, at 98°C. After evaporating most of the free water, the temperature was increased to 105°C and the crucibles were dried overnight to evaporate the remaining water. After cooling down to room temperature in the desiccator, the crucibles were weighted again and then burned at 550°C for at least 30 minutes. The final weights of the crucibles were recorded at room temperature. The following equations (Eq.1 and Eq.2) illustrate how the TS and VS were calculated and expressed in % by weight.

$$\text{Total solids } \left(\%, \frac{\text{g}}{\text{g}}\right) = \frac{Wet\ mass\ (g) - Dry\ mass\ (g)}{Wet\ mass\ (g)} * 100 \qquad \text{(Eq.1)}$$

$$\text{Volatile solids } \left(\%, \frac{\text{g}}{\text{g}}\right) = \frac{Dry\ mass\ (g) - Burned\ mass\ (g)}{Wet\ mass\ (g)} * 100 \qquad \text{(Eq.2)}$$

3.4.2 Chemical oxygen demand (COD)

In order to measure COD of digestate and feed sludge, APHA 5220 D procedure was followed (APHA 2005). Sample preparation involved the dilution of a small amount of digester effluent or feed with RO water, in a COD bottle. The dilution was performed to adjust the concentration, to fit within the maximum range of the method. The diluted sample was mixed using a Polytron benchtop homogenizer at 7000 RPM for 5 minutes and 2.5 ml of the sample was transferred to a 12 ml glass vial. Then the sample was mixed with 3 ml of digestion solution (mixture of mercuric sulfate, potassium dichromate, and concentrated H_2SO_4) and 1.5 ml of catalyst solution. It was

then incubated for 3 hours at 150°C in the Thermotron temperature controlled chamber. Afterwards, the sample was cooled down to the room temperature and sample absorbance was measured at 600 nm wavelength with a spectrophotometer. Moreover, standard solutions were prepared using potassium hydrogen phthalate. The absorbance of the standard solutions with known COD concentrations (ranging from 100 to 700 mg COD/l) were also measured. The sample COD concentration was calculated by plotting the standard curve (Appendix A, Figure A.1).

3.4.3 Capillary suction time

Capillary suction timer (CST) is a common method to measure the rate of dewaterability of a multi-phase fluid. In this research, the experimental process was followed according to APHA 2710 G (APHA 2005). This test involved the placement of 5 mL of digestate into a metal cylinder which was located on a filter paper. The liquid portion of the sample was drawn through the filter via capillary forces, while the solids were held above the filter. Two sensors were located on the filter paper and connected to a digital timer. The timer started when the liquid reached the first sensor and stopped when the liquid touched the outer sensor. CST is affected by the TS content of the sample and the recorded time was normalized by percent TS in the sample. Temperature is also an influential parameter on CST measurement; thus, all the samples were brought to room temperature (21 ± 1°C) prior to the start of the analysis.

3.4.4 pH

pH is one of the most important digester operational parameters. The pH of the feed and digester effluent were measured weekly, to monitor the digester performance and ensure that the pH was within the safe (neutral) range. It was measured using a pH electrode attached to an Accumet™ Excel XL25 pH/mV/Temperature/ISE meter, based on Standard methods 4500-H^+ B (APHA 2005).

3.4.5 Alkalinity

The procedure to measure alkalinity was based on APHA 2320 B method (APHA 2005). In this method, a sample was centrifuged at 10000 rpm for 30 minutes, roughly 5 ml of supernatant was transferred to a beaker, and the volume was recorded. Then, the supernatant was titrated with sulfuric acid solution (0.1 N). The pH was monitored during the titration with a pH probe, until it reached 4.6. The consumed acid volume was recorded to calculate the alkalinity.

3.4.6 Ammonia

To measure dissolved ammonia, two different methods were followed during this research. For the first method (ammonia selective electrode method)), a sample was first centrifuged at 10000 rpm for 30 minutes. The supernatant was then used to measure dissolved ammonia concentration in digester influent and effluent, according to Standards Method 4500 D (APHA 2005). In this analysis, an ammonia selective electrode with dual channel pH/ion was used. The ammonia nitrogen (NH_3 (aq) and NH_4^+) concentration was determined by diluting the sample with type 1 water, ultra-pure water without impurities by passing through a distillation process and ions are removed through an ion exchange resin and 0.2 µm filtration, to match the test range of the method. Sodium hydroxide was added to the sample to elevate the solution pH above 11 in order to transform dissolved ammonia into NH_3 (aq). The ammonia concentration was then measured. Then, standard curve, which was created by preparing a set of standard solutions ranging from 50 to 1000mg/l, was used to calculate the ammonia concentration in the sample (Appendix A, Figure A.2).

In the other method, ammonia was measured using the colourimetric method APHA 4500-NH_3 F (phenate method) (APHA 2005). Supernatant was used in this method as well and it was diluted with type 1 water to match the test range. The reaction of reagents, hypochlorite and phenol, with

ammonia in the presence of sodium nitroprusside as a catalyst resulted in the formation of a blue colored compound, indophenol. The absorbance of the sample was recorded at 660 nm wavelength with a spectrophotometer. Afterwards, the sample concentration was calculated by comparing sample absorbance with the standard curve, within a range of 0 to 7.5 mg/l (Appendix A, Figure A.3).

3.4.7 Volatile fatty acids

Total VFA concentration, including acetic, propionic and butyric acids, was measured by injecting the prepared sample into an Agilent 7890A GC. The GC column was 25 m (Agilent 19091F-112, HP-FFAP polyethylene glycol TPA column length x ID: 25 m, 320 μm) and was equipped with a flame ionization detector, in which helium was the carrier gas at the flow rate of 40 ml/min. The initial temperatures of oven and detector were 70 and 300°C, respectively and their final temperatures were 200 and 300°C, respectively. First, sample was centrifuged at 10000 rpm for 30 minutes and supernatant was filtered through 0.2 μm nylon filters. Then, the mixture of 0.5 ml of sample and 0.5 ml of internal standard (isobutyric acid) was injected to the column. This method was adopted from a procedure in the literature (Ackman 1972). A standard solution was also injected to GC prior to sample injection in order to test the GC recovery. The standard solution was a mixture of acetic, propionic and butyric acids with 2000 mg/l concentration each.

3.4.8 Long chain fatty acids

Major LCFAs (i.e. palmitic, oleic and stearic acids) present in the co-digester, were common inhibitory compounds for methane forming bacteria. The procedure to measure the mentioned LCFAs concentrations, was adopted from a proposed method by Ziels and his colleagues (Ziels *et al.* 2015). In this method, 1 ml of the digester effluent was transferred to a glass vial and mixed with 100 μl pentadecanoic acid as the internal standard. Sodium chloride, sulfuric acid, and 1:1

hexane: methyl-tert-butyl ether were also added to the sample for LCFA extraction. The sample was then sealed and mixed at 250 rpm in the shaker for 20 minutes. Afterwards, the sample was transferred to a 1.5 ml centrifuge tube and centrifuged at 4500 x g for 10 minutes. Then, 0.1 µl of the supernatant was injected into the GC column (Agilent 19091F-112, HP-FFAP polyethylene glycol TPA column length x ID: 25 m, 320 µm) in which helium was the carrier gas with inlet flowrate of 43.84 ml/min (with a split ratio of 1:10) and the column flowrate was 3.712 ml/min. The detector temperature was 300°C and the detector flow contained 40 ml/min H_2 and 400 ml/min air. The initial oven temperature was set at 100°C and was increased to 240°C. The overall run time was 18 minutes. The result of total LCFA concentration was expressed as mg LCFA/g TS.

3.4.9 Biogas volume

Gas produced in the automated digesters went through a Ritter milligas counter that automatically recorded the volume of the gas, before it was collected in a tedlar bag. The volume of biogas produced was recorded daily. The pressure and room temperature were also recorded daily in order to convert the biogas volume to standard temperature and pressure (STP) conditions (0°C and 1 atm). The biogas volume in the tedlar bag was measured by removing the bag from the digester and pumping the gas to a U-type manometer filled with water. Based on the liquid displacement in the manometer and the calibration curve, (created by injecting the known volume of gases), the biogas volume was calculated and corrected to STP conditions. The calculated biogas volume was also adjusted with time difference in the daily feeding time of the digester.

3.4.10 Biogas composition

Biogas sampled from the digester headspace was injected into the GC (Agilent 7820A) to quantify its contents (including methane, oxygen, nitrogen and carbon dioxide) (van Huyssteen 1967). The biogas was mainly composed of the compounds mentioned above; however, trace

amounts of moisture, hydrogen, and volatile sulphur compounds, that were assumed to be negligible, were also included. The percentage of the four main compounds (methane, oxygen, nitrogen and carbon dioxide) were measured. The GC was equipped with three meter packed column (Agilent G3591-8003/80002) and a thermal conductivity detector. The oven temperature was 70°C and helium at a flowrate of 25 ml/min was used as the carrier gas. In this test, 0.5 ml biogas was collected by a gas tight syringe from the gas line, which was attached to the tedlar bag, and manually injected into the GC. A gas mixture of 7% nitrogen, 20% carbon dioxide, and 73% methane was used to calibrate the GC.

3.4.11 Fecal coliform and heavy metals

Selective growth medium was used to quantify the concentration of pathogens in digestate samples, by using a thermoresistant coliform as an indicator organism. Based on Standard Methods 9222D (APHA 2005), the sample was first placed on a growth medium to allow the microorganisms to grow. In this regard, fresh digestate was saved in a sterilized container and it was diluted with sterilized type 1 water, to bring it to the maximum range of the method. Then, a part of the diluted sample was filtered through a 0.44 mm membrane filter and placed on a selective membrane filtration medium (m-FC Nutrient Pad Sets, Sartorius, Germany), which was the growth media for thermoresistant fecal coliform enumeration. The plates were incubated at 44.5 ± 0.1°C for 36 hours in the Thermotron temperature control chamber to let the fecal coliforms grow. After 12 hours of incubation, blue colonies were counted every hour, to record the total number of colonies and avoid missing any of the colonies which may appear and disappear gradually. The number of colonies were reported as colony forming units (CFU) and normalized by the TS content of sample so as to compare with U.S. Environmental Protection Agency (USEPA 1999) and B.C. OMRR biosolids land application regulations (OMRR 2008).

Moreover, heavy metals quantification was carried out for digestate samples by a local laboratory based on U.S. Environmental Protection Agency method 6020B using inductively coupled plasma mass spectrometry (USEPA 2014).

3.5 Statistical analysis of data

Data from the single stage digesters were used for statistical analysis. Different FOG/sludge ratios (0, 10, 20, 30 and 40% VS) were considered as the experimental levels. The responds were considered as follows: TS removal, VS removal, specific methane yield, dewaterability rate and fecal coliform concentration.

Minitab 18 statistical software was used for running the analysis of variance (ANOVA) at 95% confidence interval ($\alpha = 0.05$) and it was assumed that experimental levels have linear effect on experimental outputs. Appendix B includes normality plots for various data analyzed.

Chapter 4 . Results and discussion

The obtained results in this research and discussion are presented in this chapter and separated into three subsections: the performance of single stage anaerobic digesters with and without FOG addition, the performance of TPAD systems in anaerobic co-digestion with FOG, and the effect of biochar addition to sludge to mitigate LCFA inhibition.

4.1 Single stage AD performance

The primary objective of running single stage ADs, during the first digestion scenario, was to monitor the performance of the co-digester and control to investigate the effectiveness of the addition of FOG to municipal sludge for enhancing methane yield. Also, the FOG/sludge ratio was increased in a stepwise manner to find the optimal condition to obtain the highest biogas production. As it was mentioned in chapter 1, LCFA accumulation, as a result of the addition of FOG, can lead to process failure. Hence, inhibitory parameters: LCFA, VFA, and ammonia concentration were monitored regularly. Figure 4-1 and Figure 4-2 present daily biogas production and specific daily biogas yield during the experiment at different FOG/sludge ratios, respectively.

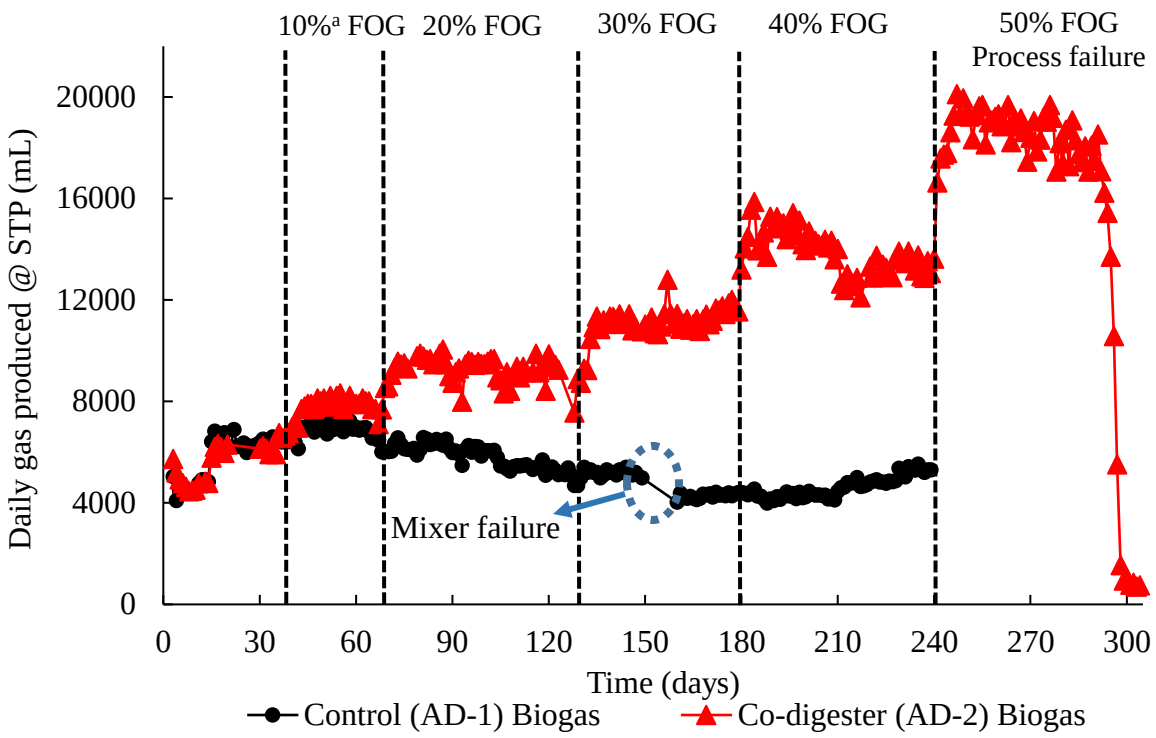

Figure 4-1: Daily biogas produced from single stage digesters ([a]FOG/sludge VS ratio)

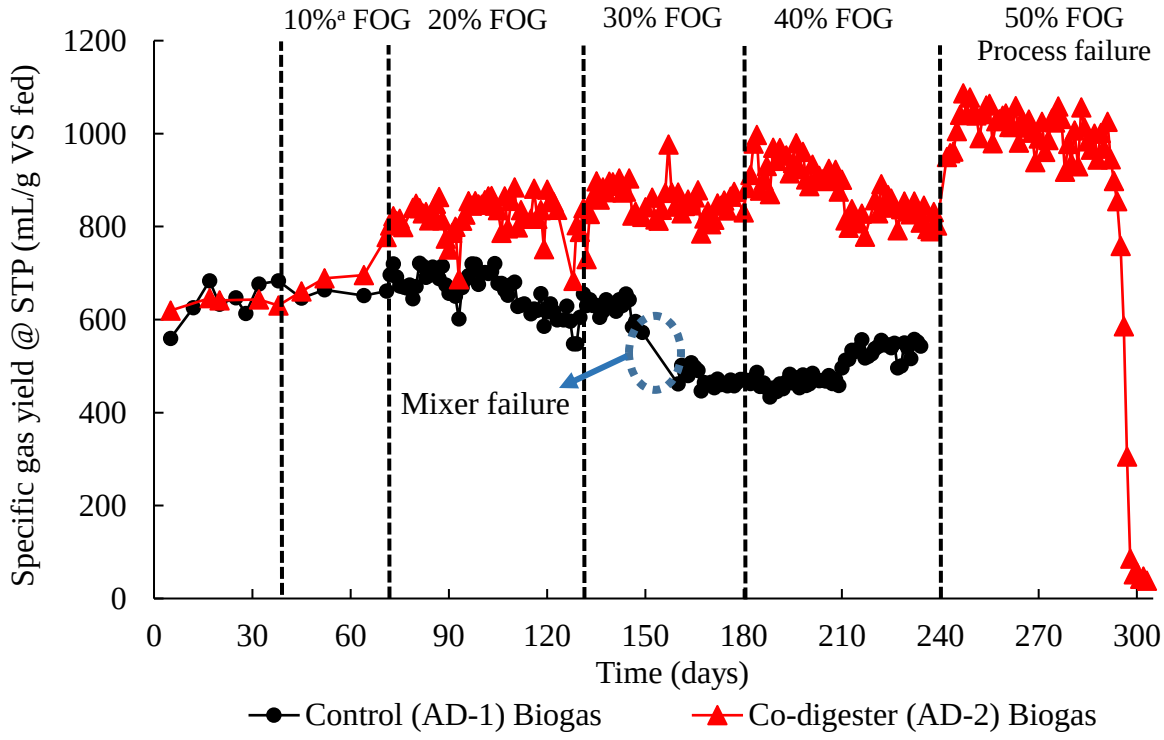

Figure 4-2: Specific daily biogas yield from single stage digesters ([a]FOG/sludge VS ratio)

As it can be seen from the figures, both digesters were fed with mixed sludge for 38 days until they reached a steady state. Then, FOG was added to AD-2 in a 10% FOG/sludge ratio (VS basis). As expected, the overall biogas production from AD-2 increased as the FOG ratio was increased; since, by increasing the FOG ratio (Figure 4-3), more organic compounds were provided for microorganisms to consume and produce biogas from. Previous studies reported that 94.8% of lipids can be converted to biogas; however, only 50.4% of carbohydrates and 71.0% of proteins are convertible to biogas (Jeganathan *et al.* 2006).

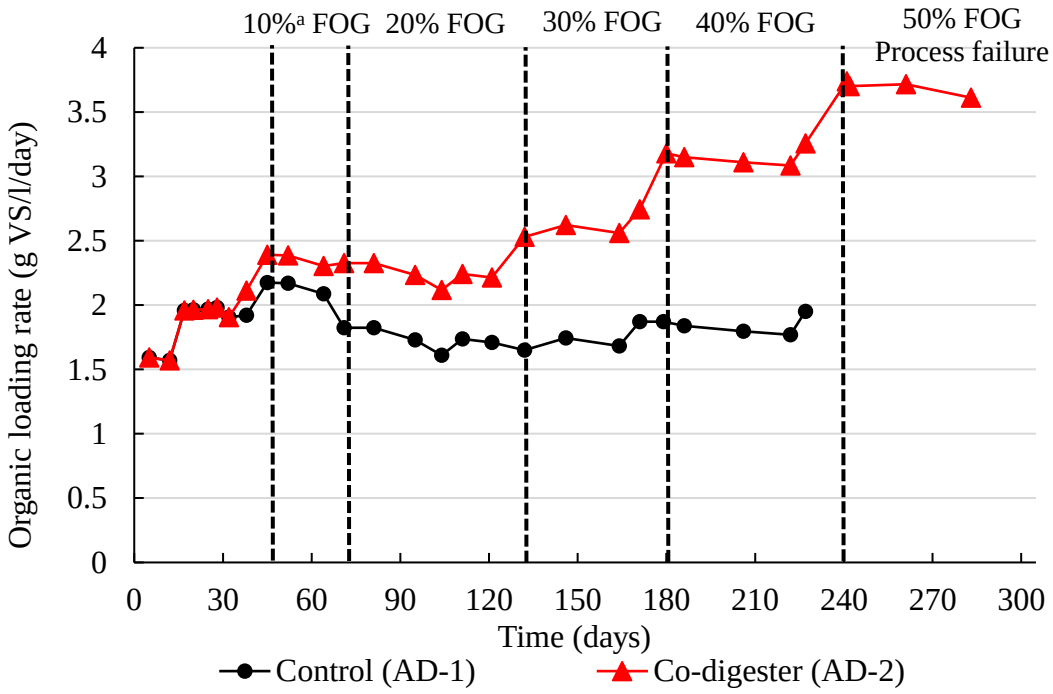

Figure 4-3: Organic loading rate ([a] FOG/sludge VS ratio)

At each stage, the ratio FOG/sludge was changed after biogas production reached a steady state. AD-2 was run for 304 days in total and after running for 52 days at 50% FOG/sludge, the signs of the failure of the digester were observed. Based on the obtained result, the highest stable biogas production was achieved at 40% FOG/sludge, and daily biogas production and specific daily biogas yield improved by 200% and 52.5% over the control digester, respectively.

An increase in OLR (up to 3.15 g VS/l/day) resulted in stable AD performance with no signs of process inhibition. These results support the finding in previous studies (Davidsson *et al.* 2008; Luostarinen *et al.* 2009; Noutsopoulos *et al.* 2013; Ziels *et al.* 2016). However, improvement in anaerobic co-digestion performance at higher OLRs was reported under lower SRTs (Martín-González, L. *et al.* 2010).

The control digester was fed with mixed sludge. It was run for 239 days and had a stable performance during the experiment at an average OLR of 1.84 g VS/l/day. In Figure 4-1 and Figure 4-2, there is a gap in biogas data for AD-1 (day 149 to 160) due to the motor replacement of the digester. After fixing the motor, AD-1 recovered and returned to the same level it previously was at. Table 4-1 summarizes the characterization of the influent and effluent of single stage ADs.

The collected data shows a decrease in pH as a result of an increase in the addition of FOG due to the acidic nature of FOG. Consequently, alkalinity and ammonia concentrations were reduced; however, after adding FOG up to 40% VS, the results were in the expected range for the AD process. The literature has also confirmed the same trend for the mentioned parameters (Davidsson *et al.* 2008; Wan *et al.* 2011). According to literature, after adding FOG at a high OLR, LCFA can accumulate and lead to VFA accumulation which cause a decrease in pH and process failure (Wan *et al.* 2011; Li, C. *et al.* 2013; Noutsopoulos *et al.* 2013). However, during this experiment, the addition of FOG up to 40% VS did not lead to VFA accumulation and a negligible VFA concentration in the digester effluent was observed. Moreover, as expected, a high COD concentration in the digestate was measured, since, a high fraction of organics in FOG leads to an increase in COD concentration (Salama *et al.* 2019).

Table 4-1: The characterization of the influent and effluent of AD-1 (control) and AD-2 (co-digester)

	AD-1	AD-2			
Parameters	Control	10%[a] FOG	20% FOG	30% FOG	40% FOG
OLR[b] (g VS/L/day)	1.84 (0.16; 26)	2.38 (0.00; 3)	2.24 (0.08; 6)	2.61 (0.09; 4)	3.15 (0.06; 5)
TS[c] (%, w/w)	2.1 (0.1; 38)*	2.2 (0.1; 5)	2.4 (0.0; 9)	2.5 (0.0; 10)	2.9 (0; 13)
VS[d] (%, w/w)	1.7 (0.1; 38)	1.8 (0.0; 5)	1.7 (0.1; 9)	1.9 (0.0; 10)	2.2 (0.0; 13)
TCOD[e] (mg/L)	26,498 (2,468; 33)	31,411 (4442; 3)	30,346 (2,118; 8)	28,619 (1,361; 10)	32,753 (2,133; 11)
Ammonia (mg/L)	1,180 (184; 37)	1,153 (55; 3)	1,027 (219; 12)	1,105 (214; 10)	1,053 (56; 13)
Alkalinity (mg/L as $CaCO_3$)	4,016 (254; 37)	4,239 (244; 3)	3,477 (183; 12)	3,413 (67; 10)	3,354 (74; 12)
VFA[f] (mg/L)	11 (5; 26)	10 (2; 3)	8 (3; 7)	9 (3; 10)	15 (5; 11)
pH	7.6 (0.2; 29)	7.5 (0.0; 3)	7.5 (0.0; 12)	7.6 (0.1; 8)	7.4 (0.2; 24)
LCFA[g] (mg/g TS)	2.1 (1; 8)	NA[h]	19.3 (3.0; 8)	21.1 (3.5; 9)	24.7 (3.8; 9)

*Data represent arithmetic mean of measurement (standard deviation; number of replicates), [a]FOG/sludge ratio (VS basis), [b]Organic loading rate, [c]Total solids, [d]Volatile solids, [e]Total chemical oxygen demand, [f]Volatile fatty acids (summation of acetic, propionic, and butyric acids), [g]Long chain fatty acids (summation of palmitic, stearic, and oleic acids), [h]Not available

4.1.1 Solids removal

Figures 4-4 and 4-5 show the average total solids and volatile solids removal efficiencies at different FOG/sludge ratios (10-40% VS) in the effluents of co-digester and control. The error bars in the figure represent standard deviation of 3-8 samples analyzed during each FOG/sludge ratio. FOG has a high solid content and by increasing its ratio in the feed, a higher OLR was introduced into the digester. As it is shown, by increasing the FOG ratio, the efficiency of solid removal

enhanced. This is due to a higher availability of organic matter for microorganisms to consume and produce biogas from.

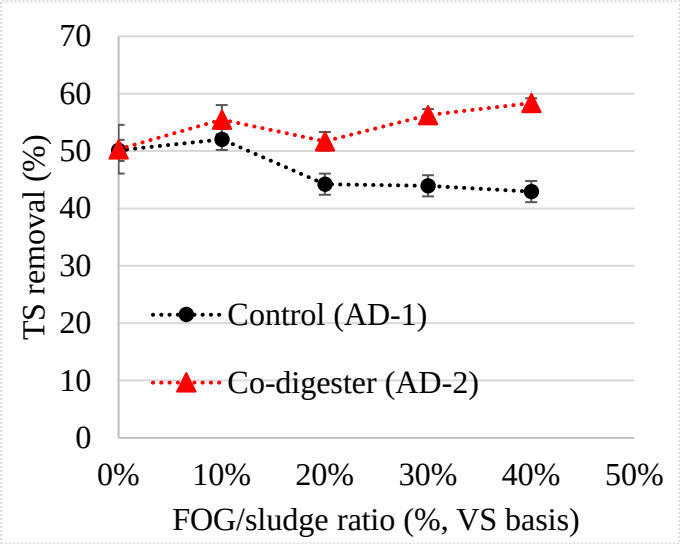

Figure 4-4: Average total solids removal efficiencies

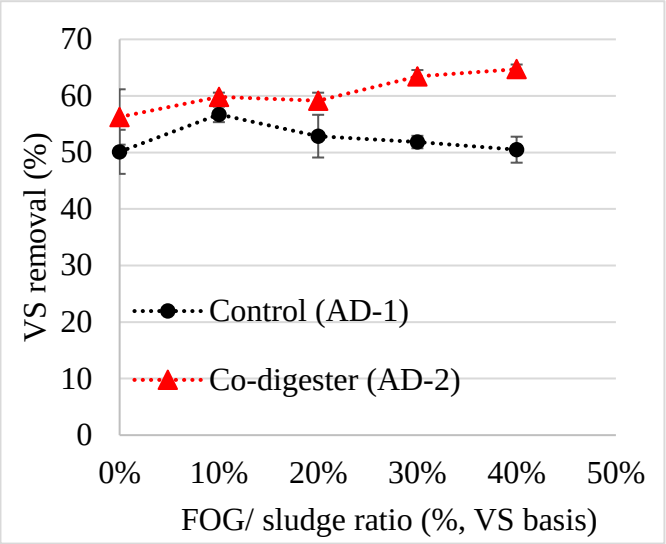

Figure 4-5: Average volatile solids removal efficiencies

The results of ANOVA test analysis are summarized in Table 4-2 and Table 4-3 to investigate the effect of the different ratios of FOG in the feed, on the performance of the digester in terms of total and volatile solids removal. According to results, the effect of the different ratios of FOG was statistically significant (p-value < 0.05) on overall solids removal in AD.

Table 4-2: Analysis of variance for total solids removal

Factor	Type	Levels	Values
FOG/sludge ratio (%)	Fixed	5	0, 10, 20, 30, 40

Source	DF[a]	Adj SS[b]	Adj MS[c]	F-Value[d]	P-Value[e]
FOG/sludge ratio (%)	4	2061.5	515.385	51.71	0.000
Error	69	687.7	9.967		
Total	73	2749.2			

[a]Degrees of freedom; [b]Sum of square; [c]adjusted mean of square; [d]observed F value; [e]probability value

Table 4-3: Analysis of variance for volatile solids removal

Factor	Type	Levels	Values
FOG/sludge ratio (%)	Fixed	5	0, 10, 20, 30, 40

Source	DF[a]	Adj SS[b]	Adj MS[c]	F-Value[d]	P-Value[e]
FOG/sludge ratio (%)	4	1777.8	444.453	54.09	0.000
Error	69	567.0	8.217		
Total	73	2344.8			

[a]Degrees of freedom; [b]Sum of square; [c]adjusted mean of square; [d]observed F value; [e]probability value

The highest VS removal (64.7%) was achieved at 40% FOG, and in comparison with VS removal in control (53.2%), therefore, it was improved by 21.5% as a result of the addition of FOG. In the literature, a similar result on VS reduction improvement was observed at 38% FOG addition (OLR = 3.13 g VS/l/day) (Luostarinen *et al.* 2009). However, lower VS reduction was observed at shorter SRTs due to the presence of slowly biodegradable materials in the sludge and FOG (Davidsson *et al.* 2008; Kabouris *et al.* 2008).

4.1.2 Methane production

In this experiment, the addition of FOG led to higher biogas production due to the higher OLR that was introduced to the system. Moreover, the methane content in the produced biogas increased from 63.1 ± 2.1% in the control to 71.5 ± 3.4% in the co-digester at 40% FOG. As it is shown in Figure 4-6, the gap of specific methane yields between control and co-digester increased as the FOG ratio increased. Therefore, the highest improvement in specific methane production was achieved at 40% FOG (68% improvement over the control).

Improvement in specific methane production was also reported in the literature, after adding FOG to mixed sludge and TWAS, respectively as a co-substrate in the AD process (Davidsson *et al.* 2008; Wan *et al.* 2011). However, the obtained results in our study, showed higher specific

methane production compared to the other studies. In our study, a higher SRT (20 days) was chosen to run the digesters and by increasing the SRT, microorganisms have more time to consume the organic fraction of the substrate and produce more biogas. Furthermore, as emphasized in the literature review, FOG characteristics vary significantly depending on the source and collection methodology, therefore improvements vary significantly.

Due to motor replacement for the control after day 160 (Figure 4-2), digester recovery to get to the same level took time and for ANOVA test analysis, methane yield data points after day 160 were not accounted for control (Figure 4-6). According to ANOVA test analysis (Table 4-4), the effect of the different ratios of FOG in the feed was statistically significant on specific methane yield of the anaerobic digestion (P-Value < 0.05) due to an increase in OLR (from 1.84 to 3.15 g VS/l/day), and higher methane obtained from FOG per gram of VS compared to other organic compounds (i.e. proteins, sugars).

Table 4-4: Analysis of variance for specific methane yield

Factor	Type	Levels	Values
FOG/sludge ratio (%)	Fixed	5	0, 10, 20, 30, 40

Source	DF[a]	Adj SS[b]	Adj MS[c]	F-Value[d]	P-Value[e]
FOG/sludge ratio (%)	4	618916	154729	59.41	0.000
Error	46	119813	2605		
Total	50	738730			

[a]Degrees of freedom; [b]Sum of square; [c]adjusted mean of square; [d]observed F value; [e]probability value

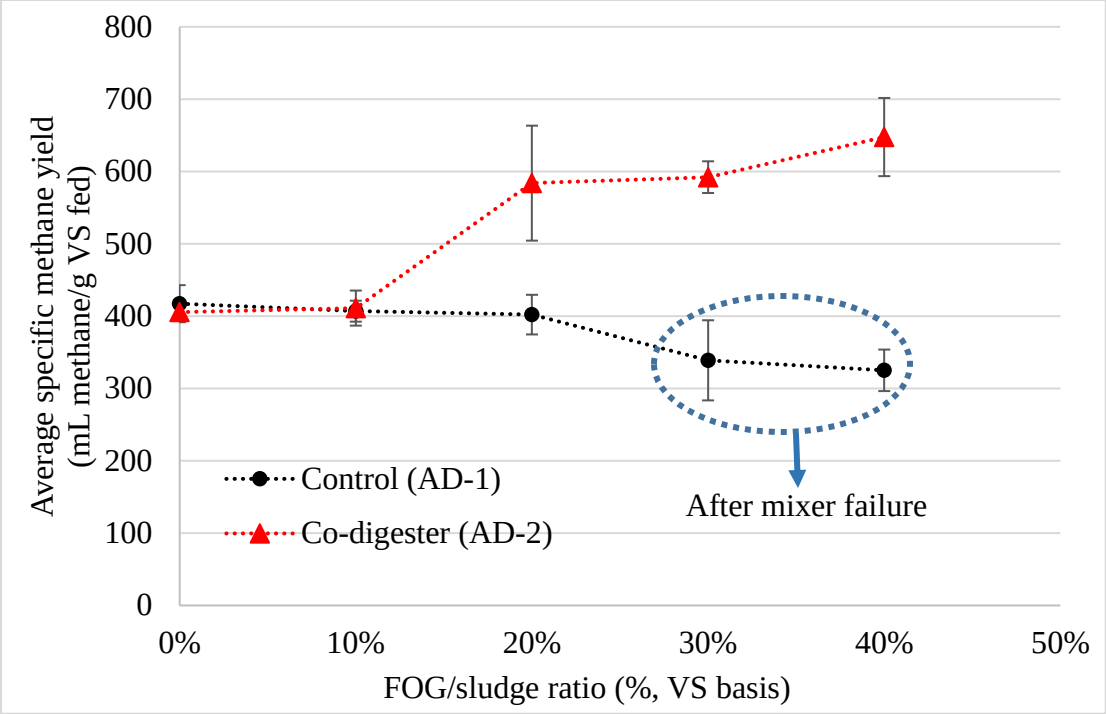

Figure 4-6: Average specific methane yield (data represent arithmetic mean and error bars represent standard deviations of 8 replicates)

4.1.3 LCFA and VFA production

Long chain fatty acids are formed through FOG hydrolysis during the AD process and degraded further to acetate and hydrogen to form methane. As it was mentioned in chapter 2, conversion of FOG to LCFAs are relatively fast but LCFAs are degraded slowly in AD process and would start to accumulate in the digester at a high FOG loading rate. Accumulated LCFA can inhibit the process and cause VFA accumulation which is detrimental to the process performance. Hence, LCFA and VFA quantification in digesters were two of the most important analytical tests to compare the efficiency and performance of the digesters.

In Figure 4-7, it is shown that by increasing the FOG/sludge ratio up to 40% VS, LCFA concentration gradually increased from 19 mg/g TS (at 20% FOG) to 24.7 mg/g TS. However, after starting to feed at 50% VS FOG, LCFA started to accumulate and after 45 days it increased

dramatically to 205 mg/g TS, and process failure (cease of biogas/methane production) was observed. In another study, LCFA was quantified under the similar process, it reached 95 mg/g TS at OLR = 2.9 g VS/l/day and decreased to 70 mg/g TS after 30 days. At this point, the experiment was stopped and the observed LCFA accumulation did not lead to process failure (Ziels *et al.* 2016). However, in our study, a higher OLR (3.7 g VS/l/day) was applied to the digester and microorganisms could not degrade the accumulated LCFA and the new substrate (FOG + mixed sludge) added daily. As a result, process failure was observed. In Figure 4-7, LCFA and VFA concentrations in the control digester are in the ranges of 1-4 mg/g TS and 5-29 mg/l, respectively, while they are in the ranges of 14-205 mg/g TS and 10-3189 mg/l in the co-digester receiving FOG, respectively. Due to significantly lower concentrations in AD-1, compared to those in AD-2, they are not easily noticeable.

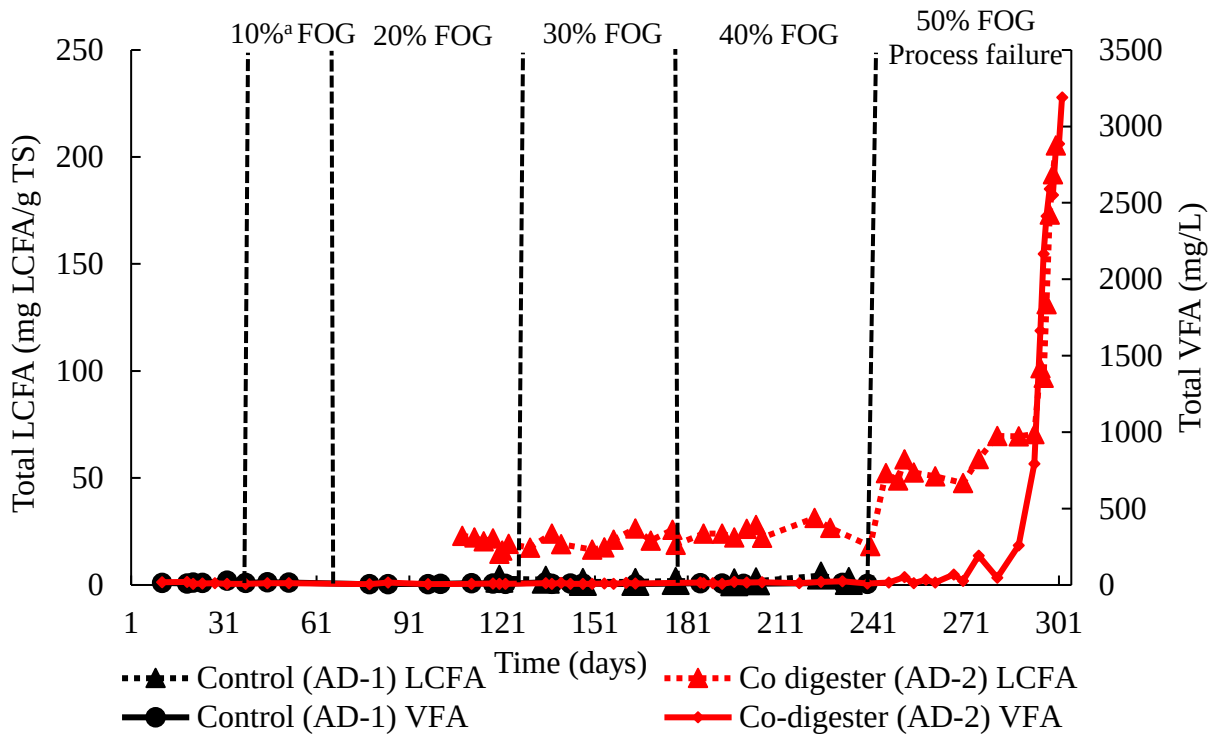

Figure 4-7: VFA and LCFA concentration ([a]FOG/sludge VS ratio)

As expected, there is a clear relationship between LCFA and VFA concentrations. During the addition of FOG (up to 40% VS), almost all the VFA produced was consumed by methanogenesis and VFA concentration left in the digestate was negligible (<15 mg/l). By increasing the FOG ratio to 50% VS, fluctuations in VFA concentration were observed leading to the instability of the process performance. After 45 days, VFA concentration suddenly increased and followed the same trend as LCFA concentration and confirmed the fact that LCFA accumulation also causes VFA accumulation due to inhibition of methanogenic archaea utilizing VFAs as substrate.

The measured concentrations of three separate forms of LCFAs (palmitic acid, stearic acid, and oleic acid) in the effluents of control and co-digester are presented in Figure 4-8. It appears that palmitic acid had the greatest portion among three LCFAs in the effluents of the co-digester. Up to 40% VS FOG, the same trend for LCFA concentrations was maintained, stearic acids had the

lowest values. Although, at 50% FOG, stearic acid concentration exceeded oleic acid concentration. This can be explained by the fact that stearic acid is degraded slower than oleic acid (Lalman & Bagley 2001). Moreover, in the digestate of the control, only palmitic acid was quantified.

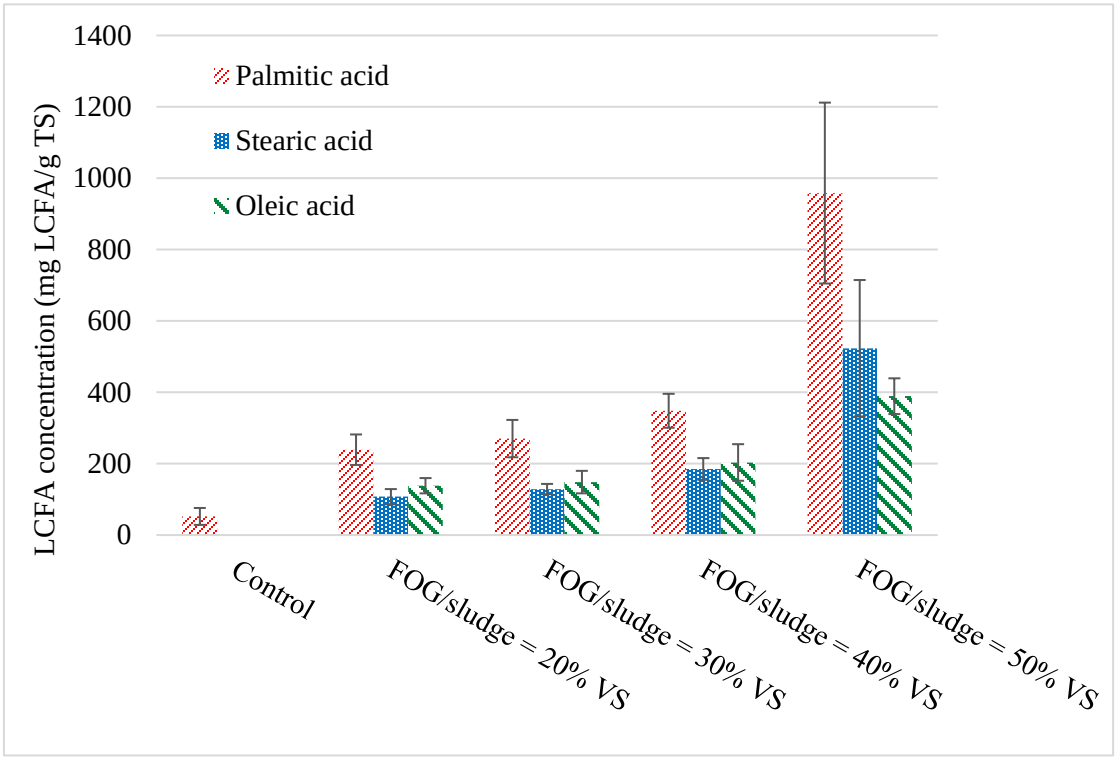

Figure 4-8: Presence of different forms of LCFAs in single stage digester and co-digester (data represent arithmetic mean and error bars represent standard deviations of 8 replicates)

It was reported that oleic acid (consists of 18 carbons with one double bond (C18:1)), is degraded to palmitic acid (C16:0) and myristic acid (C14:0) under the AD process and they are degraded further to ultimately form methane. However, there is no evidence that any LCFA is formed during stearic acid degradation as a by-product; as a result, stearic acid is degraded slower than oleic acid, as the degradation process requires more energy (Lalman & Bagley 2001).

4.1.4 Effluent dewaterability

One of the common methods to measure the rate of the dewaterability of a digestate is known as CST that is expressed in seconds and normalized by the TS (% wt.) content of the sample. The lower CST means the digestate releases its water faster, results in less volume of biosolids for disposal, and causes significant cost savings in terms of shipping/land application. Figure 4-9 shows the results of the CST test at different FOG/sludge ratios for control and co-digester. At each stage, after digesters reached to steady state, the CST test was performed three times and at each time in triplicates. The figure illustrates that there is not a discernable pattern between CST and different FOG/sludge ratios. The ANOVA test analysis (Table 4-5) shows that the effect of the addition of FOG to mixed sludge was not statistically significant on the dewaterability rate of the digestate (P-value >0.05).

Table 4-5: Analysis of variance for CST for the effluents of the digesters

Factor	Type	Levels	Values
FOG/sludge ratio (%)	Fixed	4	0, 20, 30, 40

Source	DF[a]	Adj SS[b]	Adj MS[c]	F-Value[d]	P-Value[e]
FOG/sludge ratio (%)	3	182080	60693	2.82	0.077
Error	14	301372	21527		
Total	17	483452			

[a]Degrees of freedom; [b]Sum of square; [c]adjusted mean of square; [d]observed F value; [e]probability value

In literature, there is no clear information on the effect of the addition of FOG on the dewaterability rate of the digestate in anaerobic co-digestion. However, the impact of the co-digestion of trapped grease waste from the pre-treatment unit of a WWTP on the dewaterability rate of the digestate was assessed by filterability and centrifugability tests (Silvestre *et al.* 2014). The anaerobic digesters were run under mesophilic (35°C) and thermophilic (55°C) temperatures. The results revealed that the addition of grease waste to sewage sludge at a mesophilic temperature

resulted in a better dewaterability rate of the digestate compared to the sewage sludge digestate; since by improving the nutrient balance of the substrate, less extracellular polymeric substances, directly affects the dewaterability, are produced. The authors reported that adding grease waste in a mesophilic condition enhanced the nutrient balance, increased the C/N ratio, and resulted in better dewaterability properties. Although, worse dewatering properties were also reported at a thermophilic temperature after adding grease waste (Silvestre *et al.* 2014).

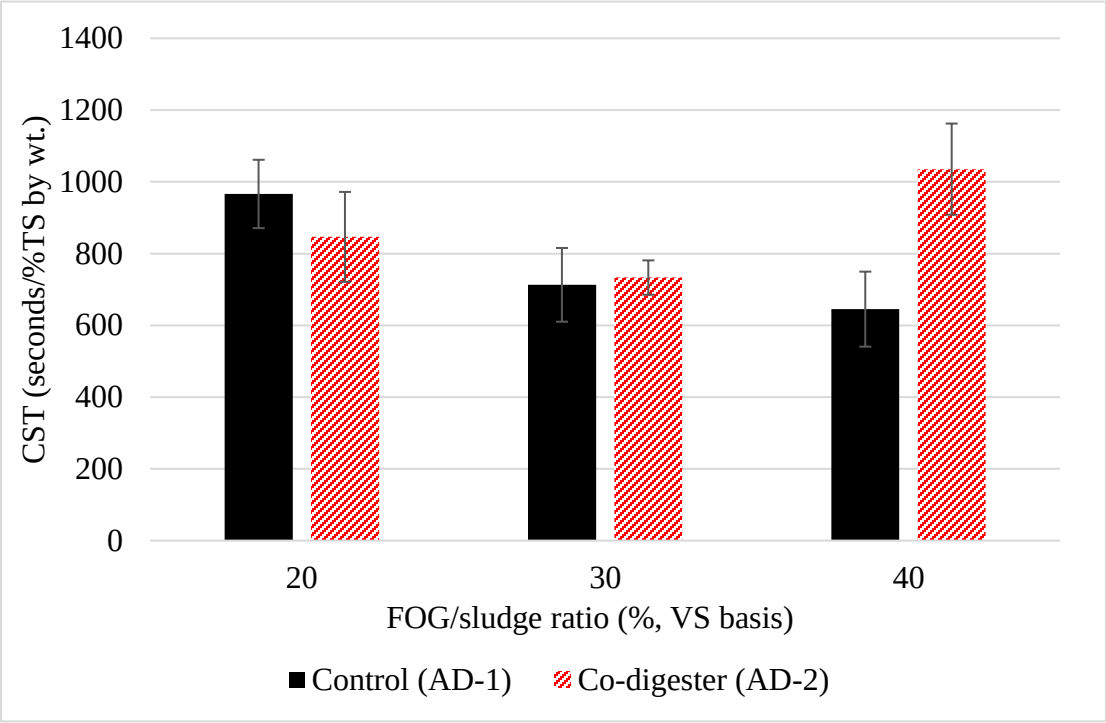

Figure 4-9: Specific capillary suction time (CST) for the final effluent of the digesters (data represent arithmetic mean and error bars represent standard deviations of 3 replicates)

4.1.5 Digestate quality for land application

Municipal biosolids from the AD process can be land applied as fertilizer if specific criteria are met. The criteria are defined based on fecal coliform levels that indicate pathogens contamination, and the concentrations of heavy metals. As described in the literature review section, in BC, biosolids are classified according to the OMRR.

4.1.5.1 Fecal coliform content

According to OMRR regulations, biosolids containing less than 1,000 most probable number (MPN)/g dry solids are categorized as class A biosolids. Class B biosolids limit the fecal coliform density to less than 2,000,000 MPN/g dry solids (OMRR 2008).

In this study, fecal coliforms were quantified using CFU to estimate the actual concentration of fecal coliforms (normalized by TS content of the sample). Figure 4-10 presents the test results of the digestates from the control and co-digester at two different FOG/sludge ratios (30 and 40% VS). The test was performed three times for each digester at steady state. Digestates from both digesters were classified as class B biosolids and there was no discernable difference between control and co-digester results.

According to ANOVA test analysis (Table 4-6), the effect of adding FOG to mixed sludge in the anaerobic co-digestion process was not statistically significant on fecal coliform removal (P-value > 0.05).

Table 4-6: Analysis of variance for fecal coliform counts in digestates

Factor	Type	Levels	Values
FOG/sludge ratio (%)	Fixed	3	0, 30, 40

Source	DF[a]	Adj SS[b]	Adj MS[c]	F-Value[d]	P-Value[e]
FOG/sludge ratio (%)	2	639512666	319756333	1.80	0.219
Error	9	1595621993	177291333		
Total	11	2235134659			

[a]Degrees of freedom; [b]Sum of square; [c]adjusted mean of square; [d]observed F value; [e]probability value

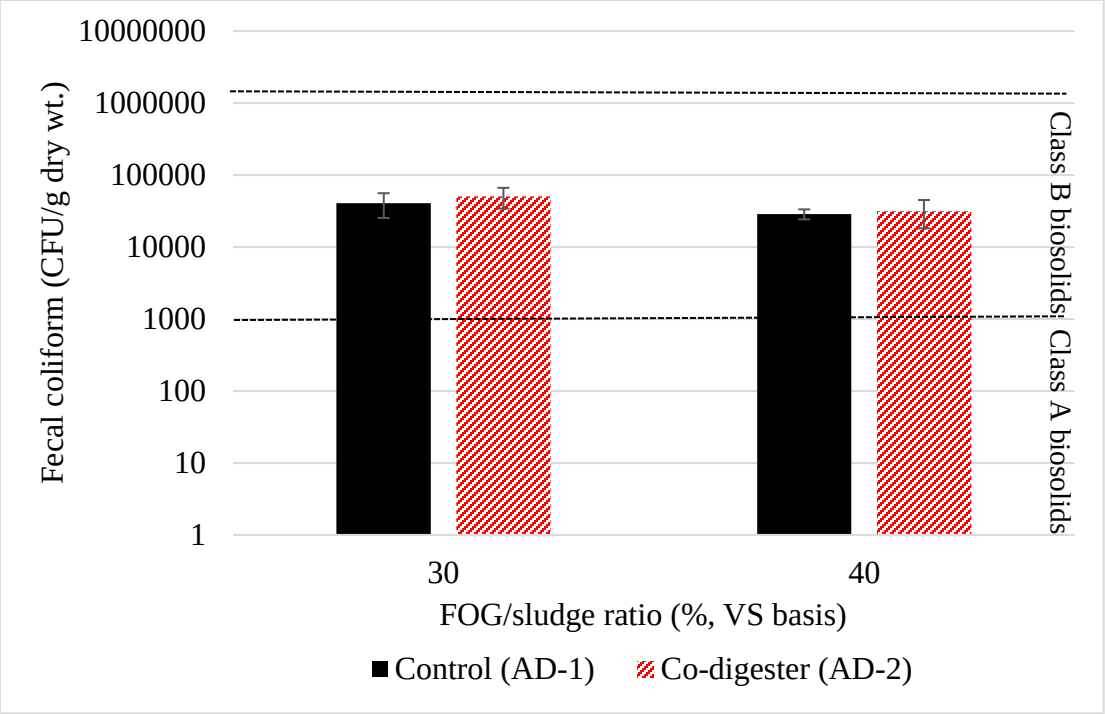

Figure 4-10: Average fecal coliforms concentration in the effluent of digesters (CFU: colony forming unit, error bars indicate standard deviation of data, number of replicates = 3)

4.1.5.2 Heavy metals content

Another parameter for categorizing biosolids for land application as fertilizer is heavy metal density. Heavy metals enter the municipal sewage sludge system through connections to industrial wastewater or from the piping system. A group of eleven heavy metals are included in OMRR regulations criteria and specified concentration ranges are defined for each heavy metal to classify biosolids into class A and class B biosolids.

The results of heavy metals analysis are summarized in Table 4-7. The analysis was performed at 20%VS FOG/sludge for the effluents of co-digester and control. Both digestates could meet the Class A biosolids in terms of heavy metals concentration, with significantly lower heavy metal concentrations than regulations. Moreover, adding FOG did not affect the heavy metal concentrations. Thus, the analysis was not repeated for the other (higher) FOG/sludge ratios.

Table 4-7: Heavy metal concentrations in digestates (FOG/sludge = 20% VS)

Parameters	OMRR[a] criteria			
	Class A biosolids	Class B biosolids	Control (AD-1)	Co-digester (AD-2)
Arsenic (mg/kg)[b]	75	75	2.39	2.26
Cadmium (mg/kg)	20	20	1.54	1.38
Chromium (mg/kg)	-[c]	1060	18.1	19.8
Cobalt (mg/kg)	150	150	1.73	1.72
Copper (mg/kg)	-[c]	2200	608	583
Lead (mg/kg)	500	500	8.98	9.16
Mercury (mg/kg)	5	15	0.926	0.888
Molybdenum (mg/kg)	20	20	9.87	9.34
Nickel (mg/kg)	180	180	14.2	14.1
Selenium (mg/kg)	14	14	5.32	5.28
Zinc (mg/kg)	1850	1850	492	474

[a]Organic Matter Recycling Regulations (OMRR 2016), [b]mg per kg of dry solids, [c]can not exceed class A levels

4.2 TPAD system performance

In order to investigate the effectiveness of the TPAD system on the performance of the anaerobic co-digestion of FOG and mixed sludge, first two bench-scale single-stage semi-continuous flow digesters with SRT of 20 days were set up and fed with 40% VS FOG/sludge for 45 days to reach a steady state (Figure 4-11). Then, the ratio of FOG in mixed sludge was increased to 50% VS. Meanwhile, two other digesters were set up at 55°C (AP-1) and 70°C (AP-2), each with SRT of 2 days, to be used as first stages (acid phases) of TPAD systems. They were fed in the same manner (50% VS FOG/sludge) to reach steady state. Once AP-1 and AP-2 reached steady state, the single-stage digesters were coupled with AP-1 and AP-2 to function as second stage (methane phases) digesters of TPAD configurations. After 75 days of operating the single stage digesters, the effluents of acid phases were introduced to MP-1 and MP-2 at SRT of 18 days. This made the overall SRT of TPAD systems 20 days, the same as the SRT of initial single stage ADs.

Biogas production from both acid and methane phases of TPADs is presented in Figure 4-11. As expected, acid phases of TPADs (AP-1 and AP-2) generated negligible values of biogas since these vessels were in charge of VFA production. Methane phases showed the same performance as the single stage vessels in terms of biogas production; however, the signs of failure were observed in MP-1 and MP-2 after 35 and 49 days respectively. Table 4-8 summarizes the characterization results of the methane acid phases before digesters failure.

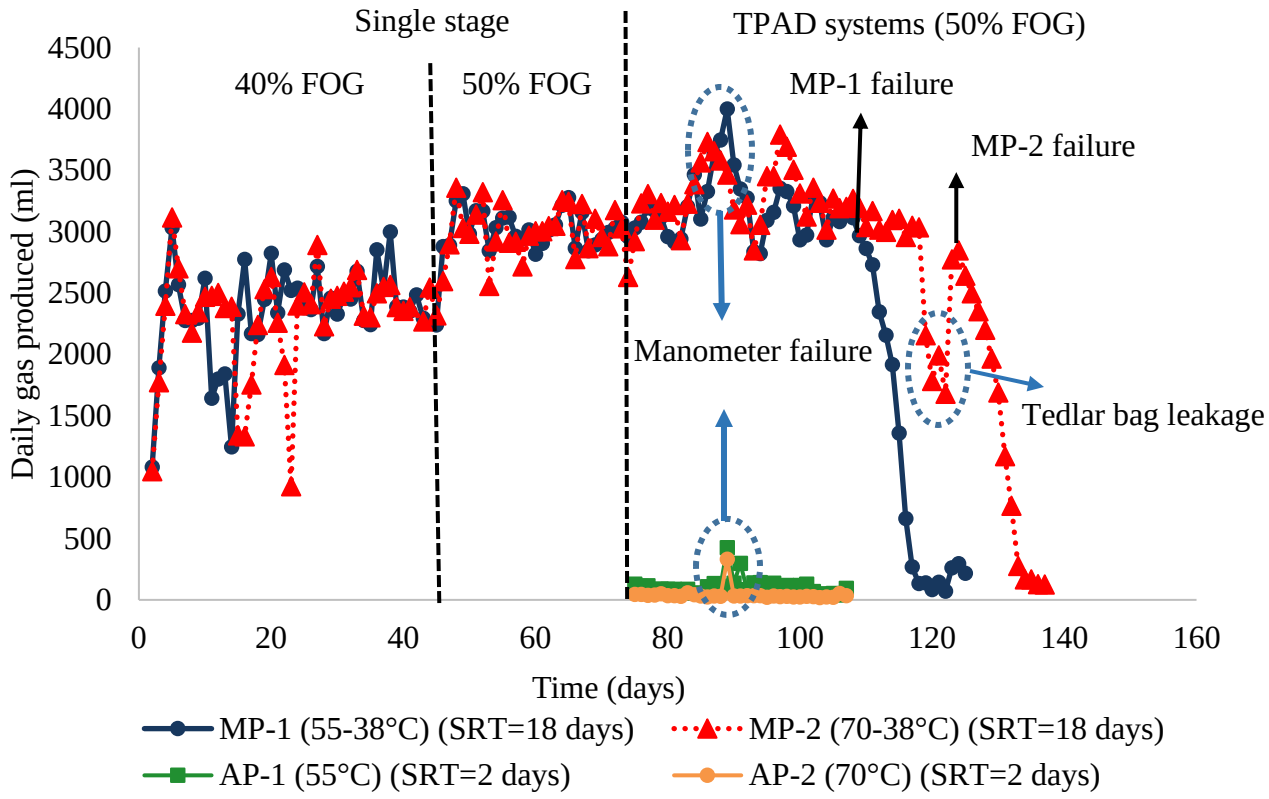

Figure 4-11: Daily produced biogas from acid and methane phases of TPADs ([a]FOG/sludge VS ratio)

Digester failure was observed in MP-1 earlier than MP-2. This can be due to higher ammonia and VFA concentrations in AP-1 in comparison with AP-2 (Table 4-8). Ammonia and VFA are inhibitory parameters that affect methanogenesis performance. AP-2 was run at a high temperature (70°C) which could lead to ammonia and VFA evaporation from liquid to gas phase. In the literature, a decrease in ammonia concentration was observed by increasing the temperature over

65°C. It was reported that the optimum temperature for the growth of protein degrading bacteria (ammonia generating process) was 65°C (Lee *et al.* 2008).

Table 4-8: The characterization of the effluent of the TPAD stages

	TPAD-1		TPAD-2	
Parameters	AP[g]-1 (55°C/2-d)	MP[h]-1 (38°C/18-d)	AP-2[g] (70°C/2-d)	MP-2[h] (38°C/18-d)
OLR[a] (g VS/L/day)	37.68 (2.03; 2)	4.08 (0.21; 7)	37.68 (2.03; 2)	4.15 (0.12; 9)
TS[b] (%, w/w)	8.0 (0.4; 7)*	3.2 (0.2; 9)	8.1 (0.2; 9)	3.3 (0.2; 13)
VS[c] (%, w/w)	7.3 (0.4; 7)	2.6 (0.2; 9)	7.4 (0.2; 9)	2.6 (0.4; 13)
TCOD[d] (mg/L)	130,958 (23,788; 6)	45,397 (2,582; 5)	152,114 (13,263; 8)	48,621 (3,299; 9)
Ammonia (mg/L)	573 (65; 10)	1,189 (69; 8)	473 (57; 11)	1,093 (59; 12)
Alkalinity (mg/L as $CaCO_3$)	1,171 (150; 7)	3,450 (142; 9)	900 (176; 8)	3,288 (134; 13)
VFA[e] (mg/L)	2,359 (282; 9)	1,145 (459; 9)	1,768 (233; 10)	1,115 (494; 13)
pH	5.26 (0.05; 10)	7.15 (0.05; 30)	5.20 (0.06; 10)	7.15 (0.04; 38)
LCFA[f] (mg/g TS)	349 (27; 9)	58.2 (9.4; 9)	339 (35; 11)	55.7 (12.3; 13)

*Data represent arithmetic mean of measurement (standard deviation; number of replicates), [a]Organic loading rate, [b]Total solids, [c]Volatile solids, [d]Total chemical oxygen demand, [e]Volatile fatty acids (summation of acetic, propionic, and butyric acids), [f]Long chain fatty acids (summation of palmitic, stearic, and oleic acids), [g]Acid phase (temp: 55 or 70°C / SRT: 2-d), [h]Methane phase (temp: 38°C / SRT: 18-d)

Long chain fatty acid is another inhibitory parameter which causes process failure. According to measured LCFA results (Table 4-8), a higher average value was observed for AP-1 than AP-2.

However, ANOVA test results showed that the effect of the temperature of the first stage was not statistically significant on LCFA concentration (P-value > 0.05).

Figure 4-12 shows the concentration of different LCFAs in digestates and feed (a mixture of mixed sludge and FOG). It is illustrated that in acid phases, a high concentration of LCFA (266-388 mg/g TS) is detected under high OLR (38 g VS/l/d) corresponding to a short SRT of 2 days and oleic acid and stearic acid had the most and the least concentrations in the digestate, respectively. As it was mentioned in previous sections, oleic acid is converted to palmitic acid under the AD process (Lalman & Bagley 2001) and it seems that in acid phases oleic acids did not have enough time to degrade and form palmitic acids. Moreover, the magnitude of these three LCFAs are in the same range for acid phases and different temperatures did not have a significant impact on the composition of the LCFAs. It is shown that the majority of produced LCFAs in the acid phase were consumed and converted to methane in the second stage. Furthermore, the composition of LCFAs in MP-1 and MP-2 showed a similar pattern to the single stage, at 50% VS FOG/sludge. However, in the end, similar to single stage ADs, TPAD systems could not maintain a stable AD performance at 50% VS FOG/sludge feed and resulted in process failure.

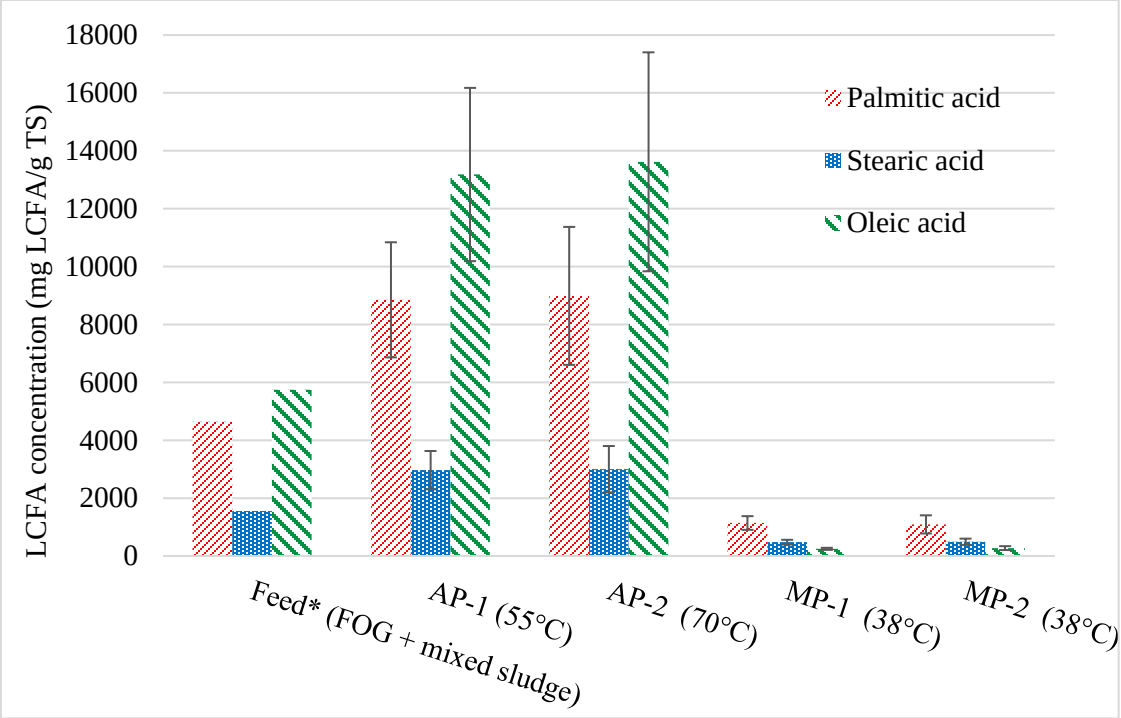

Figure 4-12: Presence of different LCFAs in TPAD stages (*FOG/sludge = 50% VS) (data represent arithmetic mean and error bars represent standard deviations of 9 replicates)

4.3 Biochar

Biochar is a relatively new adsorbent tested in the AD process that is effective in removing the inhibitors and improving methane production. In order to investigate the potential of biochar to mitigate LCFA accumulation in the AD process, three sets of batch adsorption assays tests were conducted at 55°C. Digestate from single stage digester was collected at 50% VS FOG, which had high LCFA concentrations (52-69 mg/g TS). Different amounts of biochar were added to the AD effluents (digestate) based on the TS concentration of the digestate. In each test, LCFA was quantified in the sample regularly and compared to the same sample without biochar addition. The results are presented in Figures 4-13 to 4-15.

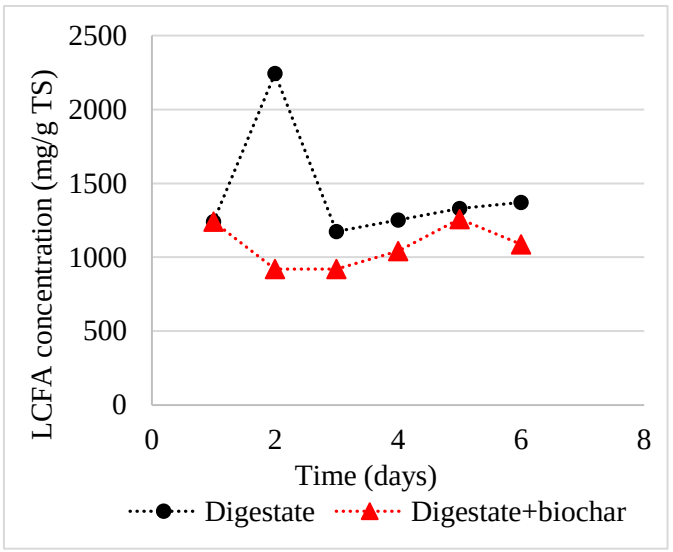

Figure 4-13: LCFA concentration of digestate with time in batch experiment (0.17 g biochar/g TS of digestate)

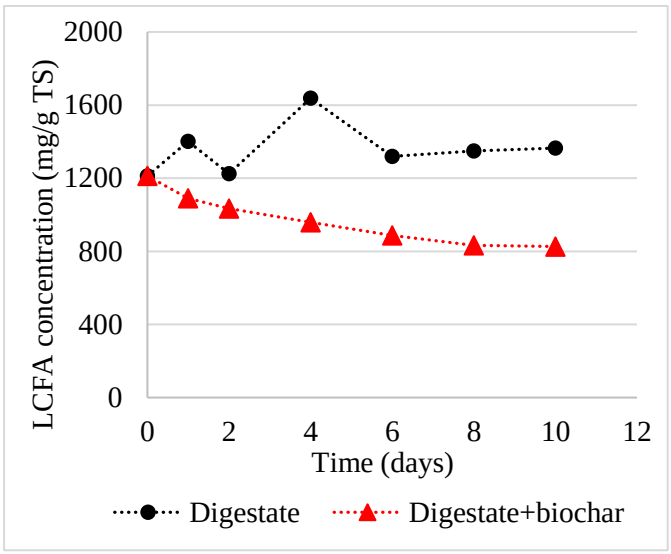

Figure 4-14: LCFA concentration of digestate with time in batch experiment (2 g biochar/g TS of digestate)

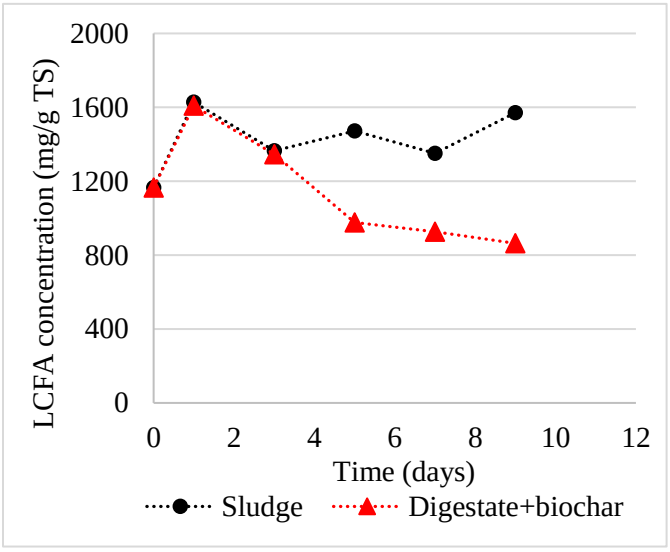

Figure 4-15: LCFA concentration of digestate with time in batch experiment (3 g biochar/g TS of digestate)

As it is shown, biochar addition had a positive effect on LCFA removal. By increasing the ratio of biochar to digestate from 0.17 to 2 and then to 3 g biochar/g TS of digestate, the gap between the LCFA concentration in the digestate with and without biochar increased. The results confirmed the biochar's availability in LCFA removal from digestate. Based on these results, 3 g of biochar was added to the failing single stage co-digester (AD-2) daily for 9 days based on a conservative

(cost effective) dose of 2 g biochar/ g TS of digestate, however no process improvement was observed. More research is needed to investigate the optimum biochar type, size, and dose addition to achieve the highest LCFA removal. Moreover, the effect of biochar addition on anaerobic co-digestion with FOG (at high FOG loading) needs to be investigated with biochar addition introduced early in the process without the first signs of process failure. It is highly likely that by the time the biochar dosing was started, methanogenesis process had been inhibited beyond the point of recovery.

Chapter 5 . Conclusions, limitations and future works

The research described in this thesis provided the results from the effect of different FOG/sludge ratios on single stage AD performance as well as on TPAD systems under the identical SRT of 20 days. Moreover, this thesis investigated the effectiveness of the biochar, as an adsorbent, to remove LCFA from digestate to mitigate AD inhibition.

Based on the experimental data of single stage digesters the following conclusions are obtained:

- FOG sampled from restaurant grease traps had high TS values (54-67% by wt.) with high VS/TS ratios (99%), low pH (~4), high COD concentration (619-742 mg/l), and high LCFA concentrations (396-527 mg/g TS).
- FOG addition to mixed municipal sludge up to 40% VS, corresponding to OLR of 3.15 g VS/l/day, led to a stable anaerobic co-digester process at mesophilic temperature. Under these conditions, specific methane production and VS removals were increased by 68 and 21.5%, respectively, over the control digester utilizing sludge only.
- Co-digester failure was observed at 50% FOG/sludge (OLR = 3.7 g VS/l/day) with LCFA and VFA values reaching up to 205 mg/g TS and 3189 g/l, respectively.
- After adding FOG to sludge, no significant impact on digestate fecal coliform concentration, dewaterability rate, and heavy metal concentration was observed. Furthermore, the biosolids produced from both digesters met the class B biosolids criteria overall.

Based on the TPAD systems performance the following conclusions are drawn:

- At the highest OLR (50% FOG/sludge), TPAD systems, with acid phases at two different thermophilic temperatures (55, 70˚C), followed by mesophilic (37˚C) methane

phases, could not achieve stable operation and failed, similar to the single stage anaerobic co-digester.

- The acid phase digester that was run at a lower temperature led to more ammonia and VFA production. Hence, the following methane phase failure was observed earlier.

Based on the batch experiments with biochar the following conclusions are drawn:

- Biochar addition to the co-digester digestate with high LCFA concentrations (52-69 mg/g TS) resulted in LCFA removal.
- By increasing the biochar addition, improvement in LCFA removal was achieved.
- However, biochar dosing to anaerobic co-digester (at 50% FOG/sludge) could not recover the failing bioreactor.

5.1 Recommendations for future work

This work presented the effect of FOG addition to a single stage digester at different ratios, and FOG addition to TPAD system at only one ratio. Moreover, the effect of biochar on LCFA removal in a batch experiment was investigated. The followings are some suggestions for future works:

- There is lack of information about different microbial enzymes that are responsible to degrade FOG in AD process. Furthermore, genomic analysis is needed to identify dominant microbial cultures to understand the challenges of degrading LCFAs and provide optimal condition to overcome these challenges.
- The optimum biochar addition to reach the highest LCFA removal should be assessed and the effect of different biochar type, particle size on the removal of LCFA should be studied. Moreover, other biochar characteristics such as conductivity, surface area, pore volume and pore distribution, hydrophobicity and water holding capacity etc. can help

to understand the mechanisms of LCFA removal and assess the optimum LCFA removal.

- The effect of the addition of biochar to a semi-continuous anaerobic digestion with FOG under high FOG loading from the point of start-up and commissioning needs to be investigated. The result would reveal a higher optimum FOG addition to reach steady state process performance.

5.2 Limitations

- FOG is semi solid and it's not soluble in water; hence, limited analytical test could be applied to it.
- LCFA adsorption test was only performed in batch mode and the optimization of semi continuous anaerobic co-digestion of FOG with biochar addition could not be investigated due to time limitation.

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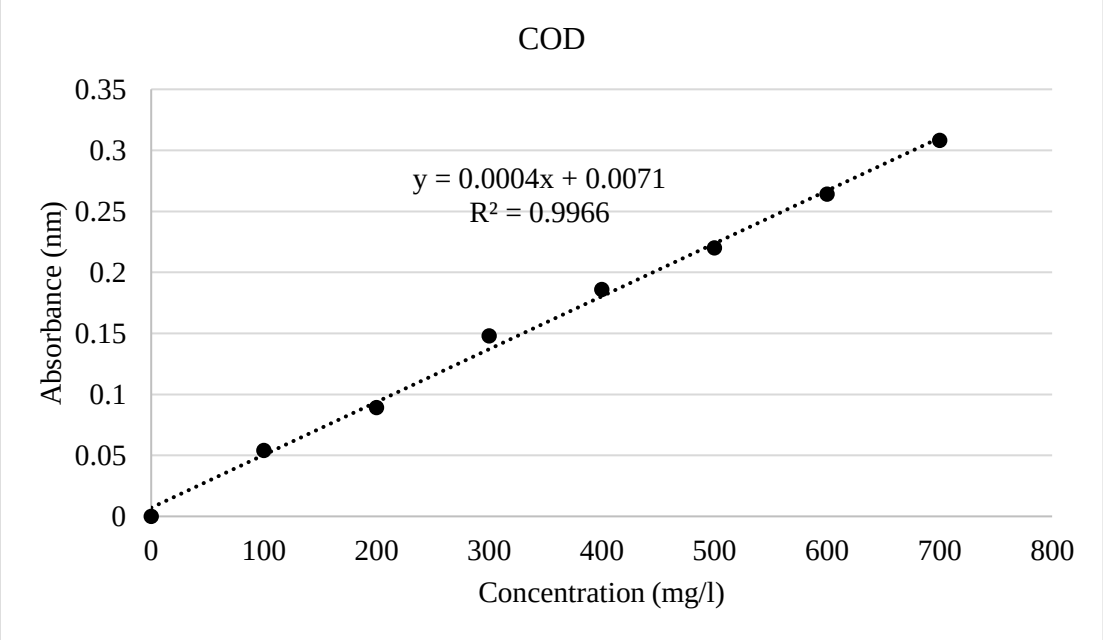
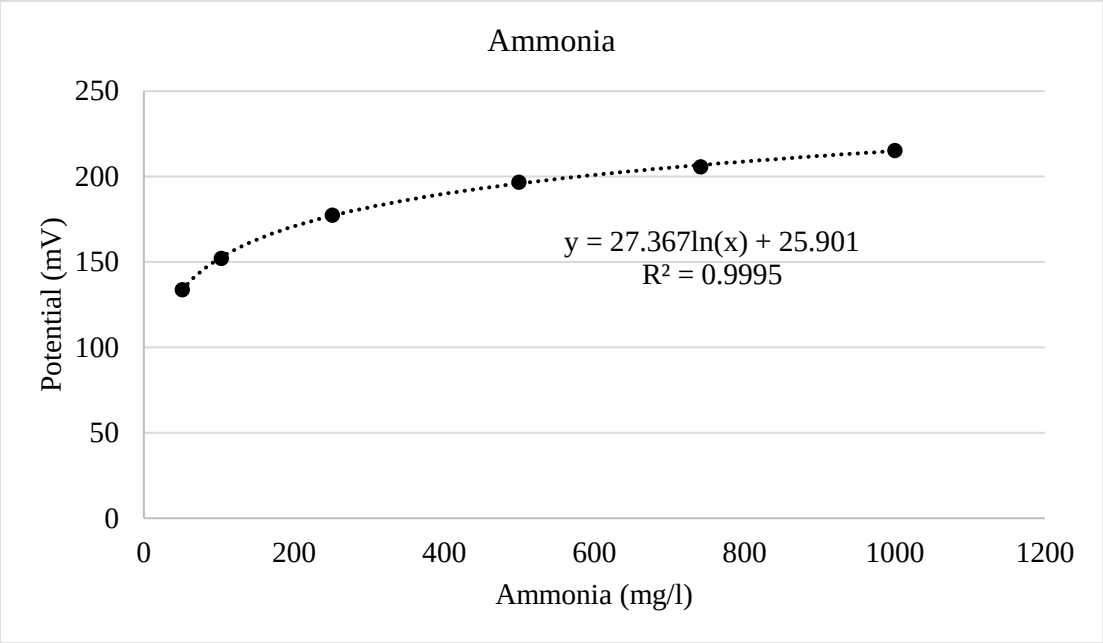
Appendices

Appendix A – Sample calibration curves

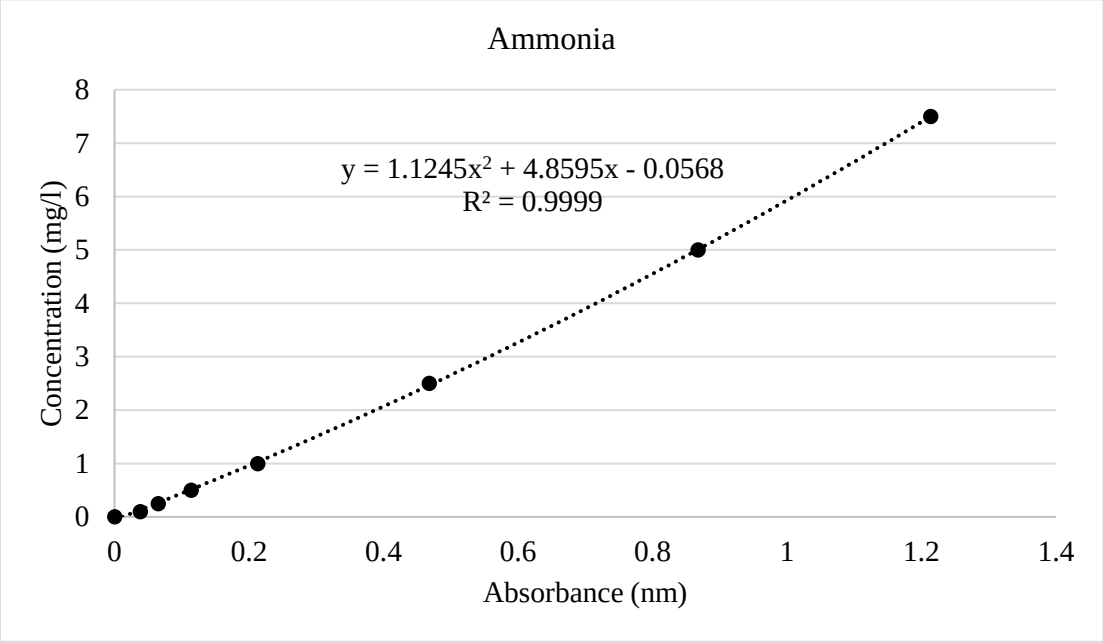
A.1 Chemical oxygen demand calibration curve (Dec 12)



A.2 Ammonia calibration curve (ammonia selective electrode method) (July 29)

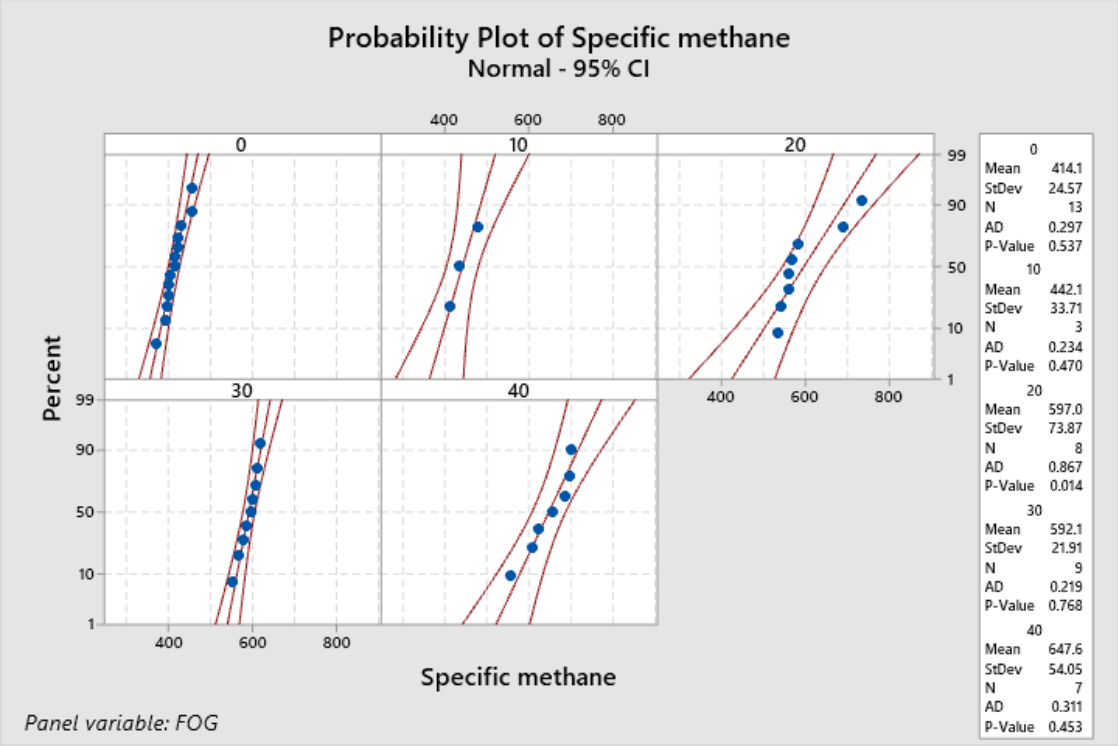


A.3 Ammonia calibration curve (phenate method) (Dec 11)

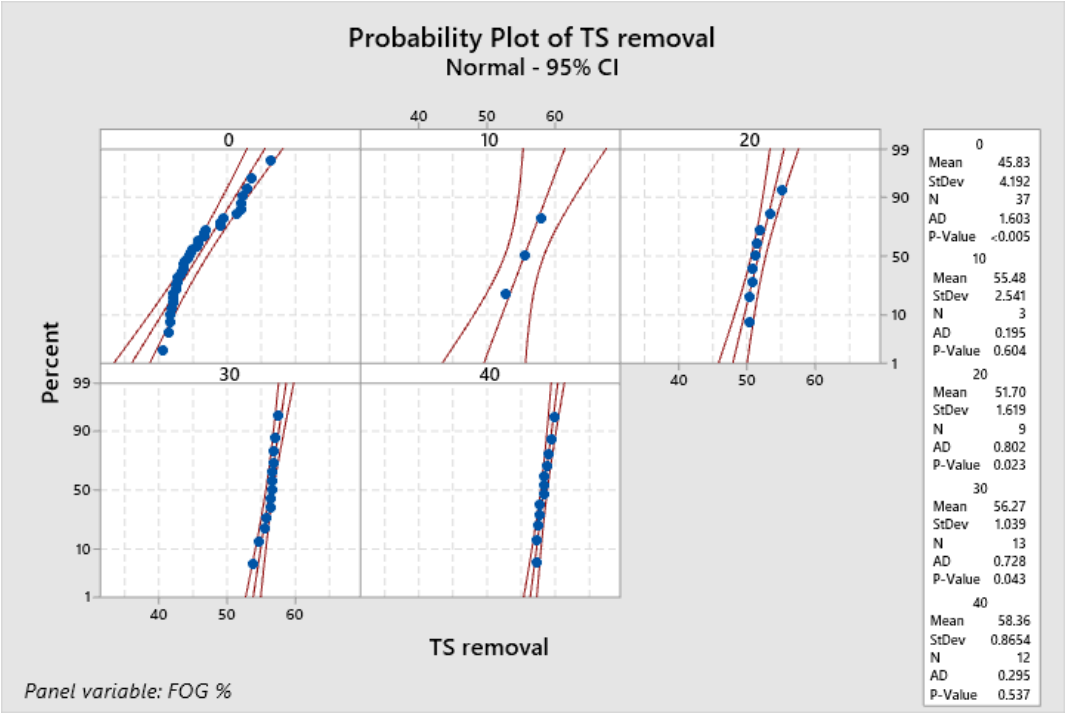


Appendix B: Representative Anderson- darling test normality plots

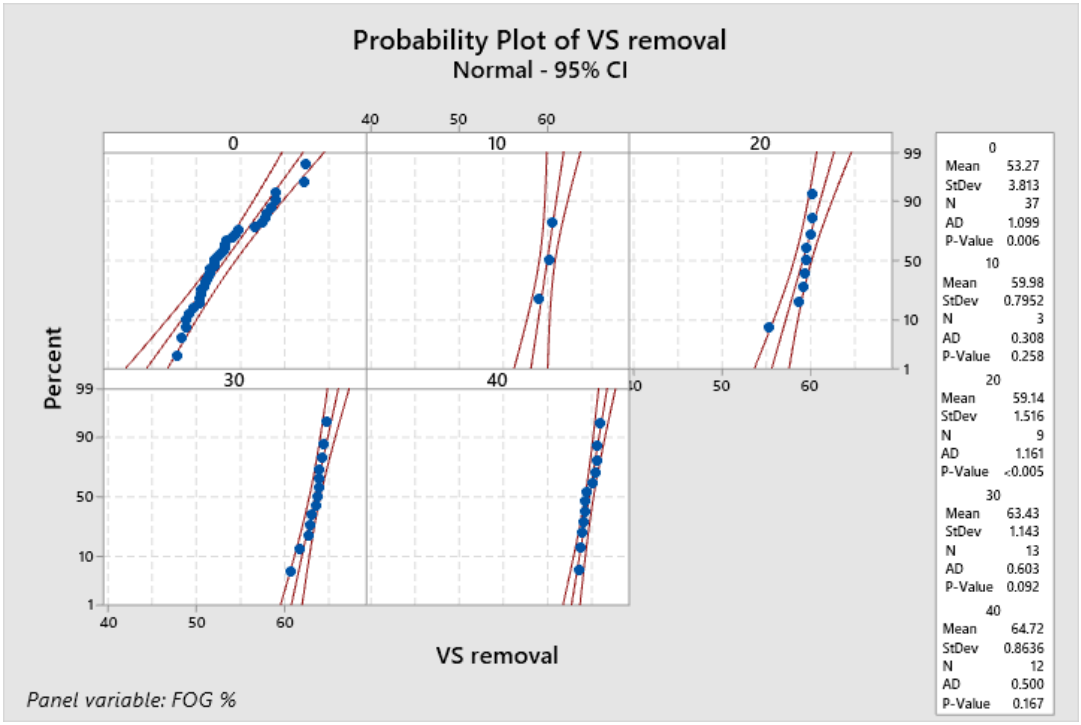
B.1: The representative Anderson-Darling test normality plot for specific methane production from single stage digesters at different FOG/Sludge ratios



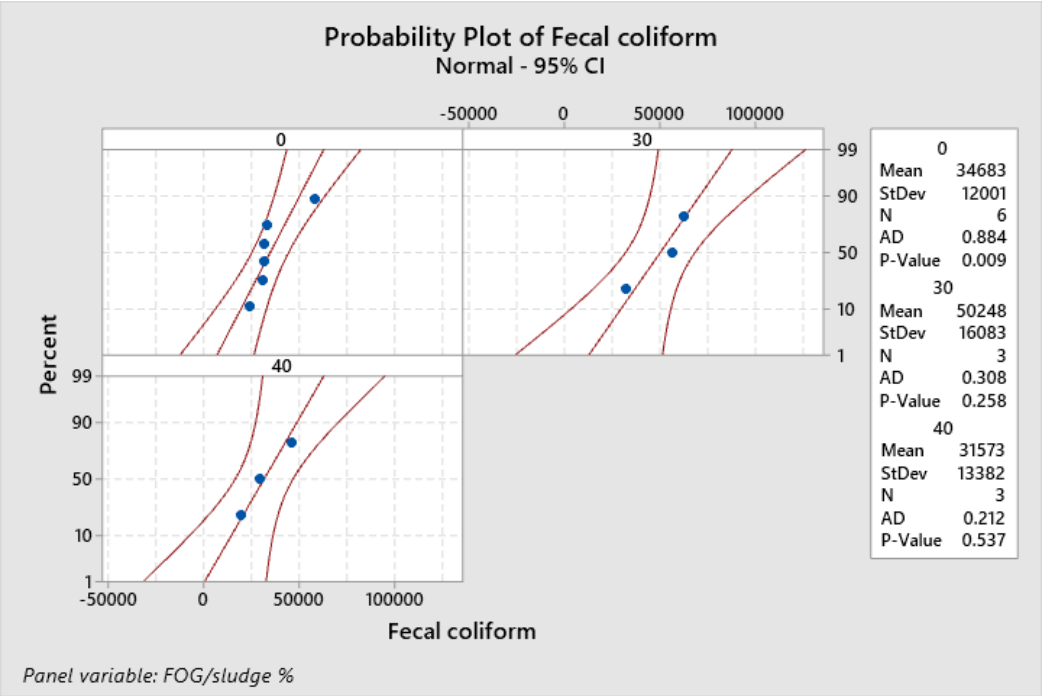
B.2: The representative Anderson-Darling test normality plot for TS removal of single stage digesters at different FOG/Sludge ratios



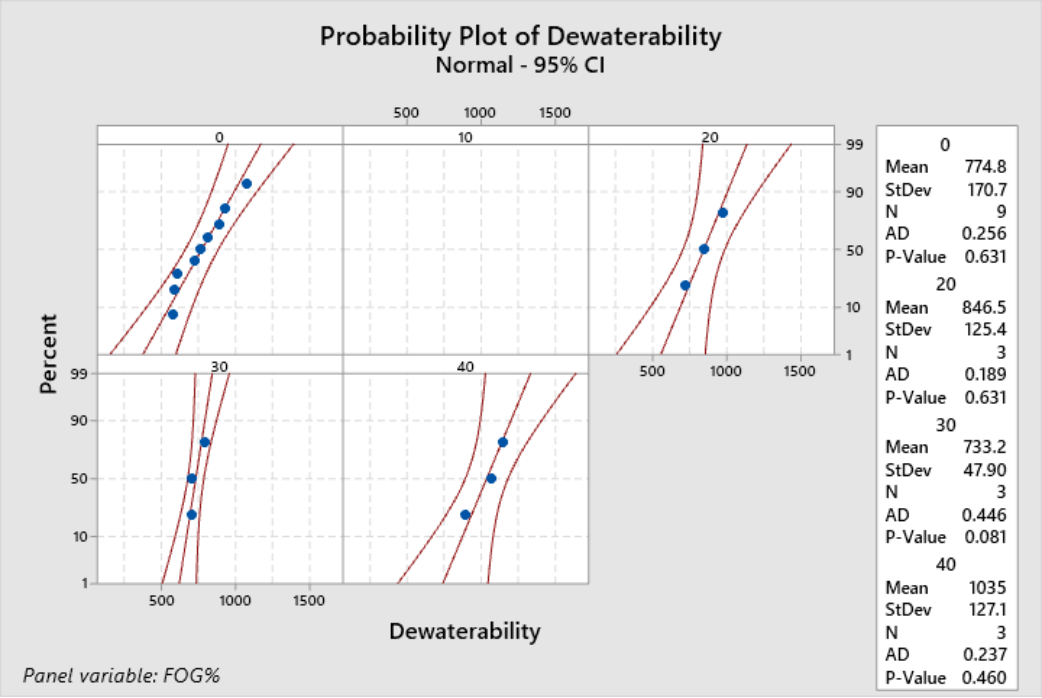
B.3: The representative Anderson-Darling test normality plot for VS removal of single stage digesters at different FOG/Sludge ratios



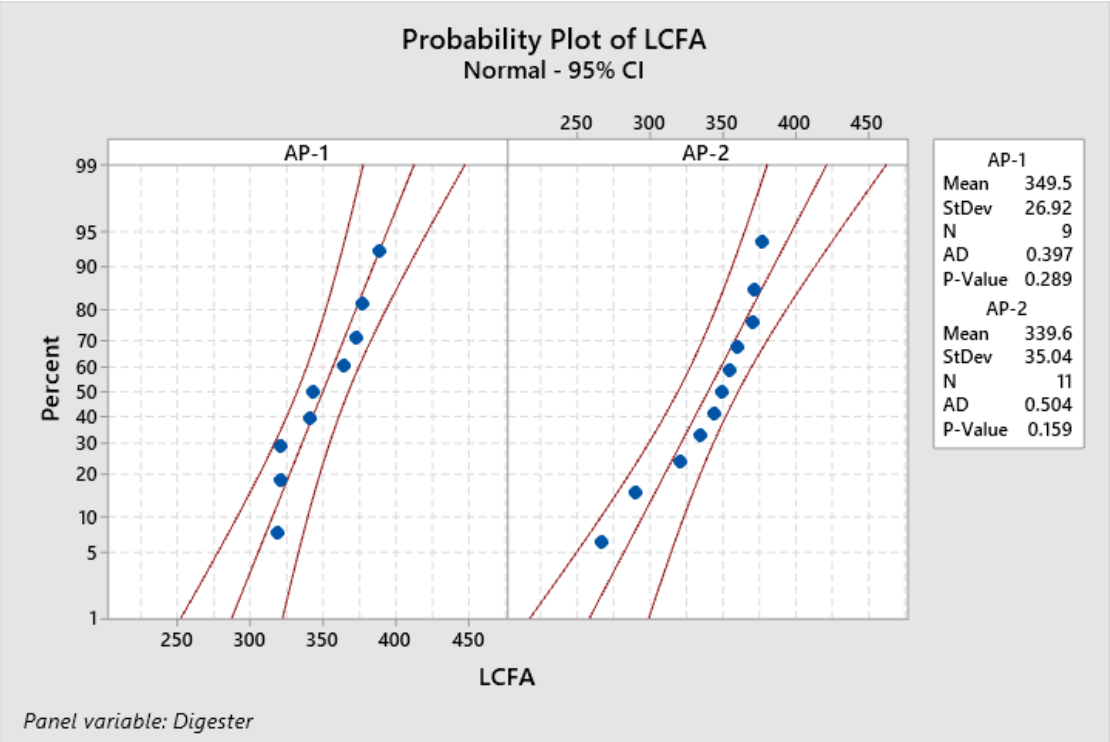
B.4: The representative Anderson-Darling test normality plot for fecal coliform concentration of digestates from single stage digesters at different FOG/Sludge ratios



B.5: The representative Anderson-Darling test normality plot for dewaterability rate of digestates from single stage digesters at different FOG/Sludge ratios



B.6: The representative Anderson-Darling test normality plot for LCFA concentration of acid phase digesters in TPAD systems



Appendix C: Biochar characteristics details

The data presented below were obtained and provided as supplementary information by Caroline Cimon, an M.A.Sc. student at UBC's Bioreactor Technology Group.

C.1: Biochar characteristics

Parameter	Average	Standard deviation
Ash content (%)	11.38	0.20
Moisture (%)	11.5	0.2
pH	8.1	0.1
Zeta potential (mV)	-26.23	1.34
Density (g/ml) (at 23°C)	1.60	0.13
Electrical conductivity (µS/cm) (powdered)	273.6	1.2
Electrical conductivity (µS/cm) (granular)	133.4	0.1

C.2: Biochar composition

Contents	Average	Standard deviation
C (%)	88.03	0.93
O (%)	11.00	0.77
Ca (%)	0.34	0.17
K (%)	0.28	0.05
Mg (%)	0.12	0.12