Thermophilic and Hyper-thermophilic Anaerobic Co-digestion of Thickened Waste Activated Sludge and Fat, Oil, and Grease

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ABSTRACT

In this thesis, the anaerobic co-digestion of thickened waste activated sludge (TWAS) and, fat, oil and grease (FOG) was investigated as a method for TWAS:FOG treatment, stabilization, reduction and conversion to bio-methane gas as a valuable source of renewable energy.

In the first phase, thermophilic and hyper-thermophilic anaerobic co-digestion of TWAS and FOG were investigated and compared. 20 – 80%FOG (based on total volatile solids) were tested using two sets of biochemical methane potential assays (BMP). Hyper-thermophilic co-digestion of TWAS with up to 60%FOG was shown to significantly increase the methane production and VS reduction as compared to the thermophilic co-digestion of the same TWAS:FOG mixture and as compared to the control (TWAS thermophilic mono-digestion). Both linear and non-linear regression models were used to represent the co-digestion results.

In the second phase, the feasibility of the thermophilic and hyper-thermophilic co-digestion of TWAS and FOG were more investigated using lab scale semi-continuous reactors. The dual-stage hyper-thermophilic reactor was introduced for the first time in this work for co-digesting TWAS and FOG. The dual stage co-digestion reactor was shown to significantly outperform the single-stage thermophilic mono-digestion reactor (the control) and the single-stage thermophilic co-digestion reactor at all three hydraulic retention times (HRTs) considered in the study namely, 15, 12 and 9 days. The dual-stage hyper-thermophilic co-digester digested up to 70%FOG at 15 days HRT without any stressing signs and produced a methane yield that was 148.2% higher compared to the control methane yield at the same HRT. It also produced a class A effluent at all three tested HRTs and positive net energy for 15 and 12 days HRT.

The effects of microwave (MW) pretreatment, and combined alkaline-MW pretreatment on the co-digestion of TWAS:FOG mixtures with 20, 40 and 60% FOG were investigated in the third phase of this study. MW pretreatment at a high temperature of 175°C was shown to be the most effective MW pretreatment option in solubilizing TWAS:FOG mixtures and in boosting the methane yield. It resulted in maximum solubilization for the 20%FOG samples and maximum methane yield for samples with 60%FOG. The combined alkaline-MW (NaOH-MW) pretreatment at a pH 10 showed to be an ineffective option for TWAS:FOG pretreatment before the anaerobic co-digestion process.

In the fourth phase, the effects of the three selected pretreatments on the solubilization of TWAS and 20%FOG mixture on the molecular scale were investigated. The pretreatments used included: (i) MW pretreatment at 175°C (since this was the best MW pretreatment condition according to the results of phase 3), (ii) Hyper-thermophilic stage @ 70°C and 2days HRT (effectively used in phases 1 and 2), and (iii) conventional heat at 70°C. The analysis involved separation of the solubilized substrates after pretreatment using ultrafiltration (UF) at four different sizes (1, 10, 100 and 300 kDa). The results showed that each pretreatment method uniquely changed the particle size distribution. These changes showed to affect the biodegradability of substrates with different class size.

Finally, two brief studies were performed using BMP tests to investigate the feasibility of FOG addition as a biogas booster in TWAS anaerobic digestion. First, the effect of FOG addition on TWAS and organic fraction of municipal solid waste (OFMSW) co-digestion was tested using hyper-thermophilic BMP tests. The addition of 30% FOG (based on total volatile solids) was

shown very effective in improving the methane yield. The 30% FOG addition to TWAS:OFMSW mixture resulted in 59.9 and 84.4% higher methane yield compared to the methane yields of TWAS:OFMSW and TWAS samples, respectively. Second, the feasibility of using the soluble part of FOG (L-FOG) as a co-digestion substrate to increase the biogas production from the thermophilic digestion of TWAS was investigated. The results showed that co-digestion of TWAS and 20 to 80% (based on total VS) of L-FOG using a substrate to inoculum ratio (S/I) of 1 improved the biogas yield by 13.5 to 83.0%, respectively. No inhibition was reported at high L-FOG %.

Co-Authorship

The work presented in this thesis starting from Chapter 2 through Chapter 5 are articles that have been published or accepted in peer reviewed scientific journals. Chapter 6 represents two conference papers that have been accepted and presented at two international conferences. All the work presented was carried out by Alqaralleh Rania, with the assistance of the indicated co-authors who provided supervision, suggestions, valuable comments and necessary revisions.

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My sweethearts Lina and Hammudi who add the happiness and fun to my everyday life, and who elevated the challenge of this journey and made it unforgettable!

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CHAPTER 1

1.1 Introduction

With the continuous growth of the world's population and the sharp increase in the demand for energy, more attention has been given to renewable and sustainable energy as an alternative to fossil fuels. Renewable energy promises to become one of the main power sources of the future. A relatively new concept for an old process for alternative energy is the biogas produced from anaerobic digestion (AD) of organic wastes or energy crops. Biogas produced from the AD process contains mainly methane gas which is considered an economically and environmentally friendly biofuel. Methane gas can be used directly to produce heat and electricity, or it can be injected into the natural gas distribution network.

Wastewater treatment plants (WWTPs) have a crucial role in decreasing the impact of anthropogenic wastewater production. Sewage sludge is the main by-product of the physical, chemical and biological processes implemented during secondary wastewater treatment (Martinez et al., 2012). The amount of sewage sludge production has increased dramatically due to the increase in urbanization and the percentage of households connected to municipal WWTPs (Coelho et al., 2011). At present, approximately 6.2 million dry metric tons of sludge is produced in US every year (Kargbo, 2010), about 10.9 million dry metric tons yearly in EU, and more than 660 000 dry metric tonnes of sludge every year from Canadian wastewater treatment facilities (Canadian Council, 2012). Therefore sewage sludge management and disposal has become a critical challenge especially with the diminishing space available for disposal in landfills, increased environmental awareness and more stringent environmental standards for sewage sludge disposal via land application (Jang et al., 2013).

AD has been widely used to treat sludge and different types of organic wastes. AD has many advantages such as providing organic mass stabilization and volume reduction, reducing pathogens and odor problems, small space requirements compared to other treatment options in addition to the production of methane gas that can be used as a potential source of energy. These advantages put AD treatment of organic wastes at an advanced place among other feasible treatment methods for sludge/waste disposal and bioenergy production (Braguglia et al., 2015; Martinez et al., 2012).

Sludge, particularly waste activated sludge (WAS) has low biodegradability which makes it hard to digest with volatile solids reduction in the range of 35-45% (Coelho et al., 2011). Waste activated sludge (WAS) is the excess portion of sludge resulted from the activated sludge process, WAS is removed from the treatment process in order to keep the ratio of biomass to food supplied (sewage or wastewater) in balance (Droste, 1996). Anaerobic co-digestion of the thickened form of WAS (TWAS) by adding a co-substrate has been proven to be useful for enhancing the AD process as it improves biogas production and concomitant organic matter degradation. Adding a co-substrate to TWAS prior to AD process offers some additional potential benefits for both substrates. This includes dilution of inhibitory compounds, increase buffer capacity, more balanced carbon to nitrogen ratio and access to essential micro and macro nutrients (Li et al., 2013).

Lipid-rich wastes usually known as fat, oil, and grease (FOG) are an attractive co-substrate to be used in anaerobic co-digestion of TWAS due to its high methane potential. The theoretical methane potential of lipids is 1014 L/kg volatile solids (VS) compared to 370L/kg for carbohydrates and 740L/kg for proteins (Mata-Alvarez et al., 2014; Wan et al., 2011). Several studies reported the potentially beneficial effects of anaerobic co-digestion of sewage sludge and FOG (Wan et al., 2011; Luostarinen et al., 2009). However, using FOG for AD co-digestion is considered to be challenging due to its inhibitory effects on the anaerobic microbial consortia, particularly methanogens. Using FOG for AD co-digestion may also create operational problems related to clogging, scum formulation and sludge flotation as a result of the adsorption of FOG on the biomass surface (Wan et al., 2011; Kim et al., 2004). Therefore, determining the appropriate percentage of FOG, as part TWAS-FOG mixture, in order to reap all its advantages while avoiding any adverse effects on the AD process is a crucial step before successfully apply the TWAS-FOG co-digestion.

AD processes are usually classified as a mesophilic AD (temperature 35^{o}C) or thermophilic AD (temperature 55^{o}C), but there is a relatively new trend, which involves operating the AD process at higher temperatures in the range of 70-80^{o}C. This is referred to as hyper-thermophilic AD. Recently, various research studies have reported the advantages of using hyper-thermophilic AD for increasing H_2 production, increasing the degradation of polylactide with organic waste, and improving stabilization for co-digestion of kitchen waste and sludge (Assawamongkholsiri et al., 2013; Cappelletti et al., 2012;Wang et al., 2012; Lee et al., 2008; Hartmann & Ahring, 2005).

Due to the above-mentioned advantages for the use of FOG, as a co-substrate, and for using the hyper-thermophilic AD, which may lead to a significant boosting of biogas production from TWAS digestion, this study focuses on investigating the anaerobic co-digestion of TWAS-FOG mixtures using thermophilic and hyper-thermophilic anaerobic digestion. The study also investigates the effect of different factors on the anaerobic co-digestion of TWAS:FOG as described in the hypothesis and objectives.

1.2 Hypothesis

This thesis proposes the use of FOG, as a potential co-substrate with TWAS, in combination with the usage of a dual-stage hyper-thermophilic reactor as a new approach that will enhance the AD process of TWAS and increase the biogas production and methane yield per unit mass/volume of TWAS treated. The general hypothesis of this study is:

- Co-digestion of TWAS using a co-substrate creates a more balanced mixture resulting in a better overall digestion process. FOG is the co-substrate suggested due to its high methane potential. Finding the correct balance between TWAS and FOG in the co-digestion mixture will increase biogas production.

- Incorporating a hyper-thermophilic stage ($70^{o}C$) before the traditional thermophilic anaerobic digestion will help in solubilizing the TWAS-FOG co-digestion mixture. This will increase substrate bioavailability and hence it will improve the anaerobic co-digestion process and biogas production.

- Microwave (MW), and combined alkaline-MW pre-treatments of TWAS-FOG mixture may further increase the solubility of the co-digestion mixture leading to a significant enhancement of anaerobic co-digestion of TWAS-FOG with and without incorporating the hyper-thermophilic stage.

1.3 Research Objectives

The specific objectives of this research can be summarized as follows:

- Study the effects of using FOG at different percentages as a potential co-substrate for TWAS co-digestion. Determine the optimum FOG percentage range to be used in the digestion mixture (best waste stabilizing and maximum biogas production) through a series of BMP tests and non-linear regression models.

- Investigate the effects of using hyper-thermophilic digestion step at 70^{o}C prior to the thermophilic co-digestion of TWAS-FOG on improving the co-digestion process and biogas production through a series of dual stage BMP tests.

- Operate a bench-scale semi-continuous flow reactors for a single stage thermophilic and a dual stage hyper-thermophilic anaerobic co-digestion of TWAS and FOG, and study the effects of different operational conditions such as FOG% and HRT on the anaerobic co-digestion process performance.

- Investigate the effects of MW, and alkaline-MW pre-treatments on TWAS:FOG mixture solubility and determining the optimum range of operational conditions for solubilizing the co-digestion mixture and maximizing the biogas production from the co-digestion process.

- Determine the effects of different pre-treatment methods (MW, hyper-thermophilic step, and conventional heat) on the distribution and digestion of the soluble organic matter from an ideal TWAS-FOG mixture.
- Investigate the viability of the hyper-thermophilic digestion for TWAS co-digestion with other solid organic wastes like Organic Fraction of Municipal Solid Waste (OFMSW).
- Examine the effect of using only the liquid part of FOG (L-FOG) as a co-substrate for boosting the biogas production from TWAS anaerobic co-digestion.

1.4 Thesis organization

This thesis contains seven chapters, and it is organized in a paper format. Chapter 1 is a general introduction that contains thesis background information, hypothesis, objectives, and organization. Chapters 2 to 6 include four separate manuscripts that have been already published or will be submitted soon for publication in peer-reviewed scientific journals, in addition to two conference papers. Chapters 2 through 6 are organized as follows:

- **CHAPTER 2**

Thermophilic and hyper-thermophilic co-digestion of waste activated sludge and fat, oil and grease: Evaluation and modeling methane production.

Rania M. Alqaralleh, Kevin Kennedy, Robert Delatolla, and Majid Sartaj

Manuscript published in *Journal of Environmental Management,* **183** (2016): 551-561, Elsevier.

- **CHAPTER 3**

Improving Biogas Production form Anaerobic Co-digestion of Thickened Waste Activated Sludge and Fat, Oil and Grease (FOG) Using a Dual-stage Hyper-thermophilic/ Thermophilic Semi-Continuous Reactor.

Rania M. Alqaralleh, Kevin Kennedy, and Robert Delatolla

Manuscript published in *Journal of Environmental Management,* **217** (2018): 416-428, Elsevier.

- **CHAPTER 4**

Microwave vs. Alkaline-Microwave Pretreatment for Enhancing Thickened Waste Activated Sludge and Fat, Oil, and Grease Solubilization, Degradation and Biogas Production.

Rania M. Alqaralleh, Kevin Kennedy, and Robert Delatolla

The manuscript was submitted to *Environmental Management Journal,* Elsevier.

- **CHAPTER 5**

Microwave, Hyper-thermophilic, and Conventional Heat Pretreatment of Thickened Waste Activated Sludge and Fat, Oil, and Grease mixture: A Comparison of Molecular Weight Distribution and Digestion of The Soluble Organic Matter.

Rania M. Alqaralleh, Kevin Kennedy, and Robert Delatolla

The manuscript was submitted to *Waste Management Journal,* Elsevier.

- **CHAPTER 6**

This chapter contains two brief studies that were performed using BMP tests to investigate the feasibility of FOG addition as a biogas booster in TWAS anaerobic digestion. Those two studies were represented as conference papers and include:

[1] **Thermophilic Anaerobic Co-digestion of Thickened Waste Activated Sludge and the Liquid Part of Fats, Oil and Grease.**

Alqaralleh, R., Kennedy, K. and Delatolla, R.

The 5th ICCE International Conference and Exhibition on Clean Energy, Montreal, ON, Canada, (Aug 22-24, 2016).

[2] **Biogas Recovery from Hyper-thermophilic Anaerobic Co-digestion of Thickened Waste Activated Sludge, Organic Fraction of Municipal Solid Waste and Fat, Oil and Grease**.

Alqaralleh, R., Kennedy, K. and Delatolla, R.

The International Conference on Renewable Energy and Resources, Vancouver, BC, Canada, (July 24-25, 2017).

- **CHAPTER 7**

Chapter 7 includes synthesis, integration and general discussion of all the research parts that have been done for this thesis.

- **CHAPTER 8**

Finally, chapter 8 contains conclusions from the entire thesis and proposes recommendations for future work.

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Abbreviations

AD	Anaerobic digestion
ANOVA	Analysis of variance
BMP	Biochemical methane potential
C/N	Carbon to nitrogen ratio
COD	Chemical oxygen demand
EPS	Extracellular Polymeric Substrates
FOG	Fat, oil and grease
HRT	Hydraulic retention time
LCFAs	Long chain fatty acids
MW	Microwave
Mw	Molecular weight
MwD	Molecular weight distribution
OFMSW	Organic fraction of municipal solid waste
OLR	Organic loading rate
PS	Primary sludge
ROPEC	Robert O. Pickard Environmental Centre
SCOD	Soluble chemical oxygen demand
S/I	Substrate to inoculum ratio
SRT	Solids retention time
SS	Suspended solids
TCOD	Total COD

TS	Total solids
TWAS	Thickened WAS
UF	Ultra-filtration
US	Ultrasound
TVFA	Total volatile fatty acids
VS	Volatile solids
VSS	Volatile suspended solids
WAS	Waste activate sludge
WWTPs	Wastewater treatment plants

CHAPTER 2

Thermophilic and Hyper-thermophilic Co-digestion of Waste Activated Sludge and Fat, Oil and Grease: Evaluating and Modeling Methane Production

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2.1 Abstract

Renewable energy and a clean environment are two crucial requirements for our modern world. Low cost, energy production, and limited environmental impact make anaerobic digestion (AD) a promising technology for stabilizing organic waste and in particular, sewage waste. The anaerobic co-digestion of thickened waste activated sludge (TWAS) and sewage treatment plant trapped fat, oil and grease (FOG) using different FOG-TWAS mixtures (20, 40, 60 and 80 % of FOG based on total volatile solids (TVS)) were investigated in this study using both thermophilic (55±1^{o}C) and two stages hyper-thermophilic/thermophilic (70±1^{o}C and 55±1^{o}C) anaerobic co-digestion . In the current study the hyper-thermophilic co-digestion approach as a part of the co-digestion process has been shown to be very useful in improving the methane production. During hyper-thermophilic biochemical methane potential (BMP) assay testing the sample with 60% FOG (based on TVS) has been shown to significantly increase

the maximum methane production to 673.1±14.0 ml of methane as compared to 316.4±14.3 ml of methane for the control sample. This represents a 112.7% increase in methane production compared to the control sample considered in this paper. These results signify the importance of hyper-thermophilic digestion to the co-digestion of TWAS-FOG field.

2.2 Introduction

With the continuous growth of the world's population and the sharp increase in the demand for energy sources, more attention has been given to renewable and sustainable energy as an alternative to fossil fuel. Renewable energy promises to become one of the main power sources of the future (Zhou et al., 2012). Biogas produced from the anaerobic digestion (AD) of organic wastes contains mainly methane gas which is considered an economical and environmentally friendly biofuel. Methane gas can be used directly to produce heat and electricity, or it can be injected into the natural gas distribution network (Alqaralleh et al., 2015).

Due to the continuous increase in population and urbanization, wastewater treatment plants (WWTPs) have a crucial role in decreasing the impact of anthropogenic wastewater production (Coelho et al., 2011; Martín-González et al., 2011). Sewage sludge is the main by-product of the physical, chemical and biological processes implemented during secondary wastewater treatment (Martinez et al., 2012). The quantity of sewage sludge production across the world has increased dramatically due to the increase in the percentage of households connected to municipal WWTPs (Coelho et al., 2011). At present approximately 6.2 million dry metric tons of sludge is produced in the US every year (Kargbo, 2010), about 10.9 million dry metric tons wastewater treatment facilities (Canadian Council, 2012). Therefore, sewage sludge management and disposal have become a critical challenge especially with the diminishing space available for disposal in landfills, increased environmental awareness and more stringent environmental standards for sewage sludge disposal via land application (Jang et al., 2013).

AD has widely been used to treat sludge and different types of organic wastes (Neves et al., 2009; Nayono et al., 2010; Liu et al., 2012; Wang et al., 2014). AD has many advantages such as providing organic mass stabilization and volume reduction, reducing pathogens and odor problems, small space requirements compared to other treatment options (such as landfilling and composting) in addition to the production of methane gas that can be used as an energy source. These advantages make AD treatment of solid organic wastes an attractive trend for sludge/waste disposal and bioenergy production (Martinez et al., 2012).

Sludge, particularly waste activated sludge (WAS) has a relatively low biodegradability which makes it hard to digest with limited volatile solids reduction in the single mesophilic anaerobic digestion (Coelho et al., 2011; Mann et al., 2013). Anaerobic co-digestion by adding a co-substrate to sludge prior to AD offers many potential benefits for both substrates such as dilution of inhibitory compounds, increase buffer capacity, more balanced carbon to nitrogen ratio and access to essential micro and macro nutrients (Li et al., 2013; Ara et al., 2015). These benefits provided by co-digestion were demonstrated to increase biogas production and concomitant organic matter degradation which can be considered responsible for increasing the economic feasibility of waste to energy treatment plants (Martinez et al., 2012). Furthermore, co-digestion has been shown to provide higher efficiency of land and equipment utilization by digesting different wastes in the same facility (Ho et al., 2010; Hoseini et al., 2014).

Fat, oil and grease (FOG) is a term commonly used to define the floating layer of lipid-rich waste generated from restaurants, slaughterhouse wastewater, dairy industry and various food processing industries. FOG can be categorized as yellow grease such as waste cooking oil and brown grease also known as trapped grease as it contains yellow grease plus food solids and water (Long et al., 2012). In the past, FOG and greasy wastes were mainly landfilled or land applied but due to increasingly strict environmental legislations in many European and North American municipalities, these types of disposal methods are no longer feasible. As such, other disposal options for collected FOG have recently emerged including composting, incineration and anaerobic co-digestion (Long et al., 2012).

FOG is an attractive co-substrate to be used in anaerobic co-digestion of sludge due to its high methane potential. The theoretical methane potential of lipids is 1014 L/kg volatile solids (VS) compared to 370L/kg VS for carbohydrates and 740L/kg VS for proteins (Wan et al., 2011). Several studies reported the beneficial effect of anaerobic co-digestion of sewage sludge and FOG (Luostarinen et al., 2009; Mata-Alvarez et al., 2014). However using FOG for anaerobic co-digestion is considered to be challenging due to its inhibitory effects on the anaerobic microbial consortia particularly methanogens mainly due to the high long-chain fatty acids (LCFA) content, as well as operational problems related to clogging, scum formulation and sludge flotation as a result of the adsorption of FOG around the biomass surface (Kim et al., 2004; Long et al., 2012).

AD processes are usually classified as a mesophilic AD (temperature 35°C) or thermophilic AD (temperature 55°C), but there is a relatively new trend which involves operating the AD process at a higher temperature range of 65-80°C, which is referred to as hyper-thermophilic AD. Recently various research studies reported the benefits of using hyper-thermophilic AD for increased H_2 production, increased degradation of polylactide with organic waste, and improved stabilization for co-digestion of kitchen waste and sludge (Lee et al., 2009; Cappelletti et al., 2012; Wang et al., 2012; Assawamongkholsiri et al., 2013).

The use of FOG as a co-substrate is not new in literature. However, there is a broad need for more research and published data from lab, pilot and full-scale anaerobic co-digestion using FOG in order to establish a comprehensive database that covers the major important conditions related to FOG co-digestion (Long et al., 2012). FOG co-digestion is often studied under mesophilic conditions as mesophilic digestion reduces expenses associated with heating (Li et al., 2012; Mata-Alvarez et al., 2014). Few studies used thermophilic instead of mesophilic co-digestion of FOG proved the advantage of thermophilic temperature on biogas production (Martín-González et al. 2011). However, thermophilic co-digestion of FOG and TWAS still requires further research especially in determining the optimum FOG% in the thermophilic anaerobic co-digestion mixture to reach the ideal co-digestion conditions that maximize methane production. On the other hand, to the best of our knowledge hyper-thermophilic stage has not yet been applied for TWAS-FOG anaerobic co-digestion. And regression has rarely been used to mathematically explain the experimental anaerobic co-digestion observations (Li et al., 2013; Mata-Alvarez et al., 2014).

Therefore, the main objectives of this study are:

- Introducing the innovative two-stage hyper-thermophilic co-digestion of TWAS and FOG at different FOG%.
- Investigate the effects of different FOG% (based on TVS) on the methane production and the volatile solids reduction during the anaerobic co-digestion process.
- Develope linear and non-linear regression models using the cumulative methane production results from the different co-digestion conditions to help better understanding, comparing and interpreting the co-digestion results.

2.3 Materials and Methods

2.3.1 Substrates and inoculum

Thickened waste activated sludge (TWAS) was obtained from the thickener centrifuge at the Robert O. Pickard Environmental Center (ROPEC), Ottawa, ON, Canada and contained 4.9±0.2% TS of which 72±1% were volatile solids. FOG samples were provided from the Organic Resources Management Inc. (ORMI), Ottawa, ON, Canada. ORMI provides grease trap cleaning services for different wastewater treatment plants in Ontario. Both substrates (TWAS and FOG) were stored at 4^{o}C prior to use. The thermophilic anaerobic inoculum (55^{o}C) was obtained from the effluent of a 10L thermophilic anaerobic digester acclimated to TWAS and operated at a 20 days hydraulic retention time (HRT) in our research lab. Hyper-thermophilic anaerobic inoculum (70^{o}C) used was the effluent from a 2L anaerobic digester acclimated to TWAS and operated for about 6 months at HRT of 2 days under hyper-thermophilic conditions (70^{o}C).

The characteristics of substrates and inoculums used in this study are shown in Table 2.1.

Table 2.1: Characteristics of substrates and inoculums.

Parameter	TWAS	FOG	Thermophilic inoculum	Hyper-thermophilic inoculum
pH	6.8±0.1	4.1±0.1	7.4±0.1	6.7± 0.1
TS (g/Kg)	49.2±2.0	290.1±1.1	29.8±0.8	34.6±0.7
VS (g/Kg)	35.4±1.9	282.8± 1.0	14.7±0.7	18.7±0.2
VS/TS	0.72±0.01	0.97±0.00	0.49±0.01	0.54±0.01

Data represents the arithmetic mean ± standard deviation. (n=4).

2.3.2 Biochemical methane potential assays (BMP)

This study included two BMP tests that were run simultaneously using the same sample mixtures (TWAS and FOG) to assess the effect of FOG% in the digestion mixture on the anaerobic co-digestion process and methane production. FOG% (based on TVS) was used, where 0, 20, 40, 60 and 80% were considered. A substrate to inoculum (S/I) ratio of 0.6 ($gTVS_{substrate}/gTVS_{inoculum}$) was used for all the serum bottles of both BMP tests. This S/I ratio was selected to be within the optimum range suggested by Li et al. (2011) for the anaerobic co-digestion using FOG as a co-substrate.

The first BMP is a standard single stage thermophilic BMP (55^{o}C) assay whereas the second is two stages hyper-thermophilic (70^{o}C)/ thermophilic (55^{o}C) BMP assay. To mitigate the pH effects, supplemental alkalinity was added to all the 250 ml BMP serum bottles, which are containing the digestion mixture (TWAS, FOG, and inoculum) using equal amounts of $NaHCO_3$ and $KHCO_3$ to provide approximately 4000-5000 mg/L alkalinity as $CaCO_3$. The pH of all BMP bottles was within the ideal range for anaerobic digestion system (6.5-7.5) (Droste, 1996). Bottles were next purged with N_2 gas for 3-5 minutes to remove oxygen gas from the headspace and maintain anaerobic conditions. Finally after sealing with butyl rubber stoppers and screw caps, bottles were kept in a temperature-controlled incubator shaker (New Brunswick Scientific Co. Inc., NB, Canada) at 55±1^{o}C and 100 rpm for the thermophilic BMP assay and in a 70±1^{o}C with 100 rpm incubator for 2 days then moved to the 55±1^{o}C with 100 rpm for the remainder of the BMP test period for hyper-thermophilic BMP assay. The temporal biogas production was measured using a gas manometer. The net biogas volumes for samples were calculated by subtracting the biogas produced in the inoculum bottles from the biogas produced in each of the samples bottles (sample + inoculum) then the net biogas volumes were corrected to standard ambient temperature and pressure (STP) conditions (25^{o}C and 1 atm). The composition of substrates in each BMP bottle is presented in Table 2.2 where: samples 00T, 20T, 40T, 60T, and 80T represent the thermophilic samples with 0, 20. 40, 60 and 80% FOG content, respectively. Whereas samples 00H, 20H, 40H, 60H, and 80H represent the hyper-thermophilic samples with 0, 20. 40, 60 and 80% FOG, respectively.

Table 2.2: Inoculum, FOG and TWAS composition in both BMP co-digestion assays.

Sample	FOG	FOG	TWAS	Inoculum 55°C	Inoculum 70°C	S/I	Initial VS
	(%)[a]	(g)	(ml)	(ml)	(ml)	ratio[b]	(g)
Thermophilic							
00T	0.0	0.0	46.9	112.4	0.0	0.6	2.6
20T	20.0	0.7	37.5	112.4	0.0	0.6	2.6
40T	40.0	1.4	28.1	112.4	0.0	0.6	2.6
60T	60.0	2.1	18.8	112.4	0.0	0.6	2.6
80T	80.0	2.8	9.4	112.4	0.0	0.6	2.6
Inoculum 55	0.0	0.0	0.0	112.4	0.0	-	1.6
Hyper-thermophilic							
00H	0.0	0.0	46.9	74.9	27.2	0.6	2.6
20H	20.0	0.7	37.5	74.9	27.2	0.6	2.6
40H	40.0	1.4	28.1	74.9	27.2	0.6	2.6
60H	60.0	2.1	18.8	74.9	27.2	0.6	2.6
80H	80.0	2.8	9.4	74.9	27.2	0.6	2.6
Inoculum 70	0.0	0.0	0.0	74.9	27.2	-	0.5

[a] FOG% (the percentage of FOG in the co-digestion mixture based on TVS). [b] The S/I ratio is based on TVS.

2.3.3 Analytical methods

The analysis in the BMP assays was done in triplicate, using standard methods (APHA, 1998). Total and volatile solids were determined according to standard method 2540G. Alkalinity analysis was done based on standard method 2320B using a Fisher Accument® XL25 pH meter. Chemical oxygen demand and soluble chemical oxygen demand (COD and SCOD) were measured using HACH TNT 822 (20-1500 mg/L) and TNT 823 (250-15000 mg/L) including spectrophotometer, heating block, and supplies. Biogas was measured using a manometer every 24 hours for the first 9 days of the experiment, and it was measured occasionally after the biogas production slowed down until the biogas production became negligible (after 60 days). The volume of the biogas was corrected to the standard ambient temperature and pressure conditions (STP, 25°C and 1 atm). The biogas composition was monitored weekly using a Hewlett Packard 5710A gas chromatograph equipped with a 3380A model integrator, a 5705A thermal conductivity detector and using Helium as the carrier gas.

2.3.4 Data analysis

For each of the BMP testing conditions, two non-linear empirical models were used to estimate three anaerobic digestion performance parameters including the lag phase duration, the ultimate methane production, and the maximum methane production rate.

The first model used is the modified Gompertz (MG) equation (Equation 2.1) (Donoso-Bravo et al., 2010; Lo et al., 2010).

$$B = B_0 \,.\, \exp\left(-\exp\left(\frac{Rm\,.e}{B_0}(\lambda - t) + 1\right)\right) \tag{2.1}$$

Where: B_0 is the ultimate methane yield (mL/g TVS), B is the cumulative methane yield (mL/g TVS), t is incubation time (h), R_m is the maximum methane production rate (mL/g TVS.h), and λ is the lag phase duration time (h).

The transference function (reaction curve model) was also used to fit our data in this study. The reaction curve model (RC) represented by Equation 2.2 has been used successfully in the literature for anaerobic digestion evaluation (Donoso-Bravo et al., 2010).

$$B = B_0\left(1 - \exp\left(-\frac{Rm(t - \lambda)}{B_0}\right)\right) \tag{2.2}$$

Where: B_0, B, t, R_m, and λ have the same definition as defined in the modified Gompertz equation above.

Finally, the first-order equation (Equation 2.3) was used to estimate the apparent hydrolysis rate coefficient (first-order methane production rate) (*k*, h^{-1}) for each case (Budiyono et al., 2014; Donoso-Bravo et al., 2010).

$$B = B_0\,(1 - e^{-kt}) \tag{2.3}$$

Where k is the first-order methane production rate (h^{-1}), B_0, B and t have the same definition as defined in the modified Gompertz equation.

Statistical analysis of the collected data includes t-test (*p*-value = 0.05), one-way analysis of variance (ANOVA), Pearson coefficient of determination (R^2) and degree of freedom.

2.4 Results and Discussion

2.4.1 Biogas production and effect of FOG% (BMP assays)

The net cumulative methane production obtained during the thermophilic and hyper-thermophilic BMP assays are represented in Figure 2.1 (a) and (b) respectively. Additional performance parameters of both BMP assays are presented in Table 2.3.

The experimental results show that all samples with different FOG% (based on TVS) in both BMP assays resulted in a significant increase ($p < 0.05$) in methane production compared to the control sample (00T, TWAS mono-digested in the thermophilic BMP assay) except for samples with 80% FOG which showed a significant lower methane production compared to the control sample (00T). In the thermophilic BMP assay, the control sample (00T) produced 316.4±14.3 ml of methane. The addition of 20, 40 and 60% FOG (based on TVS) to the co-digestion mixture significantly improved the methane production to 427.7±8.1 ($p = 0.003$), 451.1±20.2 ($p = 0.001$)

and 491.9±11.3 ($p < 0.001$) ml, respectively. This represents a 35.2, 42.6 and 55.4 % increase in methane production compared to the control, as shown in Figure 2.2. These results agree with Martín-González et al, (2011) study that reported an enhancement of 52% in biogas production and 36% increase in methane yield (L CH_4/g VS_{added}) when sewage treatment plant FOG wastes (STP-FOGW) and source-collected organic fraction of municipal solid waste (SC-OFMSW) with a ratio of 1:6 (VS:VS) were co-digested in a thermophilic semi-continuous reactor (with hydraulic retention time of 14.4 days). Also, Kabouris et al. (2009) reported a 179% improvement in methane yield (551 mL CH_4/gVS_{added} compared to 197 mL CH_4/gVS_{added} for the control) when 48% of dewatered FOG (VS basis) was co-digested with primary and thickened waste activated sludge in thermophilic two-phase CSTR (HRT 12 days).

For the current study, the improvement in methane production compared to control (Figure 2.2) was calculated using (Equation 2.4).

$$Improvement\ \% = \frac{(V_{CH_4})sample - (V_{CH_4})control}{(V_{CH_4})control} \times 100\% \qquad (2.4)$$

Where (V_{CH4}) sample is the net cumulative volume of methane for the sample at the end of the BMP assay, and (V_{CH4})control is the net cumulative volume of methane for the control sample at the end of the BMP assay.

Comparing the results of the cumulative methane production and the improvement in methane production for both thermophilic and hyper-thermophilic BMP assays, which are shown in Figures 2.1 and 2.2, reveals the effect of using the hyper-thermophilic stage (for 2 days) prior to

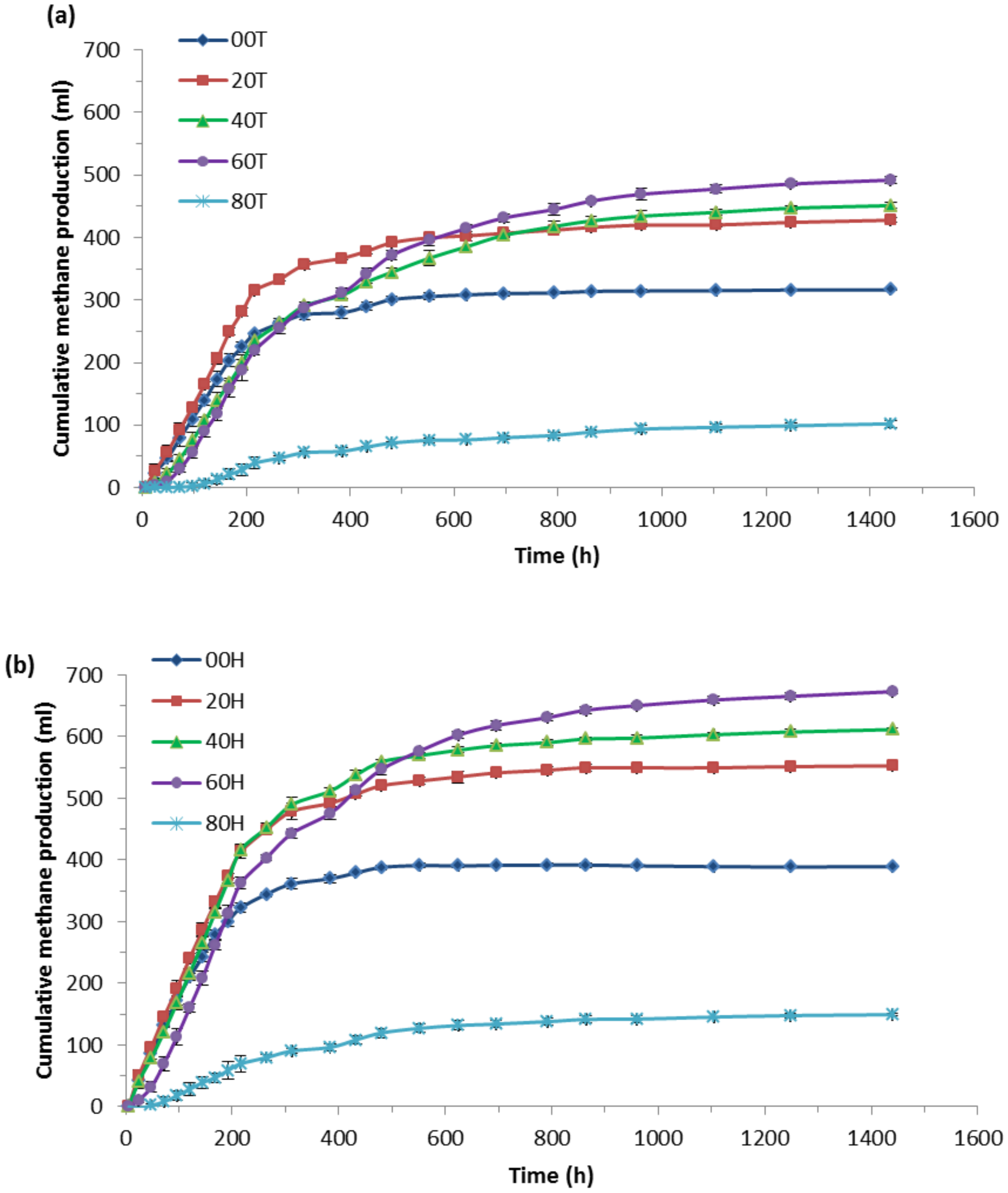


Figure 2.1: Cumulative methane production (STP) during (a) thermophilic (55°C) and (b) hyper-thermophilic (70°C) BMP assays. Data represent arithmetic mean and 95% confidence intervals (n=3).

the thermophilic digestion in increasing the methane production compared to the similar samples digested in the regular thermophilic BMP assay.

For example, sample 00H (TWAS alone/ Hyper-thermophilic BMP assay) produced 23.1% more methane (p = 0.002) compared to the control sample (00T) as shown in Figure 2.2. The highest methane production during the hyper-thermophilic BMP assay was observed for 60% FOG sample (60H) which as shown in Table 2.3 and Figure 2.2 produced 673.1±14.0 ml of methane, and this represents a 112.7% increase in methane production compared to the control sample (00T)($p < 0.001$).

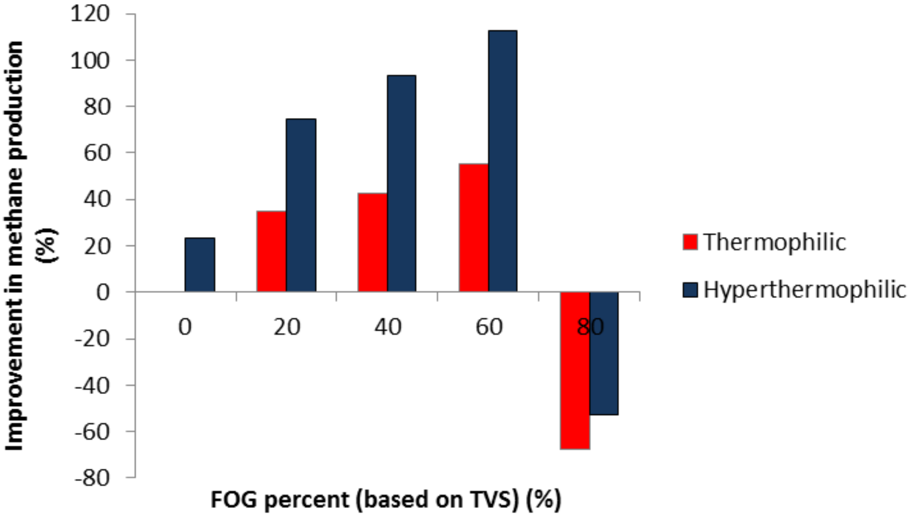


Figure 2.2: Improvement in methane production for thermophilic and hyper-thermophilic BMP assays compared to the control (TWAS only sample 00T).

Table 2.3: Thermophilic and hyper-thermophilic BMP tests results.

Parameter	Thermophilic BMP Test					Hyper-thermophilic BMP Test				
	00T	**20T**	**40T**	**60T**	**80T**	**00H**	**20H**	**40H**	**60H**	**80H**
Cumulative biogas (ml)*	513.7±23.3	655.0±12.3^{b}	685.6±30.6^{b}	744.1±17.2^{c}	205.5±22.3^{-c}	620.3±14.7^{b}	835.1±27.0^{c}	908.8±22.1^{c}	1003.1±20.9^{c}	286.9±25.1^{-c}
Cumulative CH_4 (ml)	316.4±14.3	427.7±8.1^{b}	451.1±20.2^{c}	491.9±11.3^{c}	102.1±11.1^{-c}	389.5±9.2^{b}	552.8±17.9^{c}	611.6±14.9^{c}	673.1±14.0^{c}	149.5±13.1^{-c}
CH_4 (%)	61.6±0.7	65.3±0.6^{c}	65.8±0.7^{c}	66.1±0.9^{c}	49.7±0.6^{-c}	62.8±0.7^{n}	66.2±0.9^{c}	67.3±0.6^{c}	67.1±0.8^{c}	52.1±0.6^{-c}
TS reduction (%)	62.9±0.9	64.0±1.5^{n}	65.5±1.0^{a}	67.2±1.3^{b}	39.3±1.4^{-c}	65.9±1.8^{n}	70.5±2.8^{a}	73.1±0.4^{c}	75.8±1.8^{c}	41.3±1.8^{-c}
VS reduction (%)	70.6±1.4	73.5±1.9^{n}	74.4±1.5^{a}	76.3±1.3^{b}	46.8±1.9^{-c}	76.8±1.9^{a}	78.0±1.4^{b}	79.7±0.7^{b}	82.0±0.9^{c}	49.1±1.6^{-c}
CH_4 Yield (ml/g VS_{added})	316.4±14.3	427.7±8.1^{b}	451.1±20.2^{c}	491.9±11.3^{c}	102.1±11.1^{-c}	389.5±9.2^{b}	552.8±17.9^{c}	611.6±14.9^{c}	673.1±14.0^{c}	149.5±13.1^{-c}
CH_4 Yield (ml/g $VS_{removed}$)	451.5±25.9	587.4±23.5^{b}	613.6±29.4^{b}	652.9±26.0^{b}	221.4±16.7^{-c}	507.4±10.0^{a}	711.2±17.0^{c}	771.5±13.0^{c}	825.9±15.0^{c}	307.2±32.2^{-b}

* Net biogas and methane production in (ml). Data represents the arithmetic mean ± standard deviation of replicate samples. ^{a,b,c} Represent the p-value result from the t-test for each sample compared to the control sample (00T), (^{a} for $p < 0.05$), (^{b} for $p < 0.01$) and (^{c} for $p < 0.001$). Where positive$^{a,b.c}$ represents a statistically significant increase and negative ^{a,b,a} represents a statistically significant decrease compared to control.

However, it is noteworthy that the addition of 80% FOG (based on TVS) to the co-digestion mixture had an inhibitory effect on the co-digestion process in both the thermophilic and the hyper-thermophilic BMP assays. This is shown by the significant lower methane production during the whole anaerobic co-digestion process compared to the other samples with FOG% less than 80%. There is no clear known FOG content threshold determined in the literature since the FOG content that can be used without having adverse effects on the anaerobic co-digestion process depends on many factors like the type of FOG used, the main substrate used in the digestion mixture (TWAS, primary sludge, etc.), type of digestion (mesophilic, thermophilic, hyper-thermophilic), and the type of experiment (batch tests, semi-continuous reactors, and lab scale or pilot scale) (Pastor et al., 2013). But in general, the presence of high FOG content leads to a high long-chain fatty acids concentrations (LCFAs) in the anaerobic digester, and this is believed to cause a potential inhibition on the methanogenic activity that occurs as longer lag phase and a sharp decrease in methane production (Mata-Alvarez et al., 2014). The inhibition of the anaerobic digestion process is mainly suggested to be due the toxicity effect of the high LCFAs concentrations on the anaerobic microorganisms especially the methanogens, since the LCFAs adsorption onto the methanogen cells may cause damages to the cell membrane and inhibit the transport of nutrients phenomena through the cell wall (Noutsopoulos et al., 2013; Martínez et al., 2012). However the exact nature and mechanism of the inhibitory effect of LCFAs on the anaerobic microorganisms and if this effect is reversible or irreversible is not enough clear and well understood yet (Long et al., 2012).

The total volatile solids (TVS) removals at the end of the BMP assays are represented in Table3. The results of the TVS removal (Table 2.3) are consistent with the methane production results. The thermophilic BMP assay sample that was co-digested with 20% FOG exhibited a similar TVS removal efficiency as the control sample with no significant difference ($P = 0.11$). However, as FOG % increased to 40% and 60% of the TVS removal increased significantly compared to the control (00T). Samples 40T and 60T have 5.4% ($p = 0.03$) and 8.0% ($p = 0.006$) higher TVS removal compared to the control (00T), respectively. As expected from the methane production results of sample 80T, there has been a significantly lower TVS removal ($P < 0.001$) compared to control and all other samples in this BMP assay. This can be justified by the inhibition effect of the high FOG concentration on the methanogens activity. On the other hand, and from the TVS removal results of the hyper-thermophilic BMP (Table 2.3), we may conclude that the inclusion of the hyper-thermophilic step, as a part of the BMP assay, has significantly improved ($P < 0.05$) the TVS removal for all samples with FOG% less than 80% (samples with FOG % 0, 20, 40 and 60) compared to the corresponding samples in the thermophilic assay. However, the 80H sample has recorded similar with no significant difference TVS removal as sample 80T ($p = 0.2$). The highest TVS removal in the hyper-thermophilic BMP assay was reached by the 60H sample, which was 82.0±0.9 % TVS removal, to record a 16.1% ($p < 0.001$) increase in TVS removal compared to the control sample (00T). It is worth mentioning here that the maximum TVS removal of 82.0±0.9 % achieved by the hyper-thermophilic digestion for the 60% FOG sample in the current study is significantly higher than the maximum TVS removal reported by Martín-González et al. (2011), where co-digestion of organic fraction of municipal solid wastes with FOG was compared under mesophilic and thermophilic conditions. According to Martín-

González et al. (2011), the thermophilic conditions have increased the efficiency of the co-digestion process and resulted in an improvement of 25% in biogas production, 40% in methane yield and 12% in VS reduction compared to the mesophilic conditions. In their study, they achieved a maximum of 73% TVS reduction when FOG was 48% of the total VS load.

2.4.2 Modelling of the BMP assay results

Linear regression using the first order equation can be used to estimate the hydrolysis rate coefficient k (h^{-1}) of the biogas production during the AD process. However as concluded in some studies that used modelling of biogas production in literature, linear regression cannot be used to accurately describe the full digestion process instead non-linear regression is suggested to accurately describe the AD process, estimate the biogas production and the critical digestion performance parameters of the AD process (Li et al., 2011; Donoso-Bravo et al., 2010). Hence, in this study two non-linear models (MG equation and RC model), equation 2.1 and 2.2 in section 2.3.4, were used for the non-linear regression of the methane production data resulted from the thermophilic and hyper-thermophilic BMP assays. FO equation (equation 2.3 in section 2.3.4) was used for linear regression to estimate the hydrolysis rate coefficient k (h^{-1}) for each sample. Figures 2.3 and 2.4 show the results of the nonlinear regression using MG equation and the RC model for each of the samples in the thermophilic and the hyper-thermophilic BMP assay, respectively. In Figures 2.3 and 2.4 the solid lines represent the model curve (MG equation the red line and RC model the green line), the dotted lines are the confidence intervals and the diamond marks are the experimental data (methane production during the BMP assays). Further results of the three models fitting the experimental methane production data are presented in Table 2.4. From those results (Table 2.4 and Figures 2.3 and

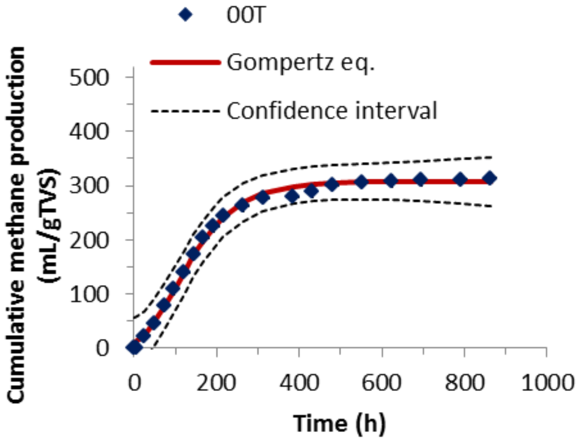
2.4) we can observe an overall agreement between the three models and the experimental data. Both MG equation and RC model had a better fit of the data compared with the FO equation by having higher R^2 values ($R^2 \geq 0.978$). To better compare the two non-linear models (MG equation and RC model), the difference between the experimental and the predicted methane yield (Δ %) was calculated for each case; results are represented in Table 4. Although modelling using MG equation resulted in higher R^2 compared to RC model for seven out of the ten cases (00T, 20T, 80T, 00H, 20H, 40H and 80H), the difference between the experimental and the model methane yield was smaller for the RC model ($0.4 \leq \Delta \% \leq 5.6$) compared to MG equation model ($0.5 \leq \Delta \% \leq 9.0$) in nine out of the ten cases. One then may conclude that RC model is more accurate in representing the methane production for the thermophilic and hyper-thermophilic co-digestion of TWAS-FOG in this study. The RC model was chosen by Donoso-Bravo et al. (2010) too as it was the best model representing their study data (biogas production during anaerobic biodegradability tests fed with primary and secondary pre-treated sludge). In their study, Donoso-Bravo compared three different non-linear models MG equation, RC model and the logistic function model. Donoso-Bravo et al. have drawn some conclusion about the shape of the modeled curves using MG equation, that is, the biogas production at t=0 is not zero. In fact, this does not have any physical meaning simply because when λ is negligible and t=0, the B_0 value in the MG model equation converges to some positive value, hence B_0 can be zero only when there is a significant lag phase (λ). Because of that, the MG equation model is more used for hydrogen production where a significant lag phase occurs. However, in this study MG equation gave satisfactory results in fitting the experimental data and predict the methane production of all the cases during the thermophilic and hyper-thermophilic anaerobic co-digestion (with error < 10%).

As far as the anaerobic co-digestion parameters are concerned, the MG equation generally gives a lower predicted methane yield (B_o, mL/gTVS) compared to the RC model. The actual experimental values for all samples in both BMP assays confirm that (Table 2.4). It can also be concluded, based on the results obtained in this study, that the MG equation gives lower values for the maximum methane production rate (R_m, mL/gTVS.h) and longer lag time (λ, h) compared to the values generated by the RC model.

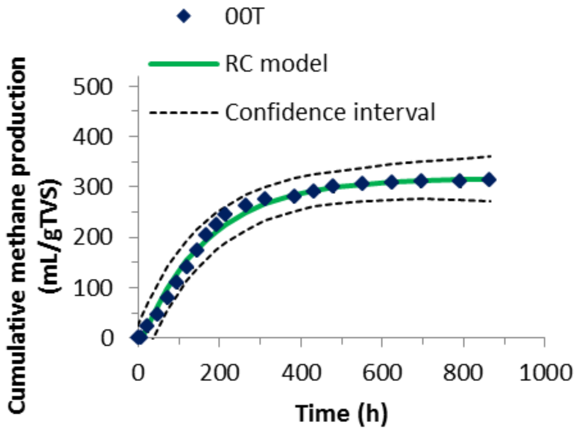
Table 2.4: Parameters estimated from linear and non-linear regression for the BMP co-digestion experiments.

Sample	**Measured CH_4 Yield**[a]	**Gompertz eq.**					**RC model**					**FO eq.**			
	(mL/g VS)	**B_o (mL/gTVS)**	**λ (h)**	**R_m (mL/gVS.h)**	**R^2**	**Δ (%)**	**B_o (mL/gVS)**	**λ (h)**	**R_m (mL/gVS.h)**	**R^2**	**Δ (%)**	**B_o (mL/gVS)**	**K (h^{-1})**	**R^2**	**Δ (%)**
Thermophilic															
00T	316.4	308.2	18.4	1.4	0.996	2.6	317.8	10.4	1.9	0.991	0.4	319.7	5.52 E-3	0.989	1.0
20T	427.7	409.1	20.5	1.7	0.995	4.3	424.4	11.5	2.3	0.990	0.8	427.6	4.91 E-3	0.989	0.0
40T	451.1	427.4	19.3	1.0	0.986	5.2	460.0	18.2	1.4	0.993	2.0	469.6	2.74 E-3	0.992	4.1
60T	491.9	467.4	34.5	1.0	0.990	5.0	511.8	25.8	1.3	0.992	4.0	529.2	2.28 E-3	0.990	7.6
80T	102.1	93.0	68.6	0.2	0.981	9.0	107.9	39.5	0.2	0.978	5.6	115.6	1.68 E-3	0.974	13.2
Hyper-thermophilic															
00H	389.5	387.7	7.7	1.9	0.997	0.5	395.6	6.8	2.8	0.995	1.6	397.0	6.72 E-3	0.995	1.9
20H	552.8	539.7	11.0	2.2	0.996	2.4	555.8	8.4	3.2	0.994	0.5	558.7	5.32 E-3	0.994	1.1
40H	611.6	589.3	17.6	2.1	0.996	3.7	613.4	11.5	2.9	0.993	0.2	618.4	4.35 E-3	0.991	1.1
60H	673.1	640.5	26.5	1.7	0.990	4.8	685.7	20.0	2.3	0.992	1.9	700.0	2.91 E-3	0.990	4.0
80H	149.5	142.7	42.1	0.3	0.991	4.5	155.2	28.5	0.4	0.990	3.8	160.5	2.41 E-3	0.986	7.4

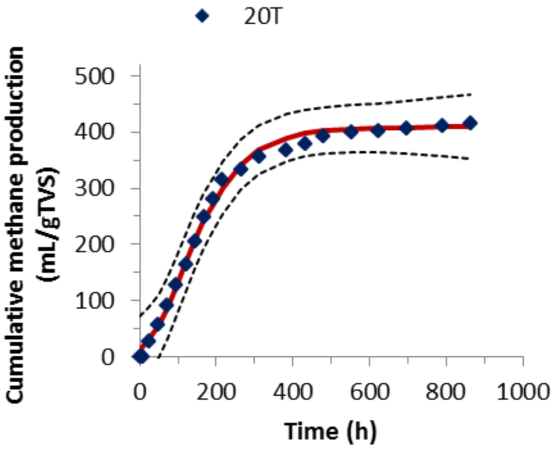
[a] Methane yield calculated from the BMP assays results (ml CH_4/g VS_{added}).


00T
Gompertz eq.
Confidence interval
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
0
200
400
600
800
1000
Time (h)

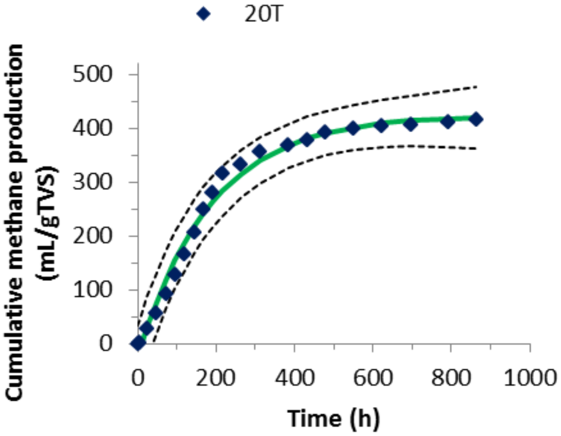
(a)


00T
RC model
Confidence interval
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
0
200
400
600
800
1000
Time (h)

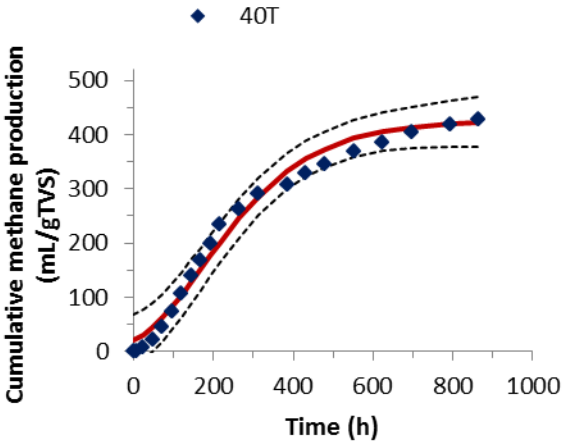
(b)


20T
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
0
200
400
600
800
1000
Time (h)

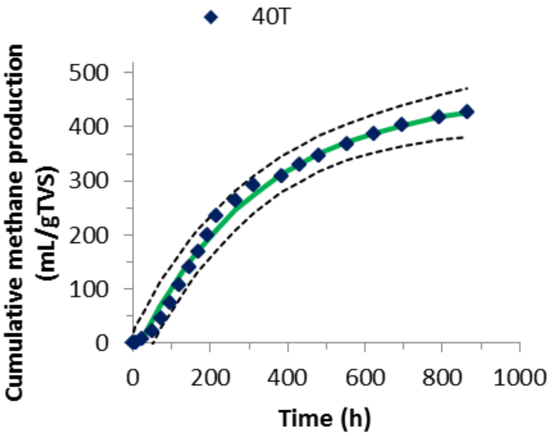
(c)


20T
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
0
200
400
600
800
1000
Time (h)

(d)


40T
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
0
200
400
600
800
1000
Time (h)

(e)


40T
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
0
200
400
600
800
1000
Time (h)

(f)

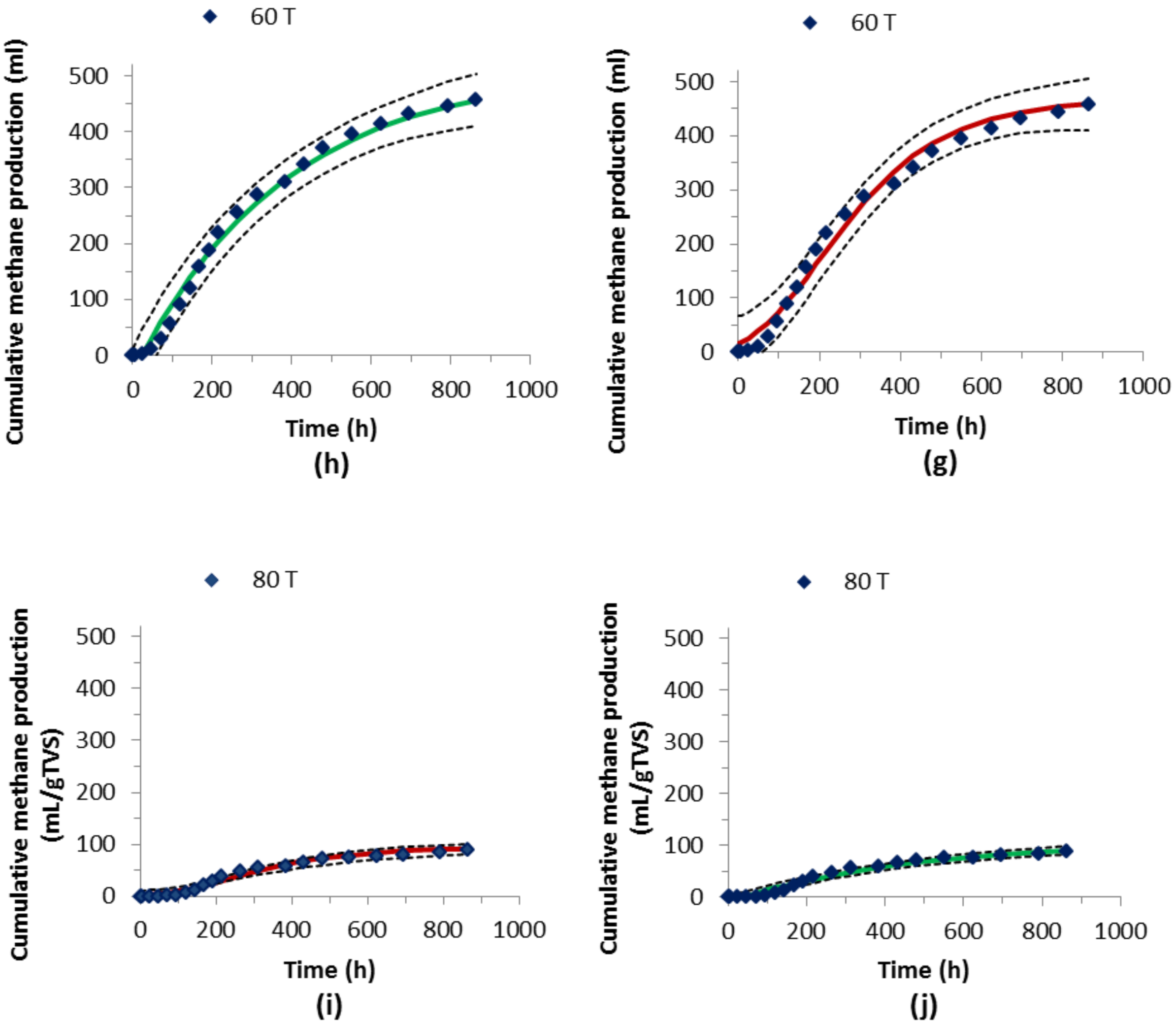
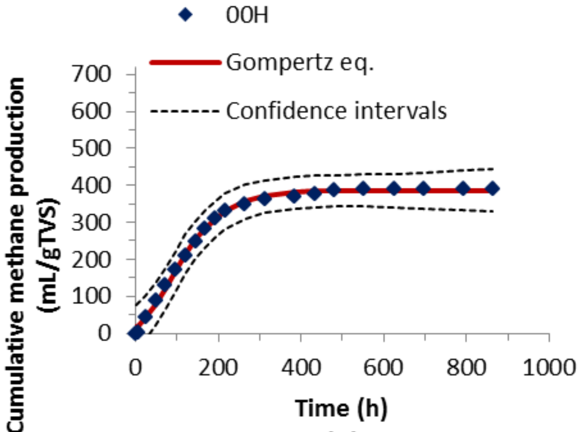
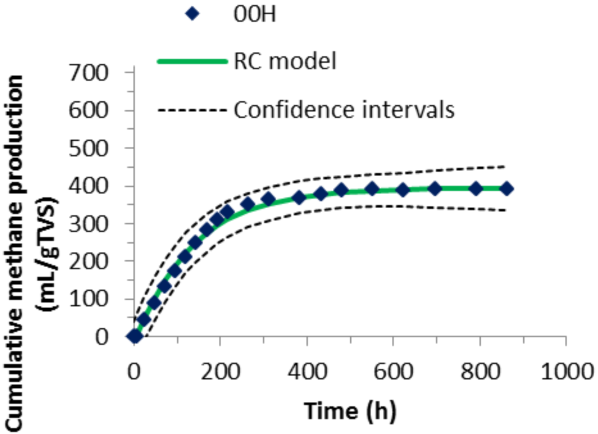


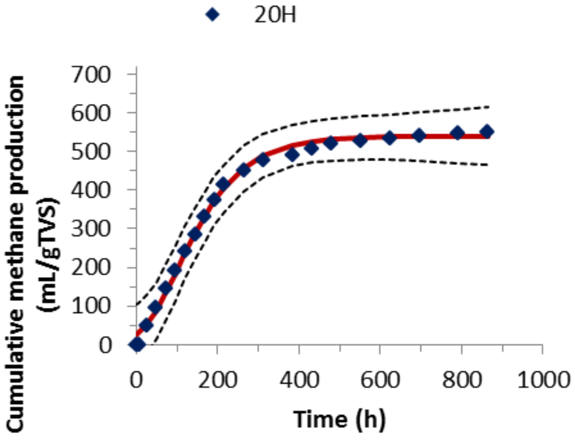
Figure 2.3: Non-linear regression for the cumulative methane yield (STP, mL/g TVS) during the thermophilic BMP assay using Modified Gompertz equation (graphs on the left hand with the red line) and RC model (graphs on the right hand with the green line).


00H
Gompertz eq.
Confidence intervals
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
Time (h)

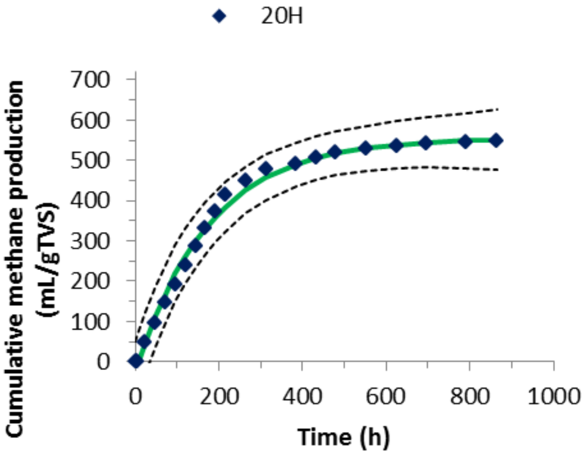
(a)


00H
RC model
Confidence intervals
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
Time (h)

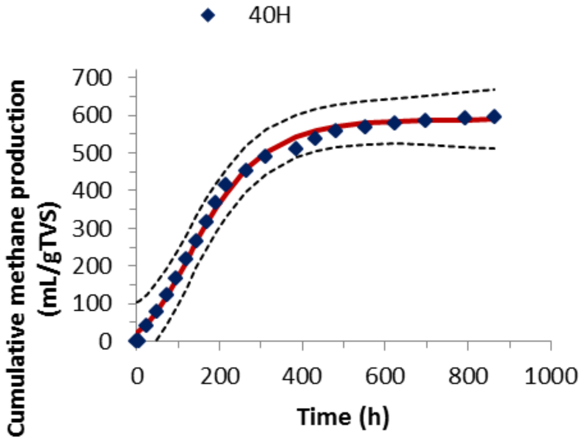
(b)


20H
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
Time (h)

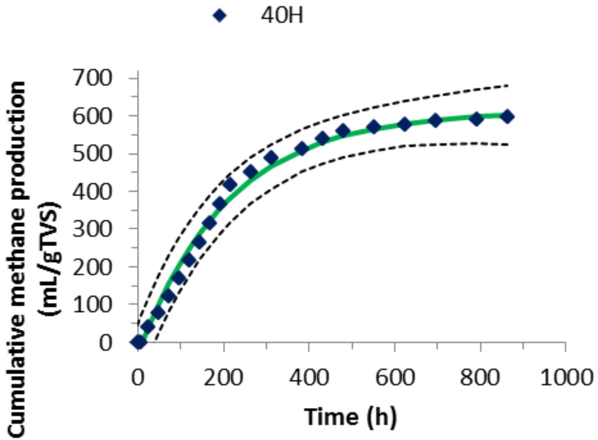
(c)


20H
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
Time (h)

(d)


40H
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
Time (h)

(e)


40H
Cumulative methane production (mL/gTVS)
0
100
200
300
400
500
600
700
0
200
400
600
800
1000
Time (h)

(f)

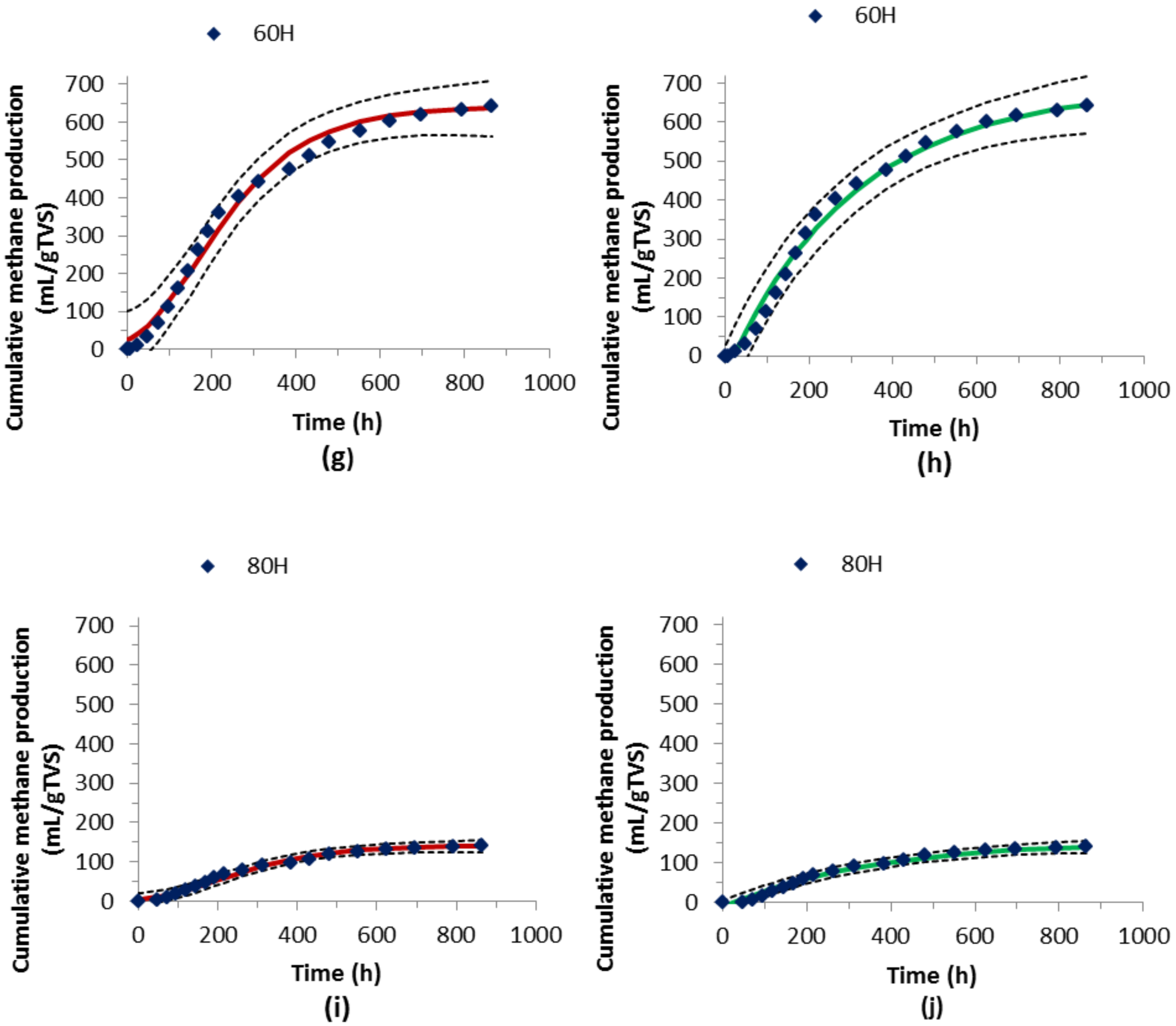


Figure 2.4: Non-linear regression for the cumulative methane yield (STP, mL/g TVS) during the hyper-thermophilic BMP assay using Modified Gompertz equation (graphs on the left hand with the red line) and RC model (graphs on the right hand with the green line).

The anaerobic co-digestion parameters determined from the model fit the data process, which are presented in Table 2.4, show that the effect of FOG addition on the co-digestion process resulted in some noticeable increase in the lag time given by both models (MG eq. and RC model). This lag time increases in a consistent fashion for both models, for instance with adding 20% FOG to the co-digestion mixture, the lag time estimated by MG eq. and RC model increased from 18.4 and 10.4 hours (for 00T sample) to 20.5 and 11.5 hours (for the 20T sample), respectively. In the thermophilic BMP assay as the FOG% increased, the lag time increased until it reached the maximum lag time for the sample with 80% FOG (it was 68.6 and 39.5 hours for the MG eq. and RC model, respectively). This may suggest that methanogens need extra time to adapt to the FOG added to the co-digestion mixture and hence as FOG% increases, the time required to adapt increases as well. In the hyper-thermophilic assay, the lag time has the same trend as in the thermophilic BMP assay (the lag time increases with increasing the FOG% in the co-digestion mixture). However, the lag time in the hyper-thermophilic BMP assay has been shorter for all the cases compared to the thermophilic BMP assay. This may explain the effect of the hyper-thermophilic stage in solubilizing the co-digestion mixture which results in more soluble organic matter available for faster digestion start and methane production at the beginning of the BMP assay (Lee et al., 2009).

Regarding the maximum methane production rate (R_m, mL/gTVS.h), it can be observed from Table 2.4 that samples with 20% FOG were the only samples having higher R_m value than the control (00T) in the thermophilic BMP assay given by both models, and as FOG% increased further the R_m value decreased. In contrast, all the samples in the hyper-thermophilic BMP assay except the 80H sample have higher R_m value compared to the control (00T). This further emphasizes the effect of the hyper-thermophilic stage in enhancing the co-digestion process by having higher R_m value compared to the control (00T) for all the cases except the 80H sample. In fact, when FOG was 80% of the co-digestion mixture, the R_m became very low (0.2-0.4) compared to R_m for the control sample (00T) and all the other samples, this explains the low methane production of the 80T and 80H samples which is caused by the inhibitory effect of the high lipid content on the methanogens and the anaerobic co-digestion process.

The results obtained using the FO equation are presented in Table 2.4. The effect of the hyper-thermophilic co-digestion manifests itself by increasing the apparent hydrolysis rate coefficient (k, h^{-1}) resulted from the FO equation (Table 4). During the same BMP test the sample with no FOG has the highest hydrolysis rate coefficient (sample 00T in the thermophilic BMP assay and sample 00H in the hyper-thermophilic BMP assay), and as FOG was added and consequently FOG% increased, the k decreased within the same BMP assay. These observations suggest that adding FOG has an inhibitive effect on the methanogens and this inhibitive effect increases with increasing the FOG% in the co-digestion mixture. It is noticeable that as far as the FOG% is less than 80%, the inhibition of the methanogens is only observed as a lower k (FO equation) and a longer lag time (GM eq. and RC model). However, the final methane production is improved and is statistically significantly higher than the control 00T ($p < 0.05$, as discussed in section 3.1) due to the high methane potential of the lipid materials (the theoretical methane potential of lipids is 1014 L/kg volatile solids (VS)) (Wan et al., 2011). But when FOG reached 80% of the co-digestion mixture, the inhibition effect was severe on the methanogens and the whole co-digestion process in both BMP assays.

Anaerobic co-digestion is considered one of the most attractive options for municipal solid waste (MSW) treatment since the anaerobic co-digestion not only provides a solution for waste management but also it provides a potential energy recovery due to the biogas production (biogas with about 60% methane on average) (Hosseini et al., 2014). Biogas is an important sustainable source of energy that can be used instead of fossil fuel. Biogas produced from anaerobic digestion can be used mainly for heat and electricity generation to be used within the anaerobic digestion plant, or it can be separated and purified as a bio-natural gas to be used as piped natural gas, compresses vehicle natural gas, raw chemical materials, etc. (Liu et al., 2012). Furthermore, at the end of the anaerobic digestion process, the resulted digestate can be improved to satisfy the standard regulations for being used as a fertilizer or soil amendment (Lo et al., 2010) and this will provide a recycle of the nutrients to the soil again plus extra economic benefit.

2.5 Conclusion

TWAS co-digestion with FOG proved to significantly improve methane production during the thermophilic and hyper-thermophilic BMP assays. Hyper-thermophilic digestion has been shown to be a very promising approach in anaerobic co-digestion of TWAS and FOG. It enhances the biodegradability of TWAS-FOG mixture and results in much higher methane production compared to the thermophilic mono-digestion of TWAS alone; 112.7% more methane has been achieved using hyper-thermophilic co-digestion of TWAS-FOG using 60% FOG. 80% FOG in the TWAS-FOG mixture (based on TVS) was found to be the threshold of FOG in the current study since this percentage of FOG limited the co-digestion process and caused a severe inhibition in the methanogens activity.

Linear and non-linear regression models were used to fit the experimental data. The linear regression using the FO equation resulted in determining the apparent rate of hydrolysis coefficient for each case in both BMP assays. This helped much in concluding the effect of hyper-thermophilic digestion on the solubilizing the co-digestion mixture (higher k (h^{-1})). Both GM eq. and RC model have been shown to have a very good fit to the experimental data from both BMP assays with a relatively small deviation between the experimental methane yields and model methane yields <10%. The use of non-linear regression with the proper model has found to be effective in providing an accurate estimation of co-digestion parameters. These parameters can help in making a more reliable comparison between the different cases and point the effect of the different conditions used in the co-digestion process and methane production.

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CHAPTER 3

Improving Biogas Production from Anaerobic Co-digestion of Thickened Waste Activated Sludge (TWAS) and Fat, Oil and Grease (FOG) Using a Dual-stage Hyper-thermophilic/Thermophilic Semi-Continuous Reactor

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3.1 Abstract

This paper investigates the feasibility and advantages of using a dual-stage hyper-thermophilic/thermophilic semi-continuous reactor system for the co-digestion of Thickened Waste Activated Sludge (TWAS) and Fat, Oil and Grease (FOG) to produce biogas in high quantity and quality. The performance of the dual-stage hyper-thermophilic (70°C)/ thermophilic (55°C) anaerobic co-digestion system is evaluated and compared to the performance of a single-stage thermophilic (55°C) reactor that was used to co-digest the same FOG-TWAS mixtures. Both co-digestion reactors were compared to a control reactor (the control reactor was a single-stage thermophilic reactor that only digested TWAS). The effect of FOG% in the co-digestion mixture (based on total volatile solids) and the reactor hydraulic retention time (HRT) on the biogas/methane production and the reactors' performance were thoroughly investigated. The FOG% that led to the maximum methane yield with a stable

reactor performance was determined for both reactors. The maximum FOG% obtained for the single-stage thermophilic reactor at 15 days HRT was found to be 65%. This 65% FOG resulted in 88.3% higher methane yield compared to the control reactor. However, the dual-stage hyper-thermophilic/ thermophilic co-digestion reactor proved to be more efficient than the single-stage thermophilic co-digestion reactor, as it was able to digest up to 70% FOG with a stable reactor performance. The 70% FOG in the co-digestion mixture resulted in 148.2% higher methane yield compared to the control at 15 days HRT. 70% FOG (based on total volatile solids) is so far the highest FOG% that has been proved to be useful and safe for semi-continuous reactor application in the open literature. Finally, the dual-stage hyper-thermophilic/ thermophilic co-digestion reactor also proved to be efficient and stable in co-digesting 40% FOG mixtures at lower HRTs (i.e., 9 and 12 days) and still produce high methane yields and Class A effluents.

3.2 Introduction

In light of climate changes, environmental challenges and rising energy demand, the quest to develop renewable energy sources has become urgent. A potential renewable energy source which has gained the interest of many researchers recently is biogas harvested from different types of organic waste. Anaerobic digestion (AD) is one of the leading methods used for generating biogas from organic waste (Alqaralleh et al., 2015; Long et al., 2012). Average biogas production in a typical wastewater treatment plant (WWTP) ranges from 15 to 28 L biogas/capita.day (Berktay & Nas, 2008). If this biogas produced using AD is efficiently utilized, it would potentially turn the WWTP into a net energy producer instead of being an energy consumer (Shen et al., 2015a; Cavinato et al., 2013). The US Environmental Protection Agency

(USEPA) has recently qualified the biogas from landfills, WWTP anaerobic digesters, agricultural digesters and separate municipal solid waste digesters as a cellulosic biofuel (category D3) under the new Renewable Fuel Standards (RFS2) (Shen et al., 2015b). However, WWTPs are still facing real challenges in deploying biogas production and utilization because of the slow biogas generation rate during the AD process, the low biogas energy content (methane%) and the high costs to upgrade the biogas (Gianico et al., 2015).

Sewage sludge, particularly waste activated sludge (WAS), is usually known for its low biodegradability due to its low carbon to nitrogen (C/N) ratio. This makes it hard to digest under traditional mesophilic conditions (35ºC) (Mahanty et al., 2014; Coelho et al., 2011). Therefore, the co-digestion of sludge with other organic wastes can offer several potential benefits for the AD process, such as improving the overall C/N ratio in the digestion mixture, diluting any toxic or inhibitory compounds, increasing the buffer capacity of the digester and improving the overall AD process. All these factors enhance the overall digestion process and increase the digester biogas production (Xu et al., 2015; Ara et al., 2014; Li et al., 2013; Alatriste-mondrago et al., 2006). Different organic materials have been used as co-substrates for the anaerobic co-digestion of sludge. Some examples on these materials are: food waste and Organic Fraction of Municipal Solid Waste (OFMSW) (Alqaralleh et al., 2017; Iacovidou et al., 2012; Esposito et al., 2011; Nayono et al., 2010; Bolzonella et al., 2006), fruit and vegetable waste (Lin et al., 2012; Park et al., 2012), different types of manure (Borowski et al., 2014; Neves et al., 2009), and fatty waste from different sources (Harris and McCabe., 2015; Martínez et al., 2012; Alves et al., 2009; Luostarinen et al., 2009).

Lipid-rich waste, usually known as fat, oil and grease (FOG), is a very attractive co-substrate option for the anaerobic co-digestion of municipal sludge because of its high methane potential, which ranges from 0.70 up to 1.43 m^3 CH_4 /kg Volatile Solids (VS) compared to sewage sludge methane potential, which ranges from 0.24 to 0.34 m^3 CH_4 /kg VS (Xu et al., 2015; Mata-Alvarez et al., 2014). FOG waste is high in biodegradable volatile solids, which can reach up to 93% (w/w, weight of VS/ weight of Total Solids (TS)), and also high in Chemical Oxygen Demand (COD), which can reach up to 1200 kg/m^3 depending on the source of the FOG (Wang et al., 2013). Several researchers have reported the advantages of anaerobic co-digestion of FOG with sewage sludge on the biogas production (Sun et al., 2014; Long et al., 2012; Luostarinen et al., 2009); however, the use of FOG for anaerobic co-digestion has been shown to have some inhibitory effects on methanogenic activity if it is used in high concentrations in the co-digestion reactor (Sun et al., 2014; Wan et al., 2011). Moreover, FOG may cause some operational problems in the digester such as foaming, clogging in the liquid or gas system, and biomass floatation due to the adsorption of lipids onto the biomass surface (Mata-Alvarez et al., 2014). For the reasons mentioned above, FOG dosing rates must be carefully determined to obtain the maximum benefit possible from the FOG addition and to avoid any unwanted adverse effects on the digestion process and the whole AD system.

In some studies, researchers have tried to determine the threshold for FOG addition for the anaerobic co-digestion of FOG and sewage sludge under mesophilic digestion conditions (35°C). For example, Silvestre et al. (2011) found that 23% FOG (w/w) co-digested with (TWAS+Primary Sludge (PS)) for 20 days resulted in a maximum methane yield of 369 L_{CH4}/kg VS_{added} (with 70.0% CH_4) and a VS reduction of 46%. Wan et al. (2011) also showed in a study on a mesophilic semi-continuous reactor co-digesting FOG and TWAS at a 15d Hydraulic Retention Time (HRT) that 36% FOG (w/w) is the proper threshold for producing a maximum methane yield of 598 L_{CH4}/kg VS_{added} (with 66.8% CH_4) and 57% VS reduction. In a similar study, Noutsopoulos et al. (2013) found that the mesophilic co-digestion of 40% FOG with (TWAS+PS) for 15d resulted in a maximum methane yield of 490 L_{CH4}/kg VS_{added} (with 70.0% CH_4) and a 59% VS reduction.

However, thermophilic digestion (55°C) has rarely been used to examine anaerobic co-digestion of TWAS and FOG, and therefore there is no accurate evaluation for the optimum FOG% that can be used safely for the thermophilic anaerobic co-digestion of FOG and TWAS in order to provide maximum methane production with a stable digestion process. Kabouris et al. (2009) performed one of those rare studies, and the authors examined thermophilic semi-continuous reactors for FOG and sewage sludge (TWAS+PS) co-digestion. In their study, the authors found that the threshold FOG% is 48% (w/w) for thermophilic anaerobic co-digestion experiments with 12d HRT. This 48% FOG resulted in a maximum methane yield of 551 L_{CH4}/kg VS_{added} (with 68.7% CH_4) and a VS reduction of 50.9%. The reason behind the prevalent use of mesophilic digestion compared to thermophilic digestion is related to the old belief that the mesophilic process is more stable and has fewer operational problems, and plus it requires less energy to attain the digester temperature as compared to thermophilic conditions (Mao et al., 2015; Kabouris et al., 2009). However, during recent years, conducting anaerobic digestion and co-digestion of sewage sludge and other organic wastes under thermophilic conditions has proved to be useful and more feasible as compared to mesophilic digestion (Coelho et al., 2011). In fact, thermophilic conditions have been shown to be efficient in treating high loading rates, operating efficiently at lower HRTs, producing higher biogas and removing more volatile solids (Martín et al., 2011; Kabouris et al., 2009).

Hyper-thermophilic digestion at high temperatures (in the range of 65-80ºC) is a relatively new digestion trend that has only been recently introduced (Wang et al., 2012). Wang et al., (2012) and few other recent studies have addressed the benefits of hyper-thermophilic digestion in increasing bio-hydrogen production, improving the degradation of polylactide and enhancing the co-digestion of shredded grass with sewage sludge (Wang et al., 2014; Cappelletti et al., 2012; Lee et al., 2009). As well, to the best of our knowledge, hyper-thermophilic digestion has not yet been used in semi-continuous reactor experimentation for the co-digestion of TWAS and FOG. Hyper-thermophilic/ thermophilic co-digestion of TWAS and FOG was proposed and tested for the first time in a Biochemical Methane Potential (BMP) batch tests in Alqaralleh et al.'s (2016) study. The results of the BMP tests showed clearly that hyper-thermophilic digestion is a promising approach that can be further investigated for the co-digestion of TWAS and FOG using semi-continuous reactors. The high temperature of 70ºC (hyper-thermophilic conditions) used in the same study helped greatly in solubilizing the co-digestion mixture, leading to a significant improvement in the anaerobic co-digestion process and recording a 112.7% increase in methane production for hyper-thermophilic co-digested samples that contained 60%FOG compared to a TWAS-only thermophilic-digested sample.

As such, the goal of the current study is to investigate the effects of applying hyper-thermophilic conditions in biogas production from the co-digesting of TWAS and FOG, as well as to determine the optimum FOG% that provides the best outcomes. This was conducted by comparing the performance of a single-stage thermophilic anaerobic semi-continuous reactor and a dual-stage hyper-thermophilic/ thermophilic anaerobic semi-continuous reactor in co-digesting TWAS and FOG mixtures at three different HRTs: namely, 15, 12 and 9 days. During the experiments, the threshold FOG% that produced the maximum possible methane yield in a stable AD process was determined for both reactors (the single-stage thermophilic reactor and the dual-stage hyper-thermophilic/ thermophilic reactor). The performance of the reactors and the biogas production, methane yield, and effluent characteristics were measured and compared to the control reactor (single-stage thermophilic reactor digesting TWAS only). This was done in order to evaluate the effects of FOG% addition, anaerobic digestion temperature, and HRT on the anaerobic co-digestion of TWAS and FOG in a semi-continuous reactor experiment.

3.3 Materials and Methods

3.3.1 Substrates and Inoculum

TWAS used for this study was provided by City of Ottawa Robert O. Pickard Environmental Center (ROPEC), Gloucester, ON, Canada. ROPEC is a secondary WWTP that performs preliminary and primary treatment followed by a conventional activated sludge process with a Solids Retention Time (SRT) of 5 days on average. TWAS sample used in this study contained 4.8±0.1% TS with 71±2% VS. The FOG sample was obtained from Organic Resources Management Inc. (ORMI) in Ottawa, ON, Canada. ORMI provides grease-trap cleaning services for different wastewater treatment plants in Ontario, Canada. Both substrates (TWAS and FOG) were stored at 4°C prior to use. The thermophilic anaerobic inoculum (55°C) was obtained from the effluent of a 10L thermophilic anaerobic digester acclimated to ROPEC-TWAS and operated at a 20d HRT for more than two years in our research lab. The thermophilic inoculum was originally obtained from Ravensview WWTP in Kingston, ON, Canada. The hyper-thermophilic anaerobic inoculum (70^{o}C) used was the effluent from a 2L anaerobic digester acclimated to ROPEC-TWAS and operated for about 10 months at an HRT of 2 days under hyper-thermophilic conditions (70^{o}C).

The characteristics of the substrates and inoculums used in this study are shown in Table 3.1.

Table 3.1: Characteristics of experimental substrates and inoculums.

Parameter	TWAS	FOG	Thermophilic inoculum	Hyper-thermophilic inoculum
pH	6.9±0.2	4.2±0.1	7.5±0.1	6.7± 0.2
TS (g/Kg)	48.4±1.1	291.4±1.6	29.0±0.9	33.5±0.9
VS (g/Kg)	34.4±1.8	282.1± 2.2	14.8±0.9	18.8±0.6
VS/TS	0.71±0.02	0.97±0.02	0.51±0.01	0.56±0.01
COD (g/Kg)	74.8±3.6	261.2±30.8	21.2±1.0	49.4±1.4

Data represent the arithmetic mean ± standard deviation. (n≥4).

3.3.2 Experimental Setup

The semi-continuous reactor study was performed for 288 days divided into ten experimental phases (I, II, III,…..to X) corresponding to different feeding strategies (depending on FOG% of the total VS in the feed) and three different HRTs, as summarized in Table 3.2 below.

Table 3.2: Summary of the experiment phases.

Phase	days	HRT (d)	Feed		
			R1	R2	R3
I	1-29	15	TWAS	TWAS	TWAS
II	30-59	15	TWAS	20% FOG*	20% FOG
III	60-89	15	TWAS	40% FOG	40% FOG
IV	90-119	15	TWAS	60% FOG	60% FOG
V	120-149	15	TWAS	65% FOG	65% FOG
VI	150-157	15	TWAS	70% FOG	70% FOG
	158-179[1]	15	TWAS	40% FOG[R]	70% FOG
VII[2]	180-186	15	TWAS	40% FOG[R]	75% FOG
VIII	187-230	15	TWAS	40% FOG[R]	40% FOG[R]
IX	231-260	12	TWAS	40% FOG	40% FOG
X	261-287	9	TWAS	40% FOG	40% FOG

* FOG % in the feed (TWAS+FOG) mixture based on total VS. [1] At day 158 R2 started a recovery phase by switching the FOG feed from 70% to 40%, whereas R3 continued with 70% FOG until the end of phase VI. [2] Phase VII is with 75% FOG for R3 only, whereas R2 was still under recovery with 40% FOG during this phase. [R] Recovery phase.

Four 2.5 L Schott-Duran® laboratory glass bottles were used in creating the lab-scale reactors. Each bottle had 3 ports, for biogas collection, feeding, and sampling. The lab-scale semi-continuous reactors were set up as shown is Figure 2.1 to study the thermophilic and hyper-thermophilic co-digestion of TWAS and FOG and compare them to the thermophilic mono-digestion of TWAS under the same HRT and organic loading rate. To start the experiments, the three reactors (R1, R2 and R3) were each seeded with 1.5 L of the thermophilic inoculum (Table1), and then a buffering capacity of 5.0-5.3 g/L Alkalinity as CaCO$_3$ was added to each reactor using equal amounts of NaHCO$_3$ and KHCO$_3$ in order to neutralize the acids produced during digestion. The final preparatory step was to purge each reactor with N$_2$ gas for 3-5 minutes to remove any oxygen from the headspace of the bottle and maintain anaerobic conditions. The three reactors started the semi-continuous anaerobic digestion operation at an HRT of 15d by daily withdrawing and feeding 100 ml of the sample (TWAS or TWAS:FOG mixture depending on the reactor and the experimental phase). Inoculating the thermophilic reactors with a thermophilic inoculum is an essential step for enabling a fast reactor start-up and a more stable operation by avoiding the temperature change shock that may occur in the case of using a mesophilic inoculum to start a thermophilic reactor (Coelho et al., 2011). Reactor H was initially inoculated with 600 ml of hyper-thermophilic inoculum, and it was run at a constant HRT of 2d by withdrawing and feeding 300ml of the sample (TWAS or TWAS:FOG mixture depending on the experimental phase) for the whole period of the study. Reactors R1, R2, and R3 were kept in a constant temperature-controlled shaker fixed at 55±1°C and 100 rpm (New Brunswick Scientific Co. Inc., NB, Canada), and reactor H was kept in a similar constant temperature-controlled shaker fixed at 70±1°C and 100 rpm. All three reactors (R1, R2, and R3) were operated under the same feeding conditions until reaching a steady state of biogas production (the steady state was considered to be achieved when the fluctuation in biogas production was less than 10%). During the study, a gradual-increase strategy was used for the FOG addition to the co-digestion mixture to accurately determine the optimum FOG% (threshold). This FOG% needed to be handled by the reactor while producing the maximum methane yields without causing adverse effects on reactor performance, biogas production or methanogens activity.

R1 was the control reactor. It was a single-stage thermophilic mono-digestion reactor that digested TWAS solely. R2 was a single-stage thermophilic co-digestion reactor that digested TWAS and FOG mixtures with different FOG percentages. The FOG% started at 20% 40%, and 60%, and then it was increased by 5% increments until reaching the optimum FOG% for maximum biogas/methane production with stable reactor performance.

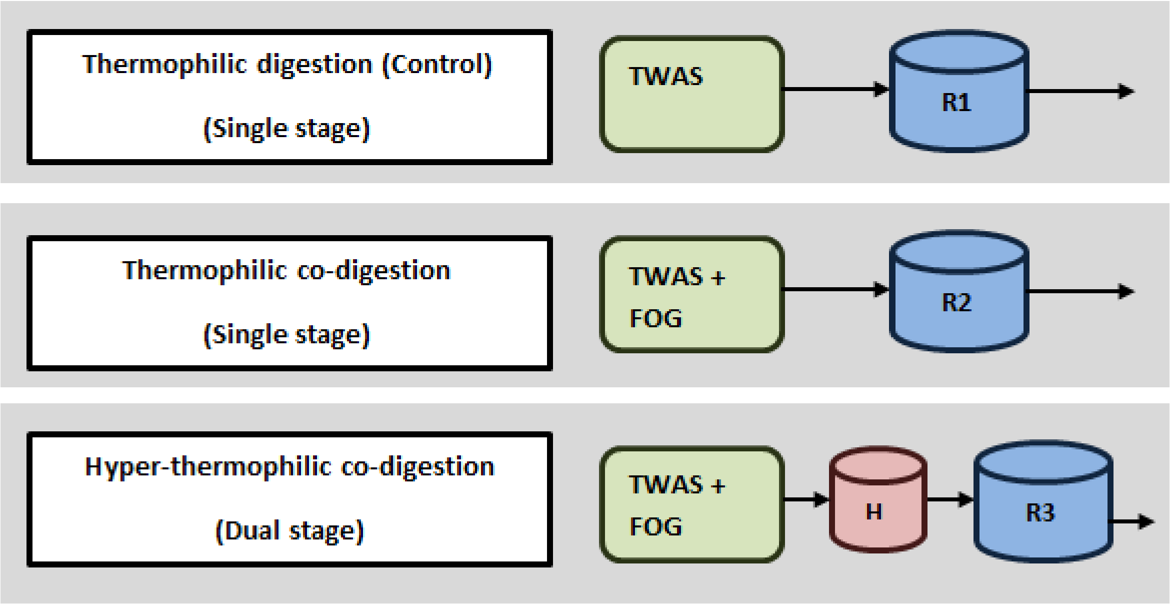


Figure 3.1: Experimental setup.

R1 and R2 both operated at three different HRTs: namely, 15, 12 and 9d. The third semi-continuous reactor system, H-R3, was a dual-stage hyper-thermophilic/ thermophilic co-digestion system that consisted of two reactors, H, and R3. Reactor H represented Stage 1 of the dual-stage anaerobic co-digestion system operating under hyper-thermophilic conditions (70° C) and had a constant HRT of 2d. Reactor R3 represented Stage 2 of the dual-stage anaerobic co-digestion system. It operated under thermophilic conditions (55°C) and had an HRT that equaled the total HRT used for reactors R1 and R2 minus 2d (2d was the HRT of reactor H). In other words, reactor R3's HRTs were 13, 10 and 7d, while the total HRTs for the complete H-R3 system were 15, 12 and 9d. All reactors were fed semi-continuously once a day, with TWAS only for R1 and TWAS-FOG mixtures for R2. As for the dual-stage H-R3 system, the feeding process was slightly different; the TWAS:FOG mixtures were first fed to the hyper-thermophilic reactor H (with 2d HRT), and then the effluent from reactor H was the feed for reactor R3.

3.3.3 Analytical Methods

To characterize and compare the performance of the three reactors (R1, R2, and R3), several parameters were measured during the study period. The biogas production from the reactors was measured twice daily using a gas manometer. The total daily biogas volumes were corrected to standard ambient temperature and pressure (STP) conditions (25°C and 1 atm). The biogas composition was monitored weekly using a Hewlett Packard 5710A gas chromatograph equipped with a 3380A model integrator, a 5705A thermal conductivity detector, and helium as the carrier gas. Effluent samples were taken weekly (or more often as needed) to measure pH, TS, VS, Alkalinity and total volatile fatty acids (TVFA). The pH measurements were performed using a Fisher Accument® XL25 pH meter. TS and VS were determined according to the standard method 2540G (APHA, 1998). Chemical oxygen demand (COD) was measured using HACH TNT 822 (20-1500 mg/L) and TNT 823 (250-15000 mg/L) including a spectrophotometer, heating block and supplies. Alkalinity and TVFA were measured using HACH TNT 870 (25-400 mg/L $CaCO_3$) and TNT 872 (50-2500 mg/L CH_3COOH), respectively. Finally, the total coliforms for samples were measured using Colilert IDEXX 24 h tests.

3.4 Results and Discussion

3.4.1 Biogas and Methane Production

The daily biogas production per L of the reactor (L biogas/L reactor.d) for reactors R1, R2, and R3 over the entire experimental period is shown in Figure 3.2. The average steady-state properties for the three reactors including HRT, organic loading rate (OLR), daily biogas production, biogas methane content (%), methane yields, VS reduction, pH, Alkalinity, and TFVA are presented in Table 3.3. The data in Table 3.3 represent the average values calculated for each stage after reaching steady state conditions ± standard deviation unless otherwise stated. The pH, Alkalinity, TVFA measurements, and TVFA/Alkalinity ratios calculated throughout the study are presented in Figures 3.3 a, b, c, and d, respectively. The values on the figures represent the average of triplicate measurements.

Phase 1- start-up (days 0-29)

During the start-up phase the three reactors R1, R2 and H-R3 were fed with TWAS only at a 15d HRT and 1.6 kg VS /m^3.d OLR. It was observed that reactors R1 and R2 had a close daily biogas production whereas R3 had a relatively lower daily biogas production, as can be seen in Figure 3.2. It is quite noticeable from Figure 3.2 that the three reactors had stable performance and smoothly reached a steady state biogas production within 11 to 16 days after starting the experiment, with an average steady-state biogas production (methane %) of 588.9±33.0 (59.7±1.0 %), 596.4±28.7 (61.0±0.3 %) and 496.5±20.2 (60.6±0.6 %) L/m^3 reactor.d for reactors R1, R2 and R3, respectively. The pH values for the three reactors ranged from 7.5 to 7.6 during this phase (the performance values for this start-up phase are not included in Table 3.3).

Phases 2, 3 and 4 (days 30-119)

Phase 2- 20% FOG (days 30-59): The second phase started on day 30 of the study. During this phase, reactor R1 (the control reactor) continued operating under the same conditions as in phase 1 (i.e., TWAS only, 15d HRT and 1.6 kg VS /m^3.d OLR). It is worth mentioning that reactor R1 continued operating under the same conditions until the end of phase 8. Overall, it was noticed that R1 had a stable performance during the HRT 15d period (from phase 2 to phase 8) and its daily biogas production increased slightly over time. The average biogas production (methane %) for R1 during the 15d HRT was 606.0±43.7 (61.6±0.4 %) L/m^3 reactor.d. To ensure an active and stable anaerobic digestion process, pH, Alkalinity, and TVFA were monitored periodically to check the process stability. All average values are presented in Table 3.3 and Figures 3.3 a, b, and c, as pointed out earlier. The safe pH and Alkalinity for a stable anaerobic digestion process are (6.5-8.2) and (2.5-5.0 g/L as $CaCO_3$), respectively (Wang et al., 2014; Speece, 1996). The TVFA/Alkalinity ratio was used as an indicator of reactor stability. The reactor is considered stable and under favorable conditions when this ratio is below 0.4; however when it ranges from 0.4 to 0.8, the reactor is considered at a slight risk of acidification. When the TVFA/Alkalinity ratio exceeds 0.8, acidification conditions are likely to occur in the reactor. For reactor R1, the pH, Alkalinity, TVFA and TVFA/Alkalinity values were stable with minimal flocculation during the entire period of HRT 15d. These average values were 7.6±0.0 for pH, 4.41±0.33 g/L as $CaCO_3$ for Alkalinity, and 0.44±0.04 g/L for TVFA. The TVFA/Alkalinity ratio was in the range 0.07 to 0.12.

As regards reactors R2 and H-R3, the feed contained 20%FOG:80%TWAS (based on the total VS content) with a total OLR of 1.59-1.60 kg VS /m^3. During this phase, R2 reached steady state biogas production after 12 days; however, R3 needed 14 days to reach steady state biogas production. As compared to the control reactor R1, the average daily biogas production (methane %) for R2 and R3 increased significantly to reach 792.6±36.3 (65.0±0.5 %) and 918.2±56.7 (67.2±0.3 %) L/m^3 reactor.d, respectively. Apparently, the 20% FOG addition to reactors R2 and H-R3 feed caused this substantial increase in daily biogas production and methane content as compared to the control reactor R1 (digesting TWAS only).

Phase 3-40% FOG (days 60-89): Day 60 marked the start of phase 3 when the FOG% was increased in the R2, and H-R3 feeds to 40%. Both reactors adapted well to the additional increase in FOG% which resulted in a further increase in daily biogas production and methane content for both reactors. In this phase, R2 needed 13 days to reach a steady state biogas production (methane %) of 856.7±73.9 (67.8±0.5 %) L/m^3 reactor.d. This represents a 53.6% increase in daily methane production as compared to the control R1 methane production. R3 reached a steady state daily biogas production (methane %) of 1007.1±50.6 (70.9±0.4 %) L/m^3 reactor.d after 14 days with a 94.9% improvement in daily methane production compared to the control R1. For both reactors R2 and R3, pH values were stable with values around 7.5±0.0, and Alkalinity values ranged from 4.54±0.12 g/L as $CaCO_3$ for R2 to 4.80±0.12 g/L as $CaCO_3$ for R3. TVFA values were 0.63±0.07 and 0.593±0.05 g/L for R2 and R3, respectively, and TVFA/Alkalinity ratios were less than 0.2 for both reactors.

Phase 4 (days 90-119): On day 90, phase 4 of the study was launched by further increasing the FOG% in the feed to 60%. It was observed that reactors R2 and R3 reached steady state daily biogas production on days 10 and 9 from the beginning of this phase, respectively. It is evident that reactors R2 and R3 required less time to reach steady state biogas production compared to the previous phases. This may indicate that the methanogens were well adapted to the FOG addition in the feed. The average daily biogas production (methane %) during this phase was 948.7±60.9 (70.6±0.2 %) and 1103.9±43.8 (71.9±0.6 %) L/m^3 reactor.d for reactors R2 and R3, respectively. This represents an 82.9 and 116.9% higher daily methane production compared to the control R1 for reactors R2 and R3, respectively. During this phase, it was observed that the effluent pH of reactors R2 and R3 ranged from 7.4 to 7.5. Alkalinity showed a slight drop to 4.17±0.25 and 4.29±0.29 g/L as $CaCO_3$ for reactors R2 and R3, respectively. Concomitantly, TVFA showed an increase to 0.90±0.09 and 0.79±0.07 g/L for R2 and R3, respectively. However, TVFA/Alkalinity ratios remained within the stable range (0.2-0.4) for reactors R2 and R3.

It can be noticed that the dual stage hyper-thermophilic/ thermophilic H-R3 system produced 18.8% more methane compared to R2 during this phase (phase 4, 60%FOG:40%TWAS mixture). This confirms the improvement in methane conversion efficiency that was mainly caused by the hyper-thermophilic stage (H) that was added prior to the single thermophilic reactor (R3) to form the dual-stage hyper-thermophilic/thermophilic reactor (H-R3). The hyper-thermophilic stage at 70°C (H) provided the required solubilization of the co-digestion mixture, resulting in higher efficiency in the conversion of the organic matter to methane in the next thermophilic stage (R3).

After this stage, the FOG addition was increased in 5% increments in order to accurately determine the limits for the FOG% addition without causing adverse effects on the reactor stability. Increasing the FOG% in small increments will help in noticing any early signs of reactor overload, hence giving enough time to change the feeding composition before any serious upset conditions take place in the reactors. This may save the microbial community in the reactors from severe damages.

Phase 5-65% FOG (days 120-149)

Phase 5 started on day 120 by increasing the FOG% in the feed mixture to 65%. The additional 5% FOG increase in the co-digestion mixture led to a slight increase in daily biogas production (not statistically significant, $p > 0.05$) for R2 and R3, as shown in Figure 3.2. Both reactors were in steady state biogas production through the whole stage, except for 3 days for reactor R2 and 4 days for reactor R3 when more than 10% fluctuation in biogas production occurred compared to the average daily biogas production. These particular days were excluded from the average calculations for this phase. The effluent pH of reactor R2 decreased to 7.3±0.1 and Alkalinity consistently decreased to 3.36±0.19 g/L as $CaCO_3$. The effluent TVFA reached 1.04±0.08 g/L, and the TVFA/Alkalinity ratio increased from 0.2 at the beginning of the phase to 0.4 at its end. Although reactor R2's daily biogas production was still stable during this phase, the changes observed in the reactor effluent properties may indicate that the reactor started to face some stress due to the further increase in FOG load (65% FOG).

As for reactor R3's performance during this phase, the 5% increase in FOG did not cause a considerable increase in daily biogas production or biogas methane content compared to the previous phase 5 ($p > 0.05$). The R3 effluent pH did not change compared to the previous phase; however, Alkalinity decreased to 3.62±0.11 g/L as $CaCO_3$. On the other hand, TVFA value increased to 0.94±0.07 g/L, and TVFA/Alkalinity ratio ranged from 0.3 to 0.4.

Phase 6-70%FOG (days 150-179)

A further increase of 5% FOG was applied in this phase, so the co-digestion mixture contained 70%FOG:30%TWAS (based on total VS). During the first week of this phase, reactor R2 showed a significant drop in daily biogas production compared to its average biogas production in the previous phase. The average daily biogas production decreased from an average of 1002.4±46.0 L/m^3 reactor.d in phase 5 to 806.8±106.5 L/m^3 reactor.d. The significant drop in daily biogas production associated with a decrease in the biogas methane content from 70.2±0.7% in the previous phase to 66.0±0.7% in this phase was considered the first warning signs that the methanogens in reactor R2 were starting to be adversely affected by the high FOG% load. Further measurements of the reactor effluent properties also showed a drop in pH to 7.0±0.2, an Alkalinity decrease to 2.75±0.25 g/L as $CaCO_3$, and a TVFA increase to 1.32±0.14, and the TVFA/Alkalinity ratio reached 0.5 by the end of the first week of this phase. These effluent properties further confirmed that reactor R2 was facing a stressing phase, and hence the decision was made to stop the 70% FOG feed on day 157 for R2 and start a recovery phase. Consequently, reactor R2 was fed with 40%FOG:60%TWAS starting from day 158 to the end of this phase and during the next 2 phases (7 and 8).

Unlike reactor R2, reactor R3 showed a significant increase in daily biogas production with the 70% FOG used in this phase, reaching an average daily biogas production of 1274.6±42.1 L/m^3 reactor.d. However, biogas methane content showed a slight decrease, to 71.7±0.4% compared to the methane content obtained in the previous phase (not statistically significant, ($p > 0.5$)). As for R3's effluent properties, the pH slightly decreased to 7.3±0.2 and Alkalinity to 3.19±0.27 g/L as $CaCO_3$, whereas TVFA increased to 1.17±0.15 g/L and TVFA/Alkalinity ranged from 0.3 to 0.4.

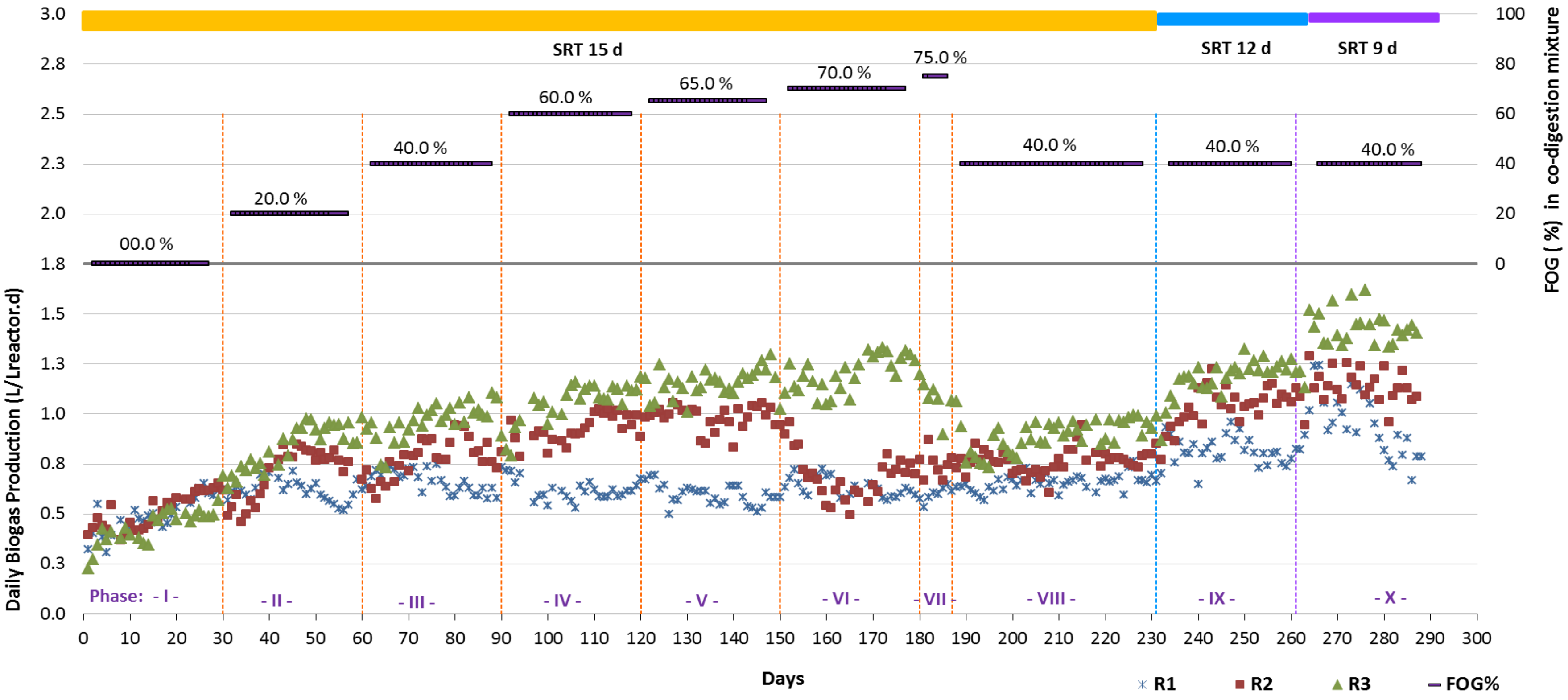
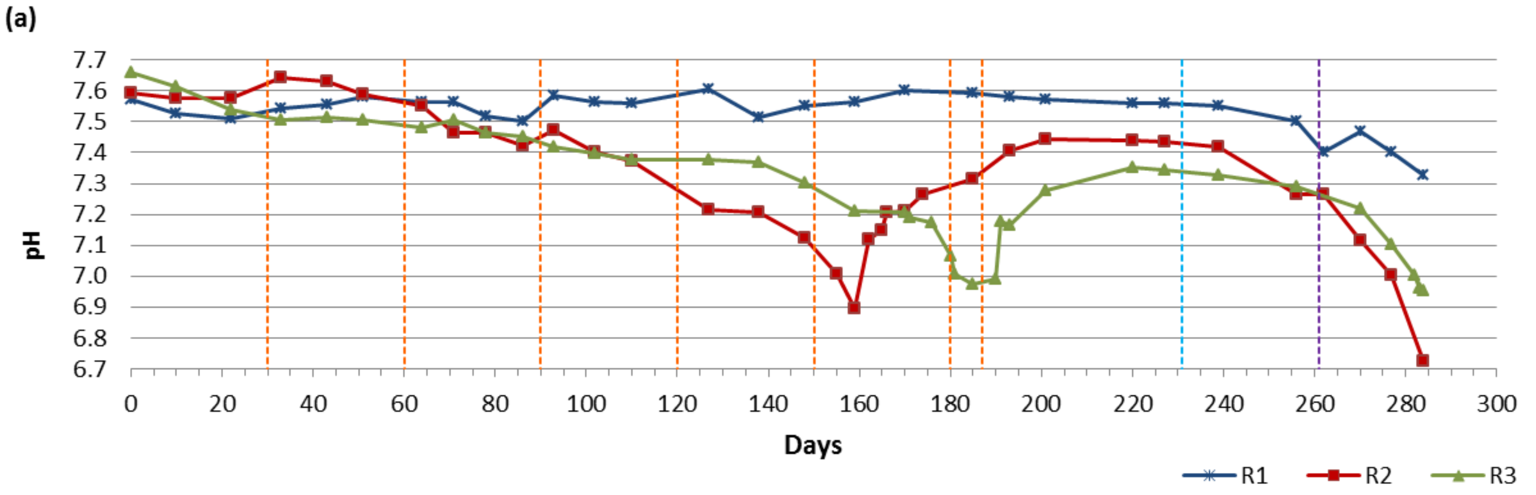


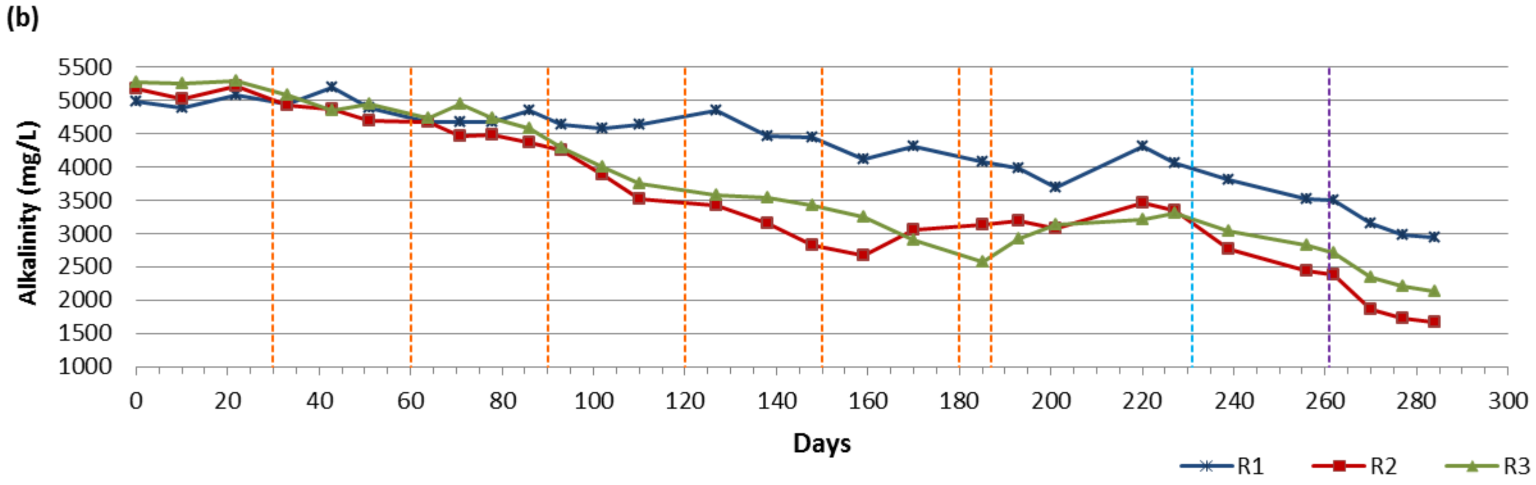
Figure 3.2: Daily biogas production for reactors R1, R2 and R3.

Table 3.3: Average steady-state properties for the semi-continuous reactors R1, R2, R3 and H.

Parameter	FOG	HRT	OLR	Biogas Production	Methane	Methane Yield	Methane Yield	VS reduction	pH	TVFA	Alkalinity
	(%)	(d)	(Kg VS/m^3.d)	(L/m^{3}reactor.d)	(%)	(L/Kg VS$_{added}$)	(L/Kg VS$_{removed}$)	(%)		(mg/L)	(mg/L as CaCO3)
R1	-	15	1.59±0.04	606.0±43.7	61.6±0.4	235.4±13.0	435.4±24.2	52.3±1.6	7.6±0.0	442.8±38.1	4412±334
	-	12	2.03±0.06	775.7±37.4	62.1±0.2	239.4±11.2	446.2±20.9	53.7±0.1	7.5±0.1	676.3±35.8	3607±172
	-	9	2.68±0.01	894.7±137.4	61.7±0.1	180.9±18.2	366.1±36.8	49.0±0.8	7.4±0.1	813.0±70.1	3028±112
R2	20	15	1.59±0.02	792.6±36.3	65.0±0.5	322.4±14.5	567.9±25.5	56.8±0.4	7.6±0.1	477.3±30.1	4748±104
	40	15	1.59±0.03	856.7±73.9	67.8±0.5	362.0±30.6	607.2±51.3	59.6±1.6	7.5±0.0	634.7±65.2	4540±122
	60	15	1.60±0.02	948.7±60.9	70.6±0.2	421.0±25.3	668.0±33.3	63.6±0.3	7.5±0.1	897.3±88.0	4165±250
	65	15	1.62±0.02	1002.4±46.0	70.2±0.7	440.4±20.2	702.1±32.2	62.7±0.8	7.3±0.1	1035.7±79.4	3360±187
	70	15	1.60±0.03	806.8±106.5	66.0±0.7	349.0±53.9	600.3±92.6	58.1±0.1	7.0±0.2	1322.5±142.1	2749±248
	40R[a]	15	1.61±0.01	770.9±25.8	66.6±0.5	319.8±9.9	533.7±16.8	60.1±0.2	7.4±0.0	801.0±47.4	3263±171
	40	12	2.04±0.05	1080.8±44.7	66.2±0.5	356.8±14.2	632.0±25.2	56.4±1.4	7.3±0.1	1177.7±75.2	2528±211
	40	9	2.69±0.03	1143.4±55.6	64.8±0.6	273.9±19.8	554.8±26.7	50.1±0.8	6.9±0.2	1446.0±58.1	1753±96
R3	20	15	1.60±0.08	918.2±56.7	67.2±0.3	387.9±13.1	649.0±22.0	59.8±0.1	7.5±0.1	462.7±31.6	4955±115
	40	15	1.58±0.10	1007.1±50.6	70.9±0.4	443.9±6.3	689.8±9.7	64.4±0.2	7.5±0.0	592.7±47.4	4795±121
	60	15	1.61±0.04	1103.9±43.8	71.9±0.6	496.8±19.4	731.1±28.6	67.9±0.3	7.4±0.0	788.0±71.9	4290±285
	65	15	1.60±0.01	1184.9±55.5	72.4±0.4	534.5±32.7	764.2±46.7	69.9±0.9	7.4±0.0	938.0±65.5	3618±109
	70	15	1.61±0.08	1274.6±42.1	71.7±0.4	576.5±19.0	831.7±27.4	69.3±0.5	7.3±0.2	1171.0±147.9	3185±266
	75	15	1.60±0.01	1063.0±64.8	68.1±0.1	436.8±49.9	662.6±75.7	65.9±1.1	7.0±0.0	1286.0±112.3	2574±131
	40R[a]	15	1.61±0.01	961.7±98.4	68.7±0.9	413.5±15.9	665.8±25.6	62.5±1.7	7.3±0.1	838.5±68.6	3216±88
	40	12	2.03±0.09	1235.6±28.9	67.8±0.4	419.4±10.5	683.2±17.2	61.4±0.4	7.2±0.1	1059.0±31.2	2853±166
	40	9	2.67±0.04	1409.0±48.1	66.7±0.7	351.0±12.1	613.8±21.1	57.2±0.2	7.0±0.1	1208.7±47.7	2223±104
H	20	2	1.60±0.08	38.1±11.6[b]	5.0±0.3	--[c]	--	--	6.8±0.0	1648.0±107.1	1657±116
	40	2	1.58±0.10	32.5±16.3	5.1±0.6	--	--	--	6.7±0.1	1828.3±116.6	1560±151
	60	2	1.61±0.04	31.9±13.1	4.3±0.4	--	--	--	6.3±0.2	1902.3±108.1	1368±78
	65-75	2	1.60-1.61	<32	4.6±.0.5	--	--	--	6.2-5.7	1947.3-2277.5	1182-915

[a] Recovery phase using 40%FOG:60%TWAS feed; [b]due to the ignorable biogas production of reactor H, the values were not included in the calculations for R3 biogas production and methane yields, and [c] values were not calculated for reactor H.


(a)
pH
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
Days
R1
R2
R3


(b)
Alkalinity (mg/L)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
Days
R1
R2
R3

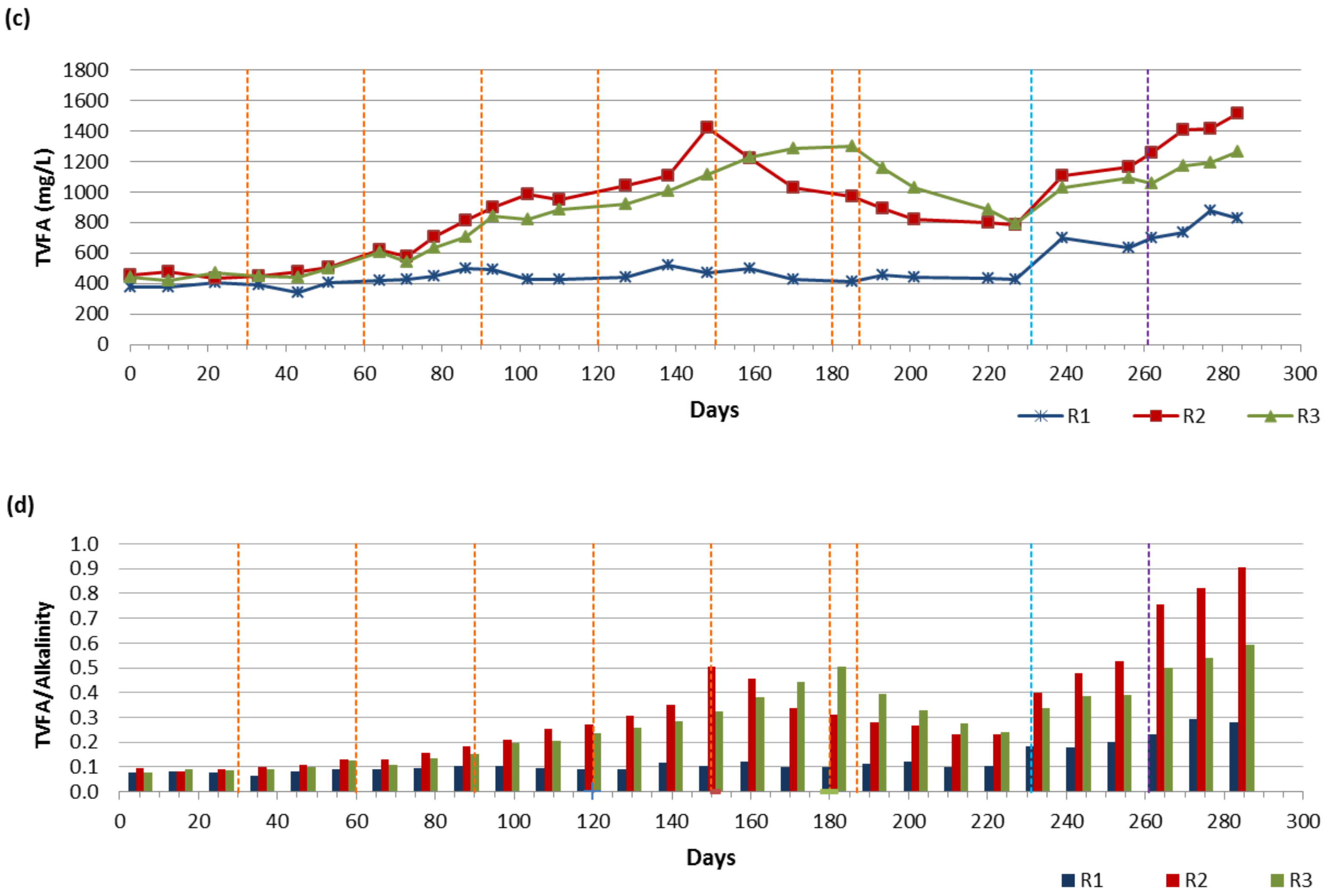


Figure 3.3: (a) pH, (b) Alkalinity, (c) TVFA, and (d) TVFA/Alkalinity ratios for R1, R2, and R3.

Phase 7-75% FOG (days 180-186)

This phase mainly examined the ability of reactor R3 to digest 75% FOG in the co-digestion mixture. By feeding reactor R3 with 75%FOG:25%TWAS (based on total VS), it was noticed that after two days from starting this new FOG load, daily biogas production started to decrease from an average of 1274.6±42.1 L/m^3 reactor.d obtained in the previous phase to 1149.0 L/m^{3}reactor.d. It was noticeable that the daily biogas production continued to decrease at a faster rate to 896.2 L/m^3 reactor.d on day 6 of this phase. The decrease in biogas production occurred along with a significant decrease in the biogas methane%, which was in the range of (68.2-67.8%) within these 6 days compared to 71.7±0.4% on average during the previous phase (phase 6). A decrease in the effluent pH to 7.0±0.0 was also noticed. Alkalinity also decreased to 2.57±0.13 g/L as $CaCO_3$. The TVFA was 1.29±0.11 g/L, and TVFA/Alkalinity ratio was 0.5. Therefore, reactor R3 started showing noticeable signs of stress in all measured parameters. As such, this phase was stopped on day 186, and a recovery phase for reactor R3 was launched using 40%FOG feed.

By the end of this phase (day 186), reactor R2 had completed 28 days of recovery and had an average daily biogas production of 729.0±29.9 L/m^3 reactor .d with 65.9±0.4% methane. This represents about 85% of the average daily methane production obtained for R2 in phase 3 (when the feed contained 40% FOG). This clearly indicates that reactor R2's microbial community was recovering from the stress incurred by the high FOG load (70%FOG during phase 6). This fast recovery was possibly achieved because the reactor did not go through severe upset conditions as the stressing feed was stopped as soon as the first signs of reactor upset appeared. Additional signs of recovery were also noticed from the increase in effluent pH from 7.0 before recovery to 7.2 afterward. Alkalinity also increased from 2.75 to 3.10 g/L as $CaCO_3$ whereas TVFA decreased from 1.32 to 0.90 g/L, and the TVFA/Alkalinity ratio decreased from 0.5 to 0.3 (these measured values for the early recovery of reactor R2 are not shown in Table 3.3).

Phase 8-recovery (days 187-230)

By day 187 of the study, a recovery phase was started for reactor R3 by lowering its FOG feed from 75% to 40%. In the open literature, the recovery phase is usually applied by switching the feed from a co-digestion mixture to the main substrate alone. For example, Wang et al. (2013) switched the TWAS and Grease Interceptor Waste (GIW) co-digestion mixture to TWAS alone, and it took the stressed reactor 36 days to recover. A different recovery approach was used here by changing the co-digestion mixture from 75%FOG:25%TWAS to 40%FOG:60%TWAS. This approach was used because the reactor did not go through severe inhibition or toxicity conditions, as the FOG overdose was determined and tackled at the first signs of reactor stress. Additionally, the goal of the study was to use the reactors for further testing at other HRTs (12 and 9d), and hence, by switching the feed to a lower FOG concentration, this would ensure maintaining the methanogen community that had adapted and acclimated to the FOG:TWAS co-digestion during the study period in reactors R2 and R3.

As a result of reducing the FOG%, it was observed that the effluent pH of R3 started to increase with time, reaching 7.3±0.1 at the end of this phase. It was also noticeable that the Alkalinity increased to 3.22±0.09 g/L as $CaCO_3$ and TVFA decreased to 0.84±0.07 g/L. By the end of this recovery phase, reactor R3 had an average daily biogas production of 961.7±98.4 L/m^3 reactor.d with 68.7±0.9% methane. This represented a 92.7% recovery of the daily methane production achieved in phase 3. Therefore, it is evident that R3 was recovering very fast as compared to reactor R2. It seems that stopping the stressing FOG load at an early stage, when the first signs of a decrease in biogas and methane content are observed, can be considered the key factor in helping save the microbial community in the reactors and hence guarantee a fast recovery for reactor performance.

During this phase, reactor R2 continued showing signs of recovery, such as a slight increase in daily biogas production to an average of 770.9±25.8 L/m^3 reactor.d with a methane content of 66.6±0.5%, a pH increase to 7.4±0.0, an Alkalinity increase to 3.26±0.17 g/L as $CaCO_3$ and a TVFA decrease to 0.80±0.05 g/L on average. At the end of this phase, reactor R2 had completed 73 days of recovery, with 94.5% recovery of its average daily methane production compared to the daily methane production of the same reactor in phase 3 (when the feed was 40%FOG).

Phase 9- HRT 12d (days 231-260)

After both reactors R2 and R3 had recovered almost completely (>92% recovery of the daily methane production), it was time to examine the reactors' performance at lower HRTs. Therefore, in this phase, the HRTs for the three reactors R1, R2, and H-R3 were reduced from 15 to 12d. This was done by keeping the same daily feed/ withdrawal volume of 100 ml/d and reducing the working volume from 1500 ml to 1200 ml for reactors R1 and R2, and from 1200 ml to 1000 ml for R3. The feed was kept at 40%FOG:60%TWAS for reactors R2 and R3 during this phase. When the HRT was reduced to 12 days, reactors R1, R2 and R3 required 14, 15 and 12 days, respectively to adapt to the new reduced HRT and achieve a stable daily biogas production.

The control R1 had an average daily biogas production of 775.7±37.4 L/m^3 reactor.d with 62.1±0.2% methane, representing a 24.2% increase in daily methane production compared to the daily methane production for R1 during the 15d HRT period. The pH was around 7.5±0.1, and the Alkalinity of the effluent decreased to 3.61±0.17 g/L as $CaCO_3$ (Alkalinity was 4.41 on average during the 15d HRT for R1). Additionally, the R1 effluent had a TVFA of 0.68±0.04 g/L and a TVFA/Alkalinity ratio of 0.18-0.20, as shown in Figure 3.3d. This TVFA/Alkalinity ratio was higher than the ratio obtained in the previous phases for reactor R1, but still within the range for stable reactor performance (TVFA/Alkalinity < 0.4).

The HRT reduction to 12 days also positively influenced reactor R2's performance, as it caused an increase in average daily biogas production that reached 1080.8±44.7 L/m^3 reactor.d with 66.2±0.5% methane. This represents a 26.8% increase in the daily methane production for R2 compared to the daily methane production of the same reactor digesting the same feed (R2, phase 3) at HRT 15d. This also represents a 48.6% higher daily methane production compared to the daily methane production of the control reactor R1 at the current HRT (12d HRT), as can be seen in Figure 3.2. This increase in daily methane production directly contributed to the increase in the daily VS load (2.04±0.05 kg VS/m^3.d OLR). It was noteworthy that although reactor R2 had stable performance during the current phase, which was manifested by the stable biogas and methane production, the effluent characteristics started to show slight signs of stress. R2's pH was 7.4±0.0 at the beginning of this phase and decreased slightly to 7.3±0.1 at the end of the phase, Alkalinity decreased from 3.26±0.17 g/L as $CaCO_3$ in the previous phase to 2.53±0.2 g/L as $CaCO_3$ on average during this phase, and TVFA increased to 1.18±0.08 g/L compared to 0.80±0.05 g/L in the previous phase. Reactor R2 had a TVFA/Alkalinity ratio that ranged from 0.4 to 0.5 during the current phase (Figure 3.3d), representing a noticeable increase in the TVFA/Alkalinity ratio compared to the ratio of the same reactor (R2) in the previous phases with 15d HRT. This may indicate that the shorter HRT (12d) added some stress to reactor R2's anaerobic co-digestion process; however, it did not affect the reactor stability or biogas production quantity and quality (methane %).

Finally, reactor R3 showed stable performance during this phase, with an increase in daily biogas production reaching an average of 1235.6±28.9 L/m^3 reactor.d with 67.8±0.4% methane. This represents a 17.6% higher daily methane production compared to the daily methane production of the same reactor (R3) digesting the same feed (40%FOG) at HRT 15d (phase 3). This increase also represents a 74.3% higher daily methane production compared to the daily methane production of the control R1 at the same HRT (12d HRT). As for the effluent characteristics of reactor R3 shown in Figure 3.3, the pH was 7.2±0.1 on average during this phase, Alkalinity decreased to an average of 2.85±0.17 g/L as $CaCO_3$, TVFA increased to an average of 1.06±0.0 g/L, and the TVFA/Alkalinity ratio ranged from 0.3 to 0.4. This TVFA/Alkalinity ratio indicates that reactor R3 had a more stable performance compared to R2 in this phase because reactor R3's TVFA/Alkalinity ratio was still within the acceptable range for healthy reactor performance.

Phase 10 (Days 261-287)

On day 261, reactors HRT's were reduced to 9d by maintaining the same withdraw/feed daily volume as in phase 9 (100ml) and decreasing the reactors' working volumes to 900 ml for R1 and R2 and 700 ml for R3. The feed mixture in this phase was also 40%FOG:60%TWAS, as in the previous phase.

As the new HRT (9d) was applied, the control reactor R1 was not able to reach steady state daily biogas production except for seven successive days which had daily biogas production with ≤10% fluctuation (days 276-282, as can be seen from Figure 3.2). The average daily biogas production for reactor R1 was 894.7±137.4 L/m^3 reactor.d with 61.7±0.1% methane. This represents a 31.7 and 18.6% increase in the daily methane production per L of reactor compared to the same reactor's daily methane production during the 15d HRT period (days 1-230) and the 12d HRT period (phase 9), respectively. The changes in pH, Alkalinity, and TVFA showed in Figure 3 indicate that R1 required more time to adapt to the new 9d HRT, as the short 9d HRT added more stress to the reactor.

As for reactor R2's performance in this phase, the decrease in HRT led to a minor increase in daily biogas production (not statistically significant, $p > 0.05$) compared to the previous phase (phase 9 with 12d HRT), as shown in Figure 3.2. Furthermore, the methane content in the produced biogas decreased from 66.2±0.5% in the previous phase to 64.8±0.6% in this phase. Although this decrease in methane% seems to be minor, it is statistically significant ($p < 0.05$). The effluent pH also dropped to an average of 6.9±0.2, Alkalinity decreased to 1.75±0.10 g/L as $CaCO_3$ on average, and the average TVFA level increased to 1.45±0.06 g/L. These changes in the effluent Alkalinity and TVFA caused a significant increase in the TVFA/Alkalinity ratio, to a range of (0.8-0.9) during this phase. The drastic increase in the TVFA/Alkalinity ratio in addition to the appearance of a thin floating layer of foam in the reactor, which was in the form of floating aggregates (most likely a mix of biomass and FOG) on the liquid's surface, clearly indicate that reactor R2 was facing stressing conditions and likely methanogens inhibition due to the high OLR (low HRT, 9d).

Finally, it was observed that R3 exhibited a further increase in daily biogas production to reach an average of 1409.0±48.1 L/m^3 reactor.d compared to its average daily biogas production in phase 9. This increase might have been mainly caused by the increase in the organic loading rate during this phase. However, the methane content of the biogas slightly decreased to 66.7±0.7% compared to 67.8±0.4% in phase 9. The effluent pH of reactor R3 decreased to 7.0±0.1, Alkalinity dropped to 2.22±0.10 g/L as $CaCO_3$ on average, and TVFA increased to 1.21±0.05 g/L. The TVFA/Alkalinity ratio ranged from 0.5 to 0.6. Although this may point to minor stress occurring in the reactor due to the short HRT (9d), R3 still had reasonably stable performance, as can be deduced from its stable daily biogas production (Figure 3.2). It is worth mentioning that reactor R3 did not have any foaming problem during the whole study period as was observed with reactor R2. At this stage, it can be concluded that the dual-reactor system H-R3 exhibited not only stable performance for all three HRTs (15, 12 and 9d) but also showed improved rates of biogas production with considerably high methane content.

3.4.2 VS Reduction and Methane Yields

The average VS% reductions and methane yields as L methane per kg VS added and per kg VS removed were calculated for the three reactors R1, R2, and R3 in order to better characterize their performance for all phases. These values are listed in Table 3.3 (values are the steady state average ± standard deviation). As can be seen from Table 3.3, reactor R1 had an average VS reduction of 52.3±1.6% and an average methane yield of 235.4±13.0 L_{CH4}/kg VS_{added} (equivalent to 435.4±24.2 L_{CH4}/kg $VS_{removed}$) during the 15d HRT period. When the HRT was decreased to 12d, the VS reduction and methane yield for R1 did not show any significant changes; however, when the HRT was further decreased to 9d, a significant decrease in VS reduction and methane yield occurred, to an average of 49.0±0.8% and 180.9±18.2 L_{CH4}/kg VS_{added}, respectively. These VS and methane yield results reveal that the control reactor R1 could perform a stable anaerobic digestion process for an HRT ranging from 15 to 12d, but could not maintain this stability with 9d HRT.

Figure 3.4 below shows the improvement (%) in reactors' R2, and R3 methane yields (as L_{CH4}/kg VS_{added}) compared to the control yields during the 15d HRT period for the different FOG:TWAS feed mixtures used in the study. The data in the figure represent the averages (number of samples, n ≥ 4), and the error bars represent the 95% confidence intervals. The improvement in methane yield (%) was calculated using Equation (3.1).

$$Improvement\ \% = \frac{(Methane\ Yield)reactor-(Methane\ Yield)control}{(Methane\ Yield)control} \times 100\% \qquad (3.1)$$

Where: *(Methane Yield) reactor* is the methane yield calculated for reactors R2 and R3 at the different FOG% as L_{CH4}/kg VS_{added} and *(Methane Yield) control* is the methane yield calculated for reactor R1. R1 is the control reactor during the current study.

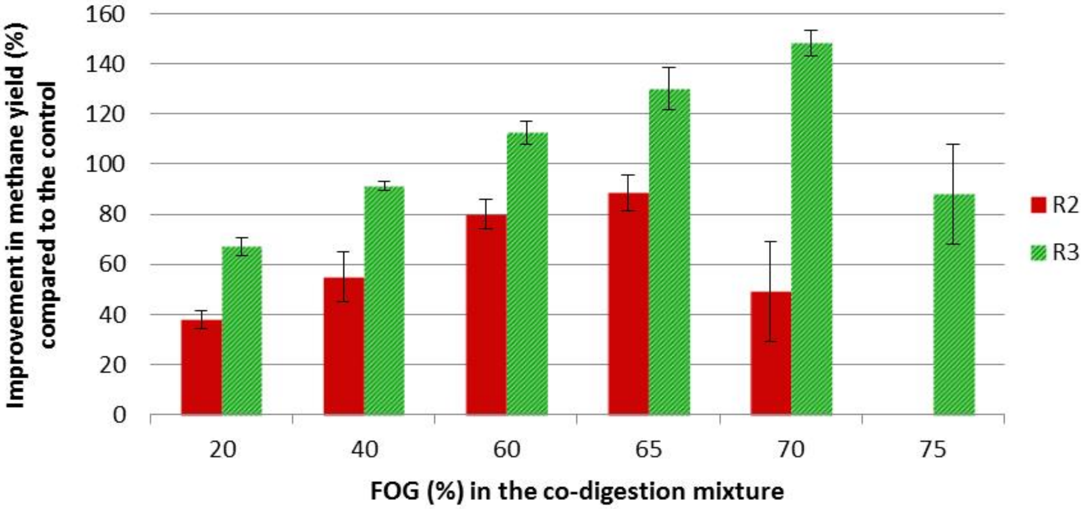


Figure 3.4: Improvement (%) in methane yield (L $_{CH4}$/kg VS_{added}) for reactors R2 and R3 compared to the control R1 during the 15d HRT.

Figure 3.4 shows clearly how the dual-stage reactor H-R3 (represented by R3 in the figure) had consistently better improvement (%) in methane yields as compared to reactor R2. Reactor R3's advantage in generating methane became more evident at higher FOG% (60%-75%); for instance, when 70% FOG was used, R3 had a 148.2% higher methane yield compared to the control R1. In comparison, the highest methane yield improvement reached by R2 was only 88.3% compared to the control, and this was reached at 65% FOG. As regards the maximum VS reduction achieved at 15d HRT, it was 69.9±0.9% at 65% FOG for R3 and 63.6±0.3% at 60% FOG for R2.

The calculated VS reduction and methane yield for R2 and R3 at other HRTs, namely at 12d and 9d, are provided in Table 3.3. R3 outperformed R2 in stability and biogas production in these HRTs, as described in detail in Section 3.1.

3.4.3 Pathogen Destruction

To evaluate reactor R1, R2 and R3's ability to produce effluents that meet Class A biosolids requirements as specified in 40 CFR Part 503 regulations (USEPA, 2016) and the CP1 Criteria for Ontario, Canada biosolids regulations (CCME, 2016), the total coliforms were measured for the feed and the reactors effluents during phases 8, 9 and 10. The feed was 40% FOG:60% TWAS at the three tested HRTs, 15, 12 and 9d. Both regulations require a concentration of Total Fecal Coliform (CFU) <1000 MPN/gTS (MPN/gTS; this represents the Most Probable Number of fecal coliform per g of TS) for the biosolids to be considered a Class A biosolids, i.e., safe for storage, sale and land application. Total coliform is a broader class of coliforms which includes fecal coliforms, and hence total coliforms are higher or in limited cases equal to the fecal coliforms in the sample (Coelho et al., 2011). Figure 3.5 summarizes the results of the coliform tests obtained in this study. The data in the figure represent the average values, and the error bars represent the 95% confidence intervals for replicates during the steady state conditions for each HRT. An important point to be considered when testing biosolids considered for Class A is that every sample must have CFU <1000 MPN/gTS, not just the average (USEPA, 2016).

As it can be seen from Figure 3.5, the control R1 was able to produce a Class A effluent that has CFU <1000 MPN/gTS when the HRT was 15 and 12d the fecal coliform concentration was decreased by 4.1 and 4.3-Log, respectively. However, at HRT 9d, one of the three R1 samples had CFU >1000 MPN/gTS (1613 MPN/gTS), and hence it was concluded that reactor R1 could not provide Class A quality effluent at HRT 9d. The same observations were found applicable for reactor R2, which co-digested a 40%FOG:60%TWAS mixture. This reactor provided a Class A effluent with CFU <1000 MPN/gTS for both HRT 15 and 12d, but it failed to provide Class A effluent at HRT 9d, as the reactor effluent had CFU that ranged from 920 to 1840 MPN/gTS. It is noteworthy that the current results agree with those of Coelho et al. (2011), who found that conventional mesophilic anaerobic digestion of TWAS was not sufficient to reduce coliforms to the 1000 MPN/gTS limit, even with HRT 20d; however thermophilic anaerobic digestion for HRT ≥ 10d was sufficient to eliminate the pathogens and produce effluent with coliforms less than the 1000 MPN/gTS limit. Another study done by Riau et al. (2010) determined the required HRT for providing Class A biosolids from the digestion of sewage sludge in a temperature-phased anaerobic digestion system (TPAD). The authors used a 4-6 day thermophilic phase followed by a mesophilic treatment in the TPAD system with a total HRT of 19d to obtain a final product that could be classed as Class A biosolids.

As for reactor R3, it is evident from Figure 3.5 that it was able to produce an effluent with undetected total coliforms for all three HRTs, 15, 12 and 9d. The total inactivation of the CFU achieved by reactor R3 confirms the effectiveness of the hyper-thermophilic/ thermophilic dual-stage system in reducing the CFU to below Class A limits for all three HRTs, 15, 12 and 9d. This confirms the advantage of the high temperature of 70° C in addition to the high TVFA concentrations resulting from solubilizing the co-digestion mixture (during the 2-day hyper-thermophilic stage (H) followed by thermophilic digestion conditions for the rest of the HRT) in achieving a Class A biosolids effluent even within an HRT of 9d (2d hyper-thermophilic plus 7d thermophilic).

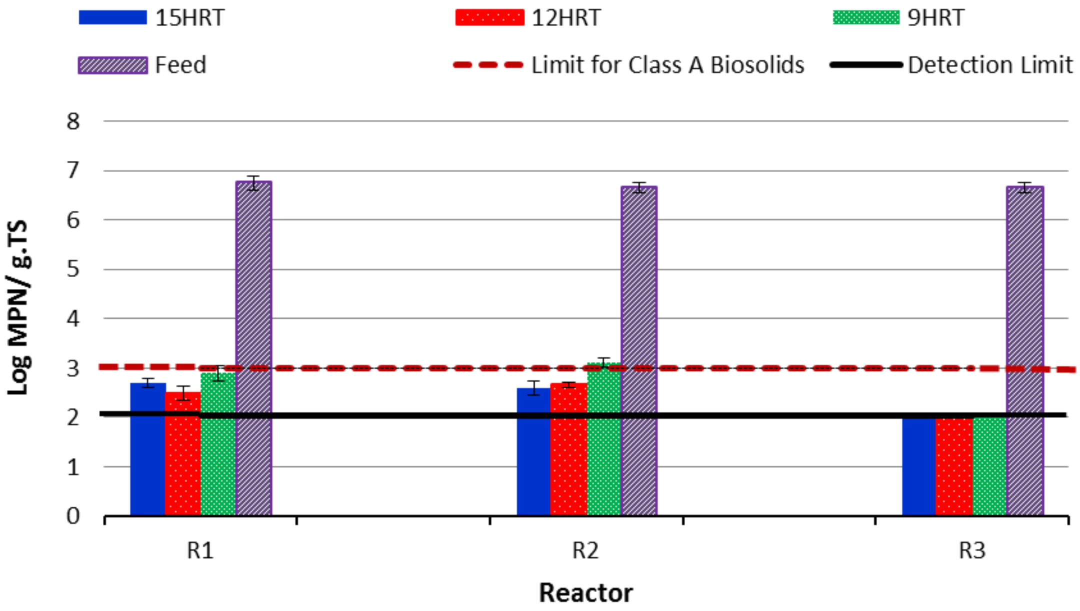


Figure 3.5: Total fecal coliform results for feed and reactors R1, R2, and R3 effluents.

3.4.4 The Net Energy Analysis

To compare the energy balance for R1, R2 and R3, the input, output and net energy per kg of VS were calculated for all HRTs and FOG% used in the study. The input energy required to heat each sample to the required temperature (55°C for thermophilic reactor and 70°C for the hyper-thermophilic reactor) was calculated using Equation (3.2) (Dhar et al., 2012).

$$Q_S = \rho_S V_s C_p (t_{final} + t_{initial}) \tag{3.2}$$

Where Q_S is the energy required to heat the sample (in kJ), ρ_S is the density of the sample (in g /L), V_s is the volume of the sample (in L), C_p is the specific heat of AD mixture (in kJ/kg °C), $t_{initial}$ is the initial temperature in °C, and t_{final} is the final temperature in °C. The following assumptions were used for the input energy calculations: (a) the initial sample temperature = 25 °C; (b) the energy balance is calculated per kg of VS in the mixture; and (c) the specific heat for samples equals the specific heat for sludge = 4.18 kJ/ kg °C (Dhar et al., 2012).

The output energy from the AD process was calculated using the methane yield and VS results for all conditions. The assumptions used for the energy output calculations were: (a) energy content of methane = 35.8 kJ/L methane (Nasr et al., 2012); and (b) Methane recovery efficiency = 90% and methane to energy conversion efficiency = 95% (Hosseini et al., 2014).

Next, the net energy production as kJ/kg VS was calculated using Equation (3.3).

$$Net\ energy = Output\ energy - Input\ energy \tag{3.3}$$

The calculated net energy for the three reactors R1, R2, and R3, is shown in Figures 3.6a and b. The data in Figure 3.6a represent the net energy calculated at HRT 15, 12 and 9d for R1, R2, and R3 when R2 and R3's feed contained 40% FOG, and Figure 6b shows the net energy production for R2 and R3 at 15d HRT using 20, 40, 60, 65 and 70% FOG.

The results in Figure 3.6a show that R1 had negative net energy for all three HRT conditions tested during the study. However, R2 and R3 had positive net energy when co-digesting mixtures with 40% FOG at 15 and 12d HRT. It is also noticeable that R2 and R3 had negative net energy for the shortest HRT (9d). These results show the advantage of FOG addition as a co-substrate in converting the AD process from negative net energy to a positive net energy process at HRT 15 and 12d. Figure 3.6b further compares R2 and R3's net energy at different FOG% within the 15d HRT period, and it is clearly shown that R3 had higher net energy at all FOG% compared to R2. The only negative net energy observed in Figure 6b was for R2, at 20% FOG. This indicates that R2 needs more than 20% FOG in the co-digestion mixture in order to reach a positive net energy status. For both reactors R2 and R3, the net energy increased with increased FOG% until reaching the threshold for each reactor. Therefore, the maximum net energy obtained was at 65% FOG for R2 and at 70% FOG for R3. The energy balance analysis for R3 confirms a promising positive net energy for HRT 15d with 40-70% FOG.

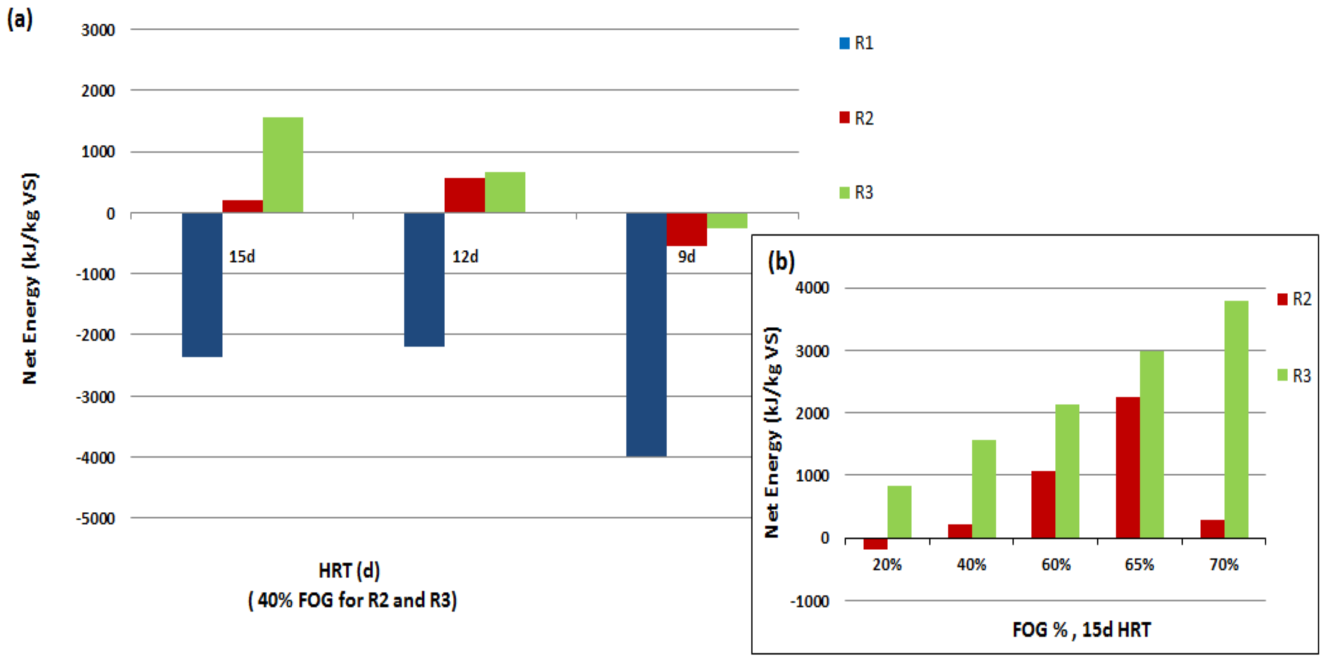


Figure 3.6: Net energy production for (a) R1, R2 and R3 at 15, 12 and 9d HRT (40% FOG for R2 and R3), and (b) R2 and R3 with different FOG% at 15d HRT.

3.5 Conclusion

The dual-stage hyper-thermophilic/ thermophilic system (H-R3) proposed in this study has been shown to be efficient in co-digesting FOG:TWAS mixtures containing up to 70%FOG at an HRT of 15d. This was apparent from the considerable improvement in biogas production quantity and quality achieved using the dual-stage hyper-thermophilic/thermophilic system as compared to the thermophilic-only reactor R2. It was also shown that the dual-stage hyper-thermophilic/thermophilic system can co-digest 40%FOG mixtures at lower HRTs of 12 and 9d, yet produce high methane yields and effluents that meet Class A biosolid standards. This has a real potential to reduce waste volume, as less time is needed for an efficient, stable co-digestion process. The energy balance achieved in this study proves the feasibility of the dual-stage hyper-thermophilic/thermophilic system, as it was consistently able to produce positive net energy at HRTs of 15 and 12d, with higher net energy values for greater FOG%.

3.6 References

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CHAPTER 4

Microwave vs. Alkaline-Microwave Pretreatment for Enhancing Thickened Waste Activated Sludge and Fat, Oil, and Grease Solubilization, Degradation, and Biogas Production

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4.1 Abstract

The effects of microwave (MW) pretreatment and combined alkaline-MW pretreatment on the co-digestion of TWAS:FOG mixtures with 20, 40 and 60% FOG were investigated. MW pretreatment at a high temperature of 175°C was shown to be the most effective MW pretreatment option in solubilizing TWAS:FOG mixtures and boosting methane yield. It resulted in maximum solubilization (%) of 68.2% for the 20%FOG samples and a maximum methane yield that was 137% higher than the control for samples with 60%FOG. The combined alkaline-MW (NaOH-MW) pretreatment at pH 10 proved to be an ineffective option for TWAS:FOG pretreatment before the anaerobic co-digestion. Despite the benefits of MW pretreatment on the TWAS:FOG samples, including significant solubilization increase, dewaterability improvement, high VS reductions and methane yields, the net energy analysis resulted in

negative values for all MW-pretreated samples due to the high energy consumption required for the MW pretreatment option.

4.2 Introduction

Massive quantities of sewage sludge and particularly Thickened Waste Activated Sludge (TWAS) are continuously produced as the main by-product from conventional wastewater treatment processes. Sludge treatment and disposal take up a significant portion of wastewater treatment plant (WWTP) operating costs, and can easily reach up to 50-60% of the total operating cost of a plant (Zhen et al., 2017; Eswari et al., 2016). TWAS contains numerous harmful substances such as heavy metals and organic and inorganic contaminants, as well as pathogens, which cause unpleasant odors and hygiene concerns. Improper disposal of TWAS can severely contaminate the environment, and it can be hazardous to public health (Alqaralleh et al., 2015; Zhen et al., 2014). Because of the aforementioned reasons, TWAS management, treatment and disposal are worldwide challenges that need immediate attention (Zhen et al., 2017; Wang and Li, 2016).

Anaerobic digestion (AD) is one of the most common stabilizing methods recognized for TWAS treatment (Braguglia et al., 2015). The biogas produced during the AD treatment is a potential source of methane (Hosseinlou et al., 2018; Lee et al., 2015). This particular advantage puts the AD process at the top of the favorite TWAS treatment methods list, as it not only stabilizes, reuses, and reduces the waste but also produces a clean renewable source of energy. However, there are some limitations associated with the AD of TWAS that reduce its feasbility: namely, the low biogas yield and the limited biodegradability of TWAS, and that TWAS usually achieve less than 45% Volatile Solids (VS) reduction through the traditional mesophilic AD, and thus more than the half of the organic matter in TWAS is either non-biodegradable or not available for biodegradation (Coelho et al., 2011; Toreci et al., 2010).

TWAS consists mainly of microbial cell biomass and extracellular polymeric substances (EPS) (Coelho et al., 2011). Cell membranes and EPS have been shown to be a physical and chemical obstruction respectively facing the anaerobic degradation of TWAS, making hydrolysis the rate-limiting step in the AD of TWAS. This makes the AD process retention time quite long (20-50days) (Wang and Li, 2016). Anaerobic co-digestion of TWAS by using Fat, Oil, and Grease (FOG) as a co-substrate has been shown to be an attractive method for increasing biogas production and methane yield from the AD process. The increase in methane yield using FOG as a co-substrate is due to the high carbon content of FOG, which causes a direct increase in methane potential compared to TWAS and other organic wastes (Alqaralleh et al., 2018; Klaucans and Sams, 2018; Li et al., 2015; Mata-Alvarez et al., 2014).

Applying an effective pretreatment method on the co-digestion mixture before the AD process can significantly improve the digestion process by enhancing solubilization and disintegration (Li et al., 2013). Pretreating TWAS before the AD process has been applied by other researchers using a wide range of pretreatment options in order to enhance the degradation process and biogas/ methane production (Li et al., 2015; Coelho et al., 2014). Some of these pretreatment methods include chemical (e.g., acid or alkaline reagents), thermal, mechanical, microwave (MW), ultrasound (US), and biological pretreatments and their combinations (Eskicioglu et al., 2017; Cella et al., 2016; Jian et al., 2016; Wei et al., 2015; Kuglarz et al., 2013; Sambusiti et al., 2013; Shahriari et al., 2012, Eskicioglu et al., 2007). Among all the mentioned pretreatment methods, the MW irradiation method has proved to be promising for solubilizing and disintegrating TWAS (Ara et al., 2014; Coelho et al., 2011; Toreci et al., 2009; Eskicioglu et al., 2007).

MW irradiation provides rapid uniform internal heating which in turn causes a significant increase in the soluble fraction of chemical oxygen demand (SCOD), and thus it increases the efficiency of digestion by increasing the soluble organic materials available for AD (Eswari et al., 2016; Coelho et al., 2014). In addition to the thermal effects, it has been proved that MW irradiation changes the dipole orientation of the polar molecules, which is known as the athermal (non-thermal) effect of MW (Coelho et al., 2014; Chi et al., 2011). The combined thermal and athermal effects of MW irradiation solubilize the sludge through three mechanisms, as suggested by Coelho et al. (2014): i) dissociating the macromolecules into smaller units; ii) liberating the organic matter enclosed within the bacterial cells; and iii) reinforcing the release of EPS, which is part of the floc matrix in the sludge.

Some studies have tested the effect of using an alkaline reagent before applying MW irradiation in order to get stronger solubilization effects from the MW pretreatment. The alkaline pretreatment prior to MW irradiation induces the swelling of particulate organics and weaken the cell walls (Zhen et al., 2014). This has been found to cause high disintegration and solubilization during the MW irradiation step. For example, in Dogan and Sanin's (2009) study on waste activated sludge (WAS), an alkaline (NaOH) reagent was used to enhance TWAS solubilization prior to MW pretreatment, and it was found that alkaline-MW pretreatment is more effective than MW pretreatment alone for TWAS solubilization. The optimum conditions for anaerobic biodegradation performance in Dogan and Sanin's (2009) study were determined to be pH 12 and MW temperature of 160°C). Combined alkaline-MW pretreatment methods have also been tested for use on TWAS and other types of organic waste prior to AD (Zhen et al., 2014; Erden, 2012). However, the use of pretreatment methods on the TWAS:FOG mixtures before the anaerobic co-digestion process is scarce in the open literature. One of the very few pretreatment applications on sludge and FOG mixtures was a study done by Li et al. (2015), who applied US and thermochemical (NaOH) pretreatments on municipal wastewater sludge and FOG mixtures before anaerobic co-digestion. Li et al. found that thermo-chemical pretreatment at pH 10 and 55ºC temperature provided the best pretreatment conditions, and resulted in 9.9±1.5 % increase in methane production compared to co-digestion of the same mixture without pretreatment (Li. et al., 2013).

MW and alkaline-MW are two effective pretreatments that have been successfully tested on single substrates, especially TWAS before AD, and have resulted in positive effects on the AD digestion process and biogas/methane production. However, to the best of our knowledge these two options have not been tested yet on TWAS:FOG mixtures as a pretreatment before anaerobic co-digestion. Therefore, the main objective of this study is to investigate the effects of applying MW and alkaline-MW pretreatments using different MW irradiation temperatures (95, 135 and 175 °C) on the solubilization, anaerobic degradation and biogas production from the anaerobic co-digestion of TWAS:FOG mixtures containing different FOG %s (20, 40 and 60%).

4.3 Materials and Methods

4.3.1 Substrates origin and characteristics

TWAS sample used for the current study was provided by the City of Ottawa’s Robert O. Pickard Environmental Center (ROPEC), Gloucester, Ontario, Canada. ROPEC is a secondary wastewater treatment plant (WWTP) that performs preliminary and primary treatment followed by a conventional activated sludge process over a solids retention time (SRT) of 5 days on average. TWAS sample contained 5.0±0.1% total solids (TS) with 72.0±1.2% VS.

The FOG sample was obtained from Organic Resources Management Inc. (ORMI), located in Ottawa, Ontario, Canada. ORMI provides grease-trap cleaning services for different wastewater treatment plants in Ontario. The FOG sample contained 28.3±1.2% TS with 98.0±0.1% VS. Both substrates (TWAS and FOG) were stored at 4ºC before use. The substrates were allowed to reach room temperature before the experiments and tests.

The thermophilic anaerobic inoculum was the effluent of a 10L thermophilic AD reactor that had been digesting ROPEC TWAS at an HRT of 20-24 days for more than three years in the lab. The thermophilic effluent had 2.9±0.1% TS with 52.3% VS.

4.3.2 MW pretreatment method

MW pretreatment was carried out using a Mars X MW unit (Microwave Accelerated Reaction System, 1250W ±15% at full power, 2450MHz, temperature range 25-260ºC, CEM Corporation, NC, USA). Mars-5 units have temperature and pressure sensors, allowing the user to accurately select the required operating conditions including temperature, pressure, intensity and holding time. For the current study, MW pretreatment for TWAS:FOG mixtures (with 20, 40 and 60%

FOG based on total VS) was applied using three different MW temperatures: namely, 95, 135 and 175°C (Figure 4.1). The intensity and holding times for the MW pretreatment were chosen to be at a low intensity of 3.5°C/min and a minimum holding time of 1 min, respectively. The selected intensity and holding time values were selected based on findings and suggestions for optimum MW intensity and holding times reported in studies in the literature that used MW pretreatment for TWAS (Koupaie and Eskicioglu, 2016; Ara et al., 2014; Toreci et al., 2011). A holding time of 1 min should eliminate any adverse effects that may occur because of longer holding times, such as enzyme inactivation, formation of refractory compounds with low biodegradability, and reaching inhibitory concentrations of ammonia (Ara et al., 2014; Coelho et al., 2011). TWAS:FOG samples with different FOG% were MW irradiated until reaching the desired temperature (95, 135 and 175°C) in 12 Teflon vessels (40 g sample in each vessel). The Teflon vessels were capped and dressed with special composite sleeves before being placed on the rotating turntable inside the MW machine. After reaching the target temperature, the samples were kept for a 1 min holding time before removal from the MW machine and were let cool to room temperature before opening in order to avoid any mass loss due to evaporation. Next, the pretreated samples were stored at 4°C until used for the experiments and tests. The samples were used within one week of pretreatment.

4.3.3 Alkaline-MW pretreatment method

Alkaline-MW pretreatment was applied using 5N NaOH solution. NaOH solution was added to the TWAS:FOG samples gradually with continuous mixing until reaching a pH of 10.0±0.1, and then the pretreated samples were distributed in 40g portions in 12 Teflon vessels for the next MW irradiation. MW irradiation was then done using the same conditions and procedure mentioned in Section 2.2. After the alkaline-MW (NaOH-MW) pretreatment, the pH of the pretreated samples was not adjusted back to neutral.

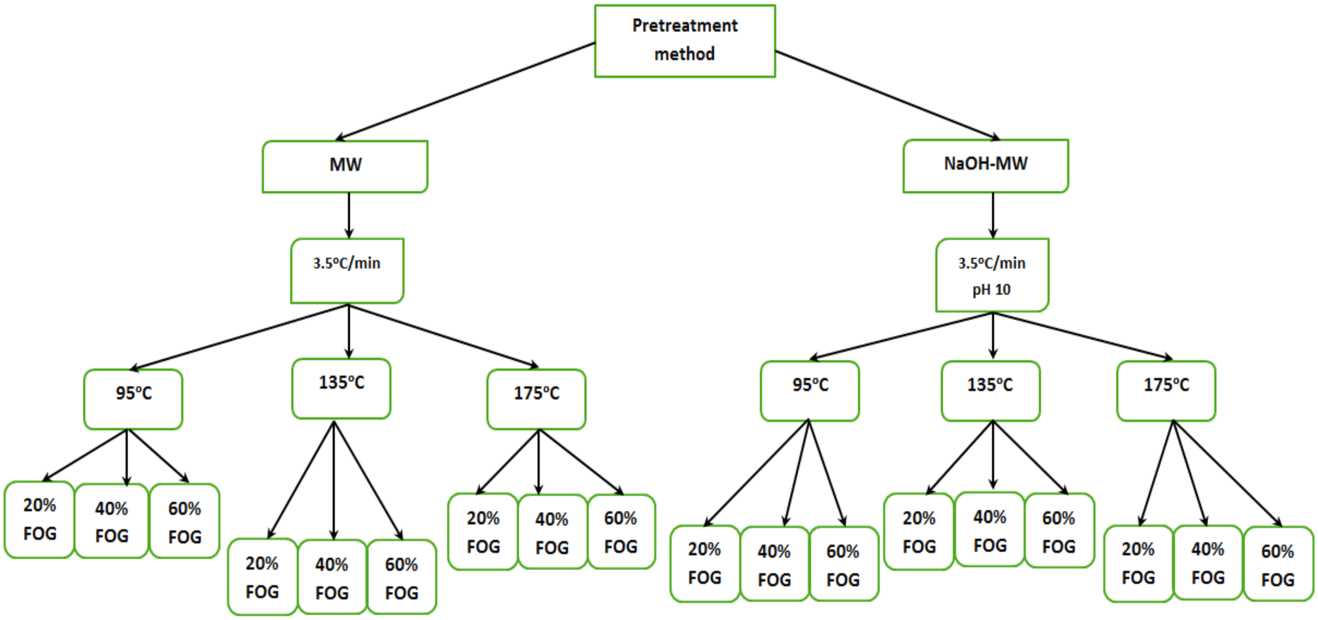


Figure 4.1: The experimental design used for the current study.

4.3.4 Batch anaerobic co-digestion tests

The MW and NaOH-MW pretreated samples were used for batch anaerobic co-digestion assays (biochemical methane potential (BMP) assays). Anaerobic co-digestion experiments were conducted in a series of 300ml glass serum bottles with working volumes (samples + inoculum) of 200ml. The TWAS:FOG samples and inoculum were added to the serum bottles to obtain a substrate to inoculum (S/I) ratio of 0.6 (g $TVS_{substrate}$/ g $TVS_{inoculum}$). This ratio has been found to be the optimum S/I ratio for TWAS:FOG co-digestion (Alqaralleh et al., 2016). Alkalinity was added to all the BMP serum bottles containing the pretreated samples using equal amounts of $NaHCO_3$ and $KHCO_3$ to provide approximately 4000±1000 mg/L alkalinity as $CaCO_3$. The pH of all

BMP bottles was kept within the ideal range for anaerobic digestion (6.5-7.5) (Droste, 1996). Next, the bottles were purged with N_2 gas for about 2-3 minutes to remove oxygen from the headspace and maintain anaerobic conditions. Finally, the bottles were capped and kept in a temperature-controlled shaker (New Brunswick Scientific Co. Inc., NB, Canada) at 55±1°C and 90 rpm. BMP tests were run in triplicate for each condition. Three BMP bottles containing only thermophilic inoculum were used as blank BMP bottles.

4.3.5 Analytical methods

The temporal biogas production in the BMP bottles was measured periodically using a gas manometer. The net biogas volumes for the samples were calculated by subtracting the biogas produced in the blank bottles from the biogas produced in each of the sample bottles (sample + inoculum). The net biogas volumes were then corrected to standard ambient temperature and pressure (STP) conditions (25°C and 1 atm). The biogas composition was monitored weekly using a Hewlett Packard 5710A gas chromatograph equipped with a 3380A model integrator and 5705A thermal conductivity detector, and using helium as the carrier gas. pH measurements were performed using a Fisher Accument® XL25 pH meter. Soluble chemical oxygen demand (SCOD) and total chemical oxygen demand (COD) were measured using HACH TNT 822 (20-1500 mg/L) and TNT 823 (250-15000 mg/L) respectively, including spectrophotometer, heating block, and supplies. Alkalinity and TVFA were measured using HACH TNT 870 (25-400 mg/L $CaCO_3$) and TNT 872 (50-2500 mg/L CH_3COOH), respectively. For measuring the total solids (TS), 20 g of samples were dried at 105 °C for 24 h before weighing the residue of each sample. The dried samples then were heated at 550 °C for 2 h to calculate the volatile solid (VS) based on the weight lost after ignition the according to the standard method 2540G (APHA, 1998). Dewaterability was determined without polymer addition using a capillary suction timer (Fann Instrument Company, Model 440, TX) the according to procedure 2710G (APHA, 1998).

4.3.6 Experimental design and data analysis

A multi-factorial design was used to investigate the effects of three different variables on pretreatment efficiency. The variables were: (i) MW Temperature, (ii) FOG% in the samples, and (iii) the addition of NaOH to provide a pH equal to 10. Table 4.1 shows the variables tested and their levels.

Table 4.1: Variables used in the statistical design and their levels.

Variable level	MW Temperature (°C)	FOG[a] (%)	NaOH
1	95	20	NaOH (@pH 10)
2	135	40	No NaOH
3	175	60	

[a] FOG % in the co-digestion sample as a percentage of total VS.

To measure the expected enhancement effect of pretreatments on the samples' hydrolysis, the percentage of SCOD increase of each sample after pretreatment was calculated using Equation (4.1).

$$\textbf{\textit{Solubilization (\%)}} = 100 * (SCOD_{final} - SCOD_{initial})/ \; SCOD_{initial} \tag{4.1}$$

Where SCOD $_{\text{final}}$ is the SCOD of the sample after pretreatment, and SCOD $_{\text{initial}}$ is the SCOD of the sample before pretreatment.

4.3.7 Central composite design

The Central Composite Design (CCD) of response surface methodology (RSM) provided by Design Expert software (Stat-Ease 11.0 version) was used to analyze the results of the MW and NaOH-MW pretreated samples and to determine the best regression model that best represents the solubilization (%) and the methane yield (ml CH_4/ g VS_{added}). CCD is a mathematical method that analyzes the relationships among the tested variable factors by first estimating the linear relationships and interactions between the variables, and second by determining the effects on the response surface (Zou et al., 2016; Rakić et al., 2014).

In the current study, MW temperature (variable A) and FOG% (variable B) were the independent variables. Their three levels (95, 135 and 175 °C for MW temperatures and 20, 40 and 60% for FOG%) were coded between +1 for the upper limit and -1 for the lower limit. The runs for the CCD are shown in Table 4.2. The functional relationship between the responses (Solubilization (%) and Methane Yield) and factors (A and B) will be given as a second-order quadratic polynomial model (the best fit for the experimental data of the current study); Equations (4.2) and (4.3) for MW and NaOH-MW pretreatments.

$$\textit{\textbf{Solubilization}} = \beta_0 + \beta_1 \mathbf{A} + \beta_2 \mathbf{B} + \beta_{11} \mathbf{A}^2 + \beta_{22} \mathbf{B}^2 + \beta 12\mathbf{AB} \tag{4.2}$$

$$\textit{\textbf{Methane Yield}} = \beta_0 + \beta_1 \mathbf{A} + \beta_2 \mathbf{B} + \beta_{11} \mathbf{A}^2 + \beta_{22} \mathbf{B}^2 + \beta 12\mathbf{AB} \tag{4.3}$$

where A and B are the MW temperature and FOG% in the current study, respectively, β_0 is the regression intercept, and β_1, β_2, β_{11}, β_{22} and β_{12} are the model coefficients.

Table 4.2 lists all samples used for the pretreatment tests, as well as the code used for each condition for the CCD analysis.

Table 4.2: Samples used for MW and NaOH-MW pretreatment tests.

Sample	**TWAS**	**FOG**		**MW**		**NaOH**
MW pretreatment	**(%)**	**(%)**	**Code**	**Temp (°C)**	**Code**	**without**
TWAS(Control)	100	0	-	--	-	--
TWAS, 20%FOG	80	20	-1	--	-	--
TWAS, 20%FOG, MW95	80	20	-1	95	-1	--
TWAS, 20%FOG, MW135	80	20	-1	135	0	--
TWAS, 20%FOG, MW175	80	20	-1	175	+1	--
TWAS, 40%FOG	60	40	0	--	-	--
TWAS, 40%FOG, MW95	60	40	0	95	-1	--
TWAS, 40%FOG, MW135	60	40	0	135	0	--
TWAS, 40%FOG, MW175	60	40	0	175	+1	--
TWAS, 60%FOG	40	60	+1	--	-	--
TWAS, 60%FOG, MW95	40	60	+1	95	-1	--
TWAS, 60%FOG, MW135	40	60	+1	135	0	--
TWAS, 60%FOG, MW175	40	60	+1	175	+1	--
NaOH-MW pretreatment						**pH with NaOH**
NaOH, TWAS, 20%FOG	80	20	-1	--	-	10
NaOH, TWAS, 20%FOG, MW95	80	20	-1	95	-1	10
NaOH, TWAS, 20%FOG, MW135	80	20	-1	135	0	10
NaOH, TWAS, 20%FOG, MW175	80	20	-1	175	+1	10
NaOH, TWAS, 40%FOG	60	40	0	--	-	10
NaOH, TWAS, 40%FOG, MW95	60	40	0	95	-1	10
NaOH, TWAS, 40%FOG, MW135	60	40	0	135	0	10
NaOH, TWAS, 40%FOG, MW175	60	40	0	175	+1	10
NaOH, TWAS, 60%FOG	40	60	+1	--	-	10
NaOH, TWAS, 60%FOG, MW95	40	60	+1	95	-1	10
NaOH, TWAS, 60%FOG, MW135	40	60	+1	135	0	10
NaOH, TWAS, 60%FOG, MW175	40	60	+1	175	+1	10

4.3.8 Net energy analysis

In order to compare the energy balances for the different pretreatment conditions, the input, output and net energy were calculated for all un-pretreated and pretreated samples. The net specific energy profit from the pretreatment for each sample E_{Net} (kJ/kg VS) was calculated using Equation (4.4) (Kuglarz et al., 2013).

$$\boldsymbol{E_{Net}} = E_B + E_{HP} - E_{HR} - E_{MW} \tag{4.4}$$

Where:

E_B is the energy produced from biogas production from the pretreated samples (kJ/kg VS),

E_{HP} is the energy produced and recovered in the form of heat (kJ/kg VS),

E_{HR} is the energy required to heat the samples to a thermophilic digestion temperature of 55°C (kJ/kg VS), and

E_{MW} is the energy required for sample MW pretreatment.

Calculations of E_B were based on the BMP test results for the methane yield of each sample. The assumptions used for the energy output calculations were: (a) energy content of methane = 35.8MJ/m^3 methane (Nasr et al., 2012); and (b) methane recovery efficiency = 90% and methane to energy conversion efficiency = 95% (Hosseini et al., 2013). The theoretical amount of energy recovered from heat E_{HP} and the energy required to heat the samples to the thermophilic temperature E_{HR} were calculated using Equation (4.5) (Kuglarz et al., 2013).

$$\boldsymbol{E_H} = M_S \,.\, C_P \,.\, \Delta T \tag{4.5}$$

Where:

M_S is the mass of wet sludge equivalent to the unit mass of VS (kg),

C_P is the specific heat capacity of the sample (kJ/kg °C), assumed to be 4.18 and 3.47 kJ/ kg °C for sludge and FOG portions, respectively (Dhar et al., 2012; Minim et al., 2002),

ΔT is the difference between the sample temperature after MW pretreatment and the temperature of the thermophilic digestion conditions (55°C).

The energy required to the heat samples to a thermophilic digestion temperature of 55°C E_{HR} was calculated only for the un-pretreated samples. Since the pretreated samples at MW temperatures of 95 to 175°C will have a temperature that is much higher than 55°C after pretreatment, they do not require the heating step before being fed into the thermophilic reactor. The energy required for the MW pretreatment E_{MW} was calculated using Equation (4.6) (Toreci, I., 2008).

$$\boldsymbol{E_{MW}} = k_{MW} \,.\, M_s \,.\, C_p \,.\, \Delta T \tag{4.6}$$

Where:

M_S, C_p, and ΔT are the same as defined for Equation 4.5.

k_{MW}: energy efficiency constant to deliver MW energy to the sample = (1/0.95).

4.4 Results and Discussion

4.4.1 Solubilization

TWAS and TWAS:FOG samples with different FOG%'s (20, 40 and 60% based on total VS) were pretreated using MW pretreatment and combined NaOH-MW pretreatment in order to determine changes in samples' properties and to measure signs of hydrolysis, such as an increase in SCOD and TVFA due to different pretreatment scenarios. The measured total and soluble chemical oxygen demand and their ratios (TCOD, SCOD, and SCOD/TCOD), as well as the VS to total solids (TS) ratios (VS/TS), in addition to the total volatile fatty acids (TVFA), alkalinity and ammonia, for all samples before and after pretreatment are represented in Table 4.3. Values in the table are the mean± standard deviation for triplicates.

It is noted from the TCOD and SCOD values shown in Table 4.3 that increasing the FOG% in the TWAS:FOG samples before pretreatment caused a direct increase in TCOD and SCOD values compared to TWAS sample. This occurred because of the high TCOD and SCOD of the FOG compared to TWAS. It is evident that after applying the different pretreatment conditions, for all pretreated samples the TCOD did not show a statistically significant change compared to the TCOD of the samples before pretreatment. However, for all the different samples and pretreatment conditions tested, the SCOD showed a noticeable increase after pretreatment compared to the SCOD of the samples before pretreatment. The increase in SCOD of the samples after pretreatment resulted in a corresponding higher SCOD/TCOD ratio for the pretreated samples compared to the un-pretreated samples. A higher SCOD/TCOD ratio indicated a higher SCOD in the sample. For MW pretreatment (without NaOH), the samples with 20% FOG achieved higher SCOD/TCOD ratios compared to the samples with 40 and 60%FOG. As the MW temperature increased, the SCOD/TCOD ratio increased as well, with a maximum SCOD/TCOD ratio of 42.6±1.2% achieved for the sample with 20%FOG and pretreated at an MW temperature of 175°C. Samples with NaOH-MW pretreatment showed a trend similar to the one observed for the MW pretreated samples; i.e., with higher SCOD/TCOD ratios achieved for the lowest FOG% (i.e., 20%FOG) and higher MW temperatures (i.e., 175°C).

To get a better understanding of the effect of different pretreatment options on the samples' disintegration, solubilization (%) was calculated for each pretreated sample as the ratio between the SCOD of the sample after and before pretreatment by using Equation (4.1) (see Section 4.3.6). It is noteworthy to mention that the increase in SCOD and hence higher solubilization (%) after pretreatment is a good sign of sample disintegration. Solubilization (%) values for the samples after pretreatment are shown in Figure 4.2. The figure shows that the solubilization (%) achieved by applying MW pretreatment increases with higher MW irradiation temperatures for each group of samples having the same FOG%. However, it is quite noticeable that as the FOG% increases, the samples achieved lower solubilization(%) compared to the samples with lower FOG%. For example, the MW-pretreated sample with 20%FOG had the highest solubilization when it was pretreated at 175°C, and it achieved a maximum solubilization of 68.2%. At the same MW temperature (175°C), the samples with 40%FOG and 60%FOG reached only 40.9% and 36.3% solubilization, respectively. Although the samples with higher FOG% achieved less solubilization(%) after pretreatment, those samples had higher overall SCOD values before and after pretreatment compared to the samples with lower FOG%, as shown in Table 4.3. This is caused by the high SCOD (compared to TWAS) of the FOG portion, which elevates the overall SCOD of the TWAS:FOG sample. The same trend discussed for the MW-pretreatment samples applies for the NaOH-MW pretreated samples. However, the range of solubilization(%) achieved by the NaOH-MW-pretreated samples was significantly smaller than the solubilization levels of the MW-pretreated samples. However, for the sample with 20%FOG pretreated with NaOH-MW at 95°C, this sample had solubilization(%) higher but not significantly different compared to the solubilization of the same sample pretreated with MW at 95°C without NaOH. The maximum solubilization within the NaOH-MW samples was 42.5% for the 20%FOG sample pretreated at an MW temperature of 175°C. This shows significantly less solubilization compared to the maximum solubilization of the same sample (20%FOG) pretreated at an MW temperature of 175 °C without NaOH (i.e., 68.2%). The lower solubilization achieved by the NaOH-MW-pretreated samples indicates that the addition of NaOH (@pH10) before MW pretreatment did not assist in increasing the TWAS:FOG samples' disintegration and solubilization compared to MW pretreatment without NaOH.

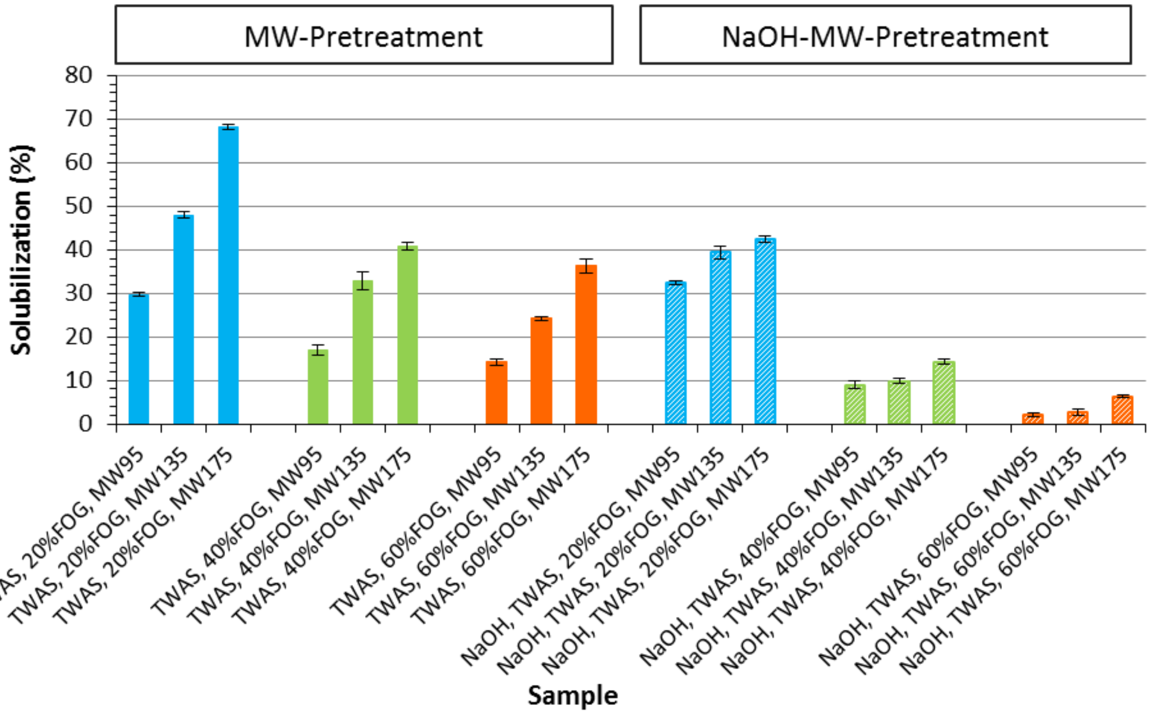


Figure 4.2: Solubilization (%) of samples after MW and NaOH-MW pretreatments.

Table 4.3: Samples properties before and after MW and NaOH-MW pretreatments.

Sample	**FOG**	**MW Temp**	**TCOD**	**SCOD**	**SCOD/TCOD**	**VS/TS**	**TVFA**	**Alkalinity**	**Ammonia**
	(%)	**(°C)**	**(mg/L)**	**(mg/L)**	**(%)**	**(%)**	**(mg/L)**	**(mg/L)**	**(mg/L)**
TWAS(Control)	0	--	47040±2786	10101±230	21.5±1.1	68.7±1.2	439.2±60.6	3890±165	676.7±58.5
MW pretreatment									
TWAS, 20%FOG	20	--	54800±1881	13669±768	25.0±1.7	70.8±0.6	696.4±62.9	3687±90	635.3±30.0
TWAS, 20%FOG, MW95	20	95	54060±2439	17723±120	32.8±1.3	71.7±0.4	783.6±42.2	3467±81	754.7±67.1
TWAS, 20%FOG, MW135	20	135	54110±2117	20229±208	37.4±2.3	72.1±0.5	950.4±84.4	3393±86	802.0±62.5
TWAS, 20%FOG, MW175	20	175	53930±1110	22992±158	42.6±1.2	72.9±0.4	1024.4±85.0	3120±60	798.0±65.9
TWAS, 40%FOG	40	--	66280±745	17528±1444	26.4±1.9	72.1±0.6	825.2±65.4	3197±169	610.7±32.3
TWAS, 40%FOG, MW95	40	95	66010±345	20480±408	31.0±0.6	72.5±0.8	1001.9±61.1	3030±118	674.7±49.4
TWAS, 40%FOG, MW135	40	135	66015±1975	23291±712	31.9±0.5	73.5±0.5	1113.8±144.5	2943±71	722.7±95.4
TWAS, 40%FOG, MW175	40	175	65650±1622	24696±291	37.6±1.0	74.6±0.3	1351.1±94.2	2700±105	749.3±63.8
TWAS, 60%FOG	60	--	76960±1715	20304±1651	26.4±1.7	73.9±1.6	945.6±90.2	2646±143	476.0±58.9
TWAS, 60%FOG, MW95	60	95	75150±959	23200±293	30.9±0.7	74.2±1.1	1055.4±74.0	2310±119	528.0±74.0
TWAS, 60%FOG, MW135	60	135	75160±2513	25232±194	33.6±0.9	74.9±0.6	1178.0±105.4	2087±81	554.7±66.5
TWAS, 60%FOG, MW175	60	175	74910±1435	27670±655	37.0±1.6	75.7±0.8	1699.4±108.3	1509±168	592.0±50.0
NaOH-MW pretreatment									
NaOH, TWAS, 20%FOG, MW95	20	95	53910±2461	18112±131	33.6±1.5	68.4±0.6	778.7±57.0	4347±81	773.3±48.2
NaOH, TWAS, 20%FOG, MW135	20	135	53770±2057	19051±416	35.5±1.9	68.9±0.3	938.2±65.5	4283±119	815.3±73.1
NaOH, TWAS, 20%FOG, MW175	20	175	53600±1005	19477±217	36.3±0.3	67.0±0.4	1100.8±48.3	3943±131	823.3±90.0
NaOH, TWAS, 40%FOG, MW95	40	95	64900±1636	19104±279	29.4±0.5	70.7±1.2	961.8±98.8	3833±145	715.3±118.7
NaOH, TWAS, 40%FOG, MW135	40	135	65060±874	19264±242	29.6±0.6	69.9±0.6	1057.9±99.3	3290±95	748.0±66.5
NaOH, TWAS, 40%FOG, MW175	40	175	64240±1504	20027±222	31.2±1.0	68.8±1.0	1190.4±44.5	2983±165	776.0±80.3
NaOH, TWAS, 60%FOG, MW95	60	95	75450±1645	20744±207	27.5±0.8	72.1±1.0	952.5±100.3	2490±177	546.7±57.2
NaOH, TWAS, 60%FOG, MW135	60	135	74550±3170	20824±300	28.0±1.1	71.7±0.8	1041.3±81.9	2243±108	570.7±47.7
NaOH, TWAS, 60%FOG, MW175	60	175	74290±2257	21592±141	29.1±1.0	70.0±0.7	1373.8±84.7	2015±92.9	638.8±82.4

The relationships between the solubilization (%) and the different MW pretreatment temperatures, as well as FOG percentages, were further analyzed using RSM for MW-pretreated samples with and without NaOH. Table 4.4 shows the results of the analysis of variance (ANOVA) for the regression parameters A: MW temperature (°C) and B: FOG (%) of the predicted response surface (quadratic model) for solubilization (%).

Table 4.4: ANOVA results for a full quadratic model for solubilization (%) of MW and NaOH-MW pretreatments.

Solubilization	**MW pretreatment**				**NaOH-MW pretreatment**			
Source	**df**	**Mean square**	***F*-value**	***p*-value**	**df**	**Mean square**	***F*-value**	***p*-value**
Model	5	1314.7	176.0	<0.0001	5	1225.4	395.2	< 0.0001
A-MW Temp.	1	3578.6	479.1	<0.0001	1	188.2	60.7	< 0.0001
B-FOG	1	2527.6	338.4	<0.0001	1	5328.6	1718.1	< 0.0001
AB	1	204.2	27.3	<0.0001	1	25.2	8.1	0.0095
A^2	1	2.94	0.4	0.5372	1	1.8	0.6	0.4573
B^2	1	260.0	34.8	<0.0001	1	583.5	188.2	< 0.0001
Residual	21	7.5			21	3.1		
Lack of Fit	3	24.0		significant	3	6.7		insignificant
Pure Error	18	4.7			18	2.5		
R^2	0.9767				0.9895			
Adjusted R^2	0.9711				0.9870			
Adeq.precision*	40.3				49.2			

* Adeq. Precision measures the signal to noise ratio; a ratio of > 4 is desirable for an adequate signal.

As shown in Table 4.4, the results of the *F*-test and *p*-value from the 5% confidence level calculation are 176.0 (p <0.0001) for the MW pretreatment model and 395.2 (p <0.0001) for the NaOH-MW pretreatment model. These values show that there is a <0.01% chance that the resulting *F*-values for both models occurred due to noise. In other words, these values indicate that both models are statistically significant in representing the solubilization (%) of the MW- and NaOH-MW-pretreated samples. However, for both models, the term A^2 had a *p*-value that was >0.05, indicating that A^2 is not a significant term. To get better-fitting models, A^2 was eliminated, and a new analysis for a reduced quadratic model for both cases was performed. The ANOVA results for the reduced quadratic models are shown in Table 4.5.

Table 4.5: ANOVA results for a reduced quadratic model for solubilization (%) of MW and NaOH-MW pretreatments.

Solubilization	**MW pretreatment**				**NaOH-MW pretreatment**			
Source	**df**	**Mean square**	***F*-value**	***p*-value**	**df**	**Mean square**	***F*-value**	***p*-value**
Model	4	1642.6	226.2	<0.0001	4	1531.4	503.6	< 0.0001
A-MW Temp.	1	3578.6	492.7	<0.0001	1	188.2	61.9	< 0.0001
B-FOG	1	2527.6	348.0	<0.0001	1	5328.6	1752.4	< 0.0001
AB	1	204.2	28.1	<0.0001	1	25.2	8.3	0.0087
A^2								
B^2	1	260.0	35.8	<0.0001	1	583.5	191.9	< 0.0001
Residual	22	7.3			22	3.0		
Lack of Fit	4	18.8	4.0	significant	4	5.4	2.17	insignificant
Pure Error	18	4.7			18	2.5		
R^2	0.9763				0.9892			
Adjusted R^2	0.9719				0.9872			
Adeq.precision*	44.8				54.5			

* Adeq. Precision measures the signal to noise ratio; a ratio of > 4 is desirable for an adequate signal.

The new reduced models have statistically significant F-values and *p*-values of 226.2 (p <0.0001) and 503.6 (p <0.0001) for the MW and NaOH-MW pretreatments, respectively. This indicates that the models are adequate in representing solubilization (%) for both pretreatments (MW and NaOH-MW). The reduced models have an adjusted R^2 of 0.9719 and 0.9872 for the MW and NaOH-MW pretreatments, respectively. Adjusted R^2 values ensure that both quadratic models reasonably fit the experimental data. The term “Adeq. Precision” in the ANOVA table measures the signal to noise ratio. This ratio should be >4 for an adequate signal (Erden, 2013). The predicted Adeq. Precision values were 44.8 for the solubilization (%) model of MW pretreatment and 54.5 for the solubilization (%) model of NaOH-MW pretreatment. These values indicate adequate signals, and this means that the models can be used to accurately navigate the design space. Finally, the lack of fit values for both reduced models show that the reduced model for MW pretreated samples still has a significant lack of fit, with *p* =0.0174. This suggests that the reduced regression model might not be adequate for describing the functional relationship between the experimental factors and the response variables. However, since the reduced quadratic model for the MW-pretreated samples has a high R^2 (>0.95) and an adjusted R^2 of 0.9763 and 0.9719, the reduced model can be considered acceptable even with a significant lack of fit (0.05 > p =0.0174) (Akindele and Sartaj, 2018; Ding and Sartaj, 2015; Erden, 2013).

Figures 4.3 (a) and (b) represent the predicted vs. observed values for the solubilization (%) of the MW- and NaOH-MW-pretreated samples, respectively. Equations (4.5) and (4.6) are the regression models that represent the solubilization (%) of TWAS:FOG samples with different FOG% after MW and NaOH-MW pretreatment, respectively.

$$\textbf{\textit{Solubilization (\%)}} = 30.19 + 14.1\,\boldsymbol{A} - 11.85\,\mathbf{B} - 4.13\,\boldsymbol{AB} + 6.58\,\boldsymbol{B}^{2} \tag{4.5}$$

$$\textbf{\textit{Solubilization (\%)}} = 11.06 + 3.23\,\boldsymbol{A} - 17.21\,\boldsymbol{B} - 1.45\,\boldsymbol{AB} + 9.86\,\boldsymbol{B}^{2} \tag{4.6}$$

Where: ***A*** is the MW temperature (°C) and ***B*** is the FOG (%) in the TWAS:FOG sample.

Figures 4.3 (a) and (b) show that the predicted values represent a good approximation of the corresponding observed (actual) values.

The positive correlation observed between the TWAS:FOG samples solubilization (%) and MW temperature for the MW pretreatment samples in the current study is in agreement with the findings of Coelho et al. (2011), who reported higher solubilization (%) of TWAS with higher

MW temperature. Toreci et al. (2010) also concluded that MW pretreatment at a temperature higher than boiling point up to 175°C significantly improves TWAS solubilization. An MW temperature higher than 175°C is not recommended, as it may cause the formation of inhibitory compounds that adversely affect the AD process (Ara et al., 2014; Toreci et al., 2010).

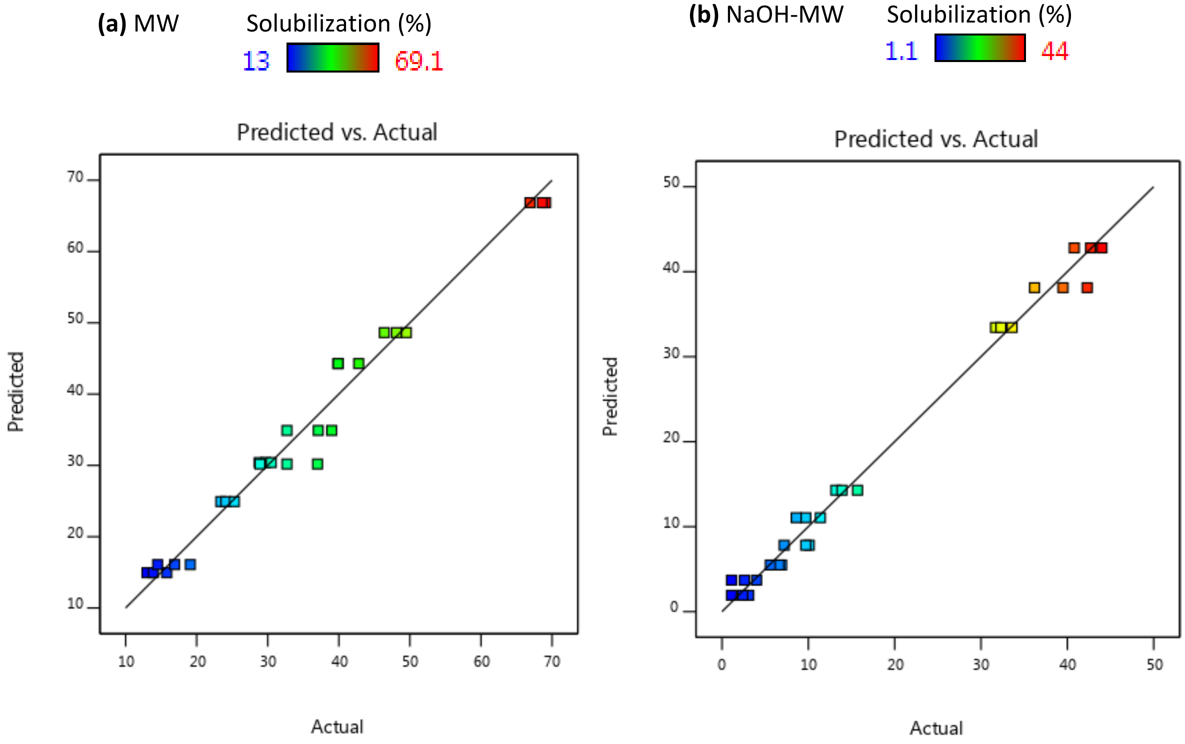


Figure 4.3: Predicted vs. observed values for solubilization (%) of: (a) MW pretreatment and (b) NaOH-MW pretreatment.

It worthy of mention that the effect of NaOH-MW pretreatment in the current study does not agree with some of the observations in other studies in the literature, where the combined alkaline-MW method was used to pretreat other organic wastes. For example, Erden's (2013) study showed that NaOH addition played an important role in enhancing the disintegration efficiency of MW pretreatment for meat-processing wastewater sludge. Another study done by

Wang and Li (2016) on the effect of CaO_2 combined with MW pretreatment on the anaerobic digestion of waste activated sludge (WAS) showed that the combined CaO_2/MW pretreatment enhanced sludge disintegration and solubilization, and even caused an 80.2% increase in the methane yield relative to the control (WAS without pretreatment). According to the aforementioned studies, NaOH-MW was expected to significantly improve TWAS:FOG sample solubilization; however, the results of the current study show that solubilization (%) for all NaOH-MW-pretreated samples under the tested conditions were less than the solubilization (%) achieved by the corresponding samples pretreated with MW alone (i.e., without NaOH).

The other parameter values that were measured for the samples before and after pretreatment, including TVFAs, alkalinity, and ammonia, are presented in Table 4.3. The TVFA values for the TWAS:FOG samples showed a drastic increase due to MW pretreatment, especially at the highest MW temperature of 175°C for all three FOG% tested. The TVFAs after MW pretreatment at 175°C for the samples with 20, 40 and 60% FOG increased by 1.8-, 2.0- and 1.7-fold, respectively, compared to the TVFA values of the corresponding samples before pretreatment. As for the NaOH-MW pretreatment, a similar effect was observed, but with a smaller increase in TVFAs after pretreatment for the TWAS:FOG samples. The TVFAs after NaOH-MW pretreatment at 175°C for the samples with 20, 40 and 60% FOG increased by 1.6-, 1.4- and 1.5-fold, respectively, compared to the corresponding samples' TVFAs before pretreatment. It is evident that the TVFA results are in agreement with the solubilization (%) results that were just discussed. This proves that there is no extra positive effect on solubilization of TWAS:FOG samples caused by NaOH addition before MW pretreatment.

On the other hand, while the ammonia value was 676.7±58.5 mg NH_3-N / L for raw TWAS sample, and ranged from 476.0±58.9 to 635.3±30.0 mg NH_3-N/L for the TWAS:FOG samples before pretreatment, both MW pretreatments (i.e., with and without NaOH) caused an increase in ammonia concentration in all TWAS:FOG samples as compared to the ammonia concentrations for the corresponding samples before pretreatment, as shown in the last column of Table 4.3.

As for the alkalinity levels of the TWAS:FOG samples, in general, the samples with higher FOG% had much lower alkalinity levels compared to the samples with lower FOG%. This low alkalinity is due to high concentrations of long-chain fatty acids (LCFA), which cause FOG to have a slightly acidic pH and much lower alkalinity compared to TWAS (Klaucans and Sams, 2018). After MW pretreatment, the alkalinity levels for the pretreated samples were shown to decrease, and that became more significant with MW temperature increase. For example, as shown in Table 4.3, the sample with 60%FOG had a 2646±143 mg $CaCO_3$/L alkalinity before pretreatment, and this value dropped to 1509±168 mg $CaCO_3$/L after MW pretreatment at 175ºC. It is worth noting that this trend of alkalinity level decrease matches the trend of TVFA value increase for the samples after pretreatment. However, the samples with the combined NaOH-MW pretreatment had overall higher alkalinity levels compared to the corresponding samples with MW pretreatment. This happened due to the alkalinity added to the samples from the NaOH.

4.4.2 Dewaterability

Dewaterability of sludge is directly related to its physical structure and the water distribution within the sludge particles, which are affected by applying different types of pretreatment (Wang and Li, 2016). In this study, the dewaterability characteristics of all samples, including the raw TWAS and TWAS:FOG samples (with 20, 40 and 60%FOG), before pretreatment, after NaOH addition (at pH 10), after MW pretreatment, and after NaOH-MW pretreatment, were determined using the Capillary Suction Time (CST) method. Any decrease in the CST indicates an improvement in dewaterability (Toreci et al., 2009). Figure 4.4 shows the results of the CST tests for all samples. All samples before pretreatment had similar CSTs with no significant difference between values, including TWAS sample and the TWAS:FOG samples with 20, 40 and 60% FOG. The addition of NaOH (at pH 10) for the different TWAS:FOG samples led to a significant increase in CST compared to the CST before pretreatment (this means a decrease in the dewaterability). The observed deterioration effect of NaOH on the samples' dewaterability, as shown in Figure 4.4, is in agreement with what has been reported in other studies where NaOH was used for alkaline pretreatment. Those studies found that the addition of NaOH to sludge has an adverse impact on its dewaterability (Erden, 2013; Li et al., 2008). In Wang and Li's (2016) study, the authors found that the addition of other alkaline reagents like CaO_2 to sludge samples also worsened the samples' dewaterability.

After applying MW pretreatment without NaOH, all samples showed a significant decrease in CST compared to TWAS and TWAS:FOG samples before pretreatment. The highest MW temperature of 175°C led to the lowest CST achieved, shown as the highest improvement in dewaterability in Figure 4.4. On the other hand, TWAS:FOG samples pretreated with NaOH-MW

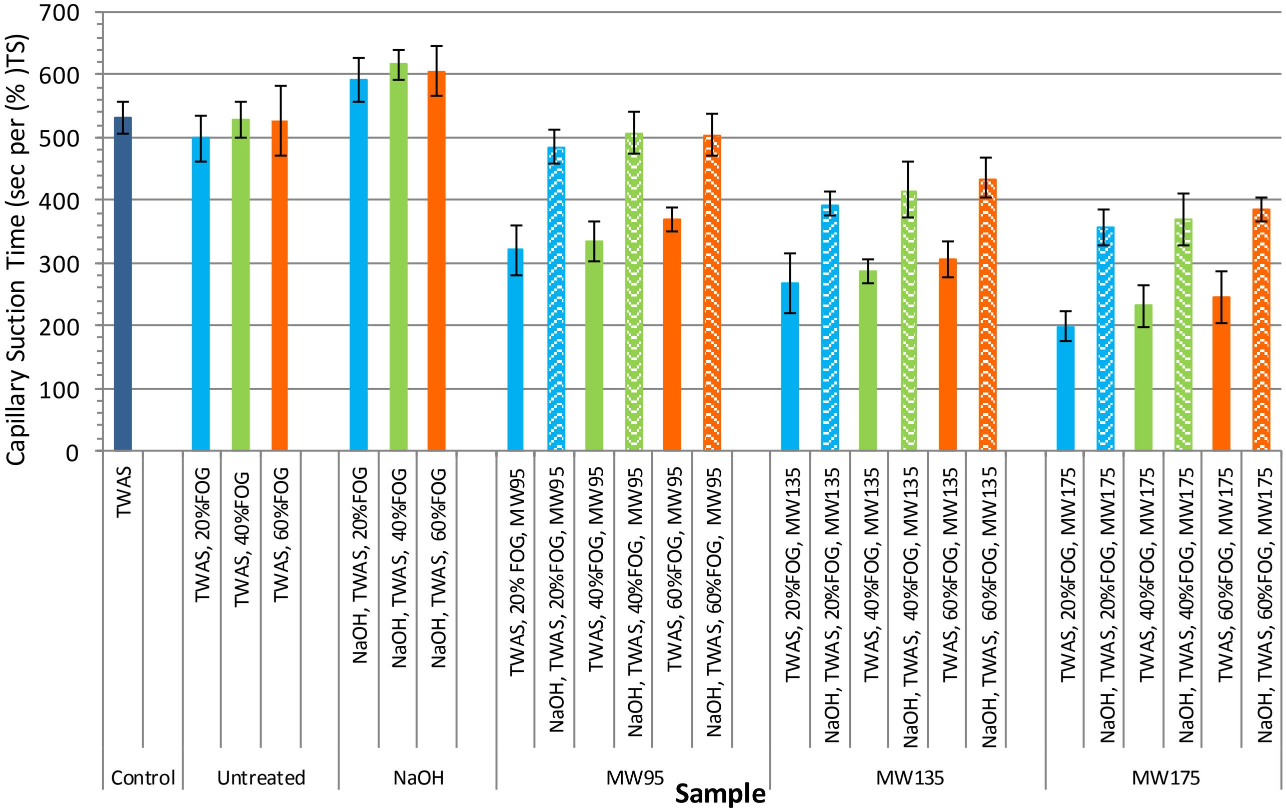


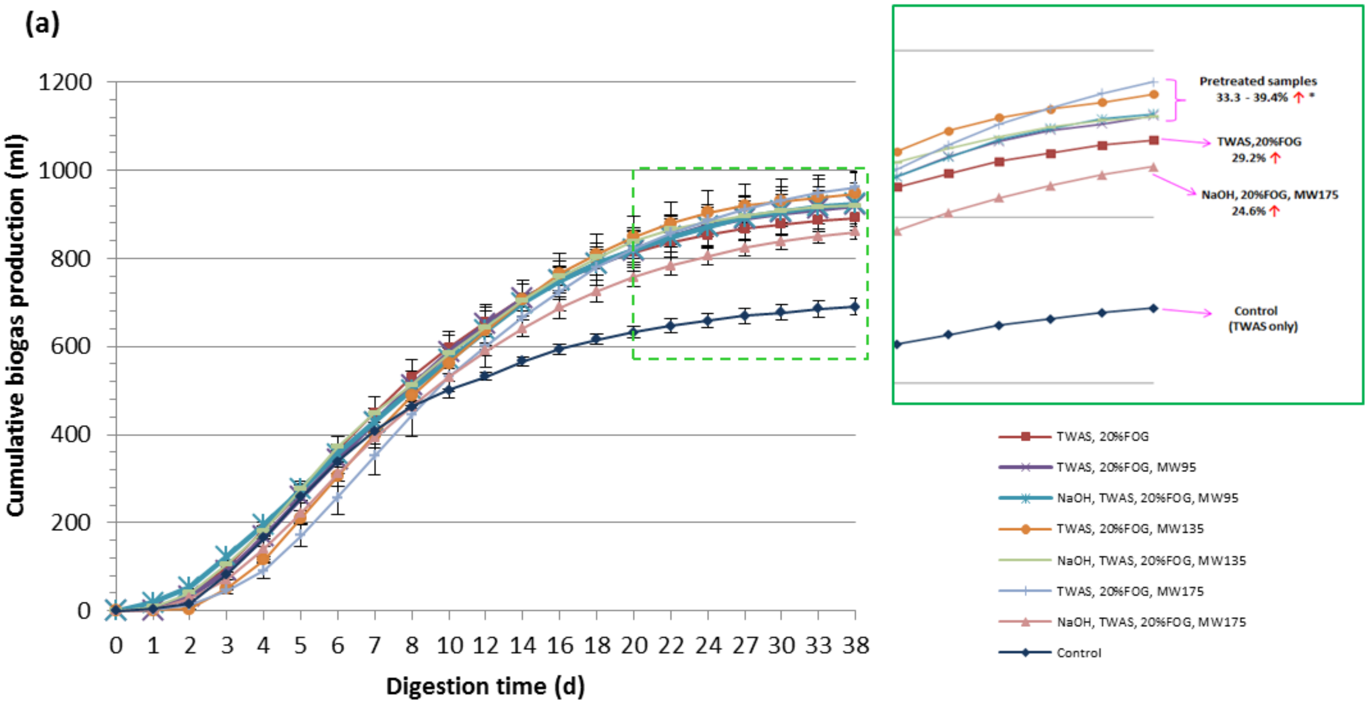
Figure 4.4: Capillary Suction Time for samples before and after MW and NaOH-MW pretreatment.

did not show any significant improvement in dewaterability when the MW temperature was 95° compared to the samples before pretreatment. However, these NaOH-MW-pretreated samples required an MW temperature ≥ 135°C to show a considerable improvement in dewaterability. Although NaOH addition reduces dewaterability, the next MW irradiation step in the combined NaOH-MW pretreatment fixes that problem and improves final dewaterability after pretreatment. The highest dewaterability improvement (i.e., lowest CST) for both the MW and NaOH-MW samples was at the 175°C MW irradiation temperature. Generally, the improvement in dewaterability for the NaOH-MW-pretreated samples was significantly lower when compared to the MW-pretreated samples without NaOH.

4.4.3 Anaerobic biodegradability

4.4.3.1 BMP assay results

In this phase, the raw TWAS and all other TWAS:FOG pretreated samples with MW pretreatment and NaOH-MW pretreatment (listed in Table 4.1) were used for the BMP assay. The cumulative biogas production from the samples with 20%, 40%, and 60% FOG during the 38-day BMP assay are represented in Figures 4.5 (a), (b) and (c), respectively.



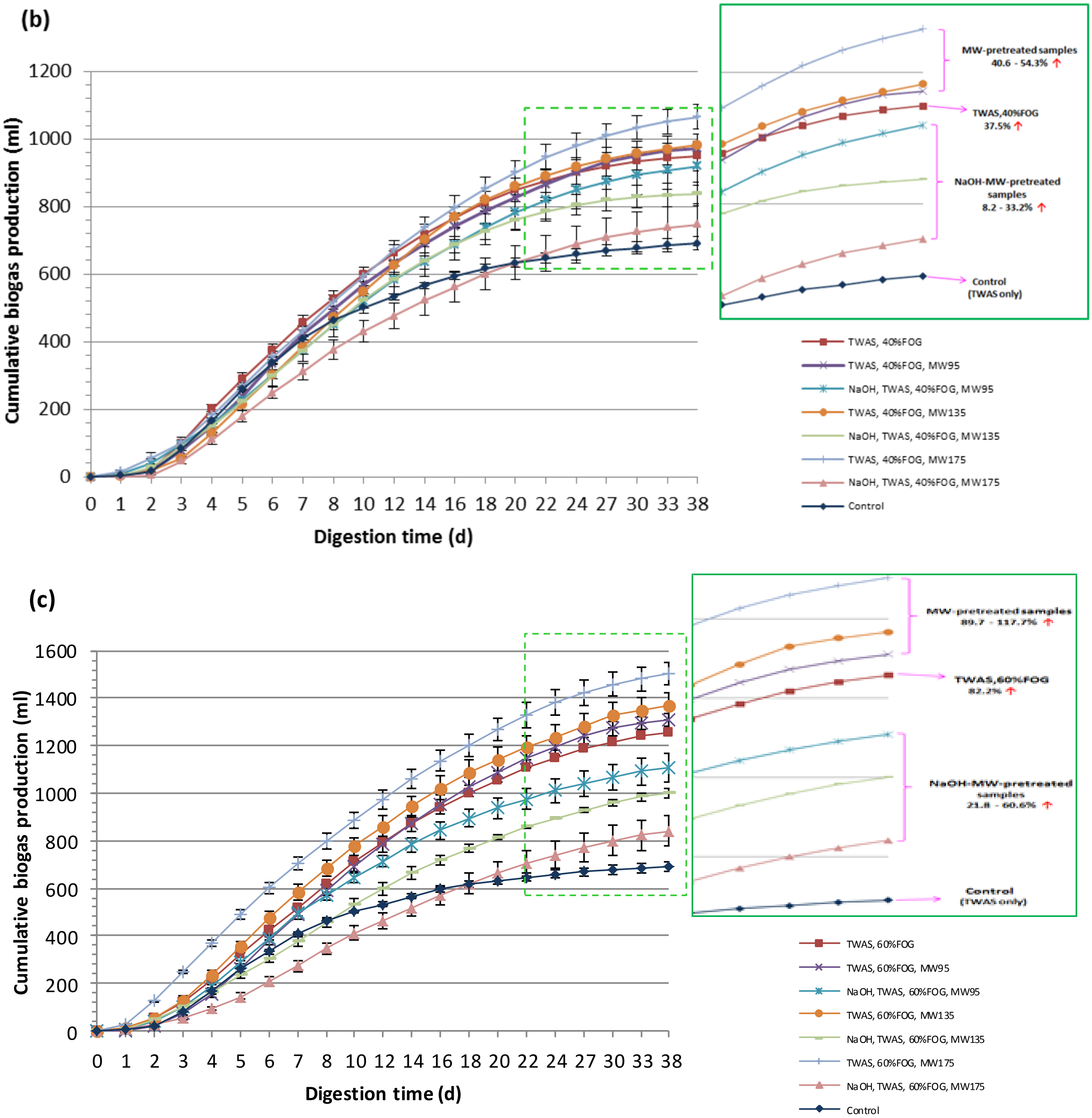


Figure 4.5: Cumulative biogas production (ml) from the BMP tests for the control and samples with (a) 20%FOG, (b) 40%FOG, and (c) 60%FOG.

↑Values shown in the magnified portion of the graph are the improvement % in the cumulative biogas compared to the control.

As observed from Figures 4.5 (a), (b) and (c), cumulative biogas production for all pretreated samples were higher than the cumulative biogas production of the control sample (TWAS alone). The fact that all samples other than the control contained from 20 to 60% FOG (based on the total VS) is the main reason why they all had a higher biogas production compared to the raw TWAS sample. FOG is known for its high biogas potential as well as its high methane yield, which can be 2- to 4-fold higher than the methane yield of TWAS (Alqaralleh et al., 2018; Mata-Alvarez et al., 2014).

MW pretreatment in general improved the cumulative biogas production of the pretreated samples compared to the control and also compared to the un-pretreated samples. Thus, one can conclude as a general observation that the higher the MW irradiation temperature, the greater the improvement in biogas production. The maximum cumulative biogas production for the 20, 40 and 60% FOG MW-pretreated samples were 39.4±9.5, 54.3±10.6 and 117.7±14.1% higher respectively than the cumulative biogas production for the control. These maximum biogas numbers were achieved by the samples pretreated at an MW temperature of 175°C. The observed effect of the MW temperature on biogas production is in agreement with the results of several studies in which MW temperatures in a range of 50°C to 175°C were used. Most of those studies found a positive correlation between increasing the MW temperature and biogas production (Ara et al., 2014; Coelho et al., 2011; Toreci et al., 2009; Eskicioglu et al., 2007).

A summary of the BMP test results, including cumulative biogas production (ml biogas), CH_4 %, methane yield (as ml CH_4/g VS_{added}) and reduction in VS (%), resulting from the BMP assay and predicted from the regression model (to be discussed next) are listed in Table 4.6. It can be noted from Table 4.6 that increasing the FOG% in the samples caused a corresponding significant increase in methane content (CH_4%) in the produced biogas. However, within the same samples (i.e., the same FOG%), the use of different pretreatment scenarios (MW or NaOH-MW pretreatments) at different MW temperatures did not cause any significant change in the CH_4% content of the produced biogas. The maximum methane yield among all the pretreated samples, with and without NaOH, was for the TWAS:60%FOG sample. When this sample was MW pretreated at 175°C (without NaOH), it achieved 720.3±65.4 ml CH_4/g VS_{added}, and this methane yield was 2.3-fold higher than the methane yield of the control sample.

It is worth noting that all the pretreated samples had a statistically significant higher VS reduction compared to the control sample. Increasing the FOG% in the pretreated samples led to this corresponding increase in VS reduction. This observation agrees with the higher methane yields just discussed. The presence of FOG in the pretreated samples boosted the anaerobic co-digestion process and resulted in higher biogas production and methane yield and VS reduction (Alqaralleh et al., 2017; Li et al., 2015). Table 4.6 also shows that for the same FOG% samples, as the MW temperature increases, the VS reduction increases as well. The samples pretreated at the MW temperature of 175°C achieved the highest VS reductions, of 74.1±1.0, 76.2±0.4 and 79.4±1.0 for 20, 40 and 60% FOG, respectively. A similar trend was observed for the NaOH-MW pretreated samples, with slightly higher but not statistically significantly different VS reductions as compared to the VS reductions of the corresponding MW-pretreated samples (for the same FOG% and the same MW pretreatment temperature). The high VS reduction results of the NaOH-MW pretreated samples are in contrast with the lower biogas production and methane yield results achieved with this type of pretreatment (compared to MW-pretreated samples). The presence of alkaline conditions with the MW pretreatment might cause a saponification reaction that converts the FOG to by-products that might have lower biogas and methane potential compared to FOG before alkaline-MW pretreatment.

According to some studies, Millard reactions occur during MW pretreatment at elevated temperatures, resulting in the production of melanoidins (Koupaie and Eskicioglu, 2016). Melanoidins are high-molecular-weight nitrogen polymers produced as a by-product of the polymerization process of low-molecular-weight intermediates like sugars and amino acids (Shahriari et al., 2012). Melanoidins are responsible for the dark brown color of the MW pretreated samples (especially at the high MW temperature of 175°C) compared to the lighter color of non-pretreated samples (e.g., TWAS, sludge, organic food waste, etc.). Figure 6 shows the changes in color for the soluble portion (< 0.45 µm) TWAS:20%FOG samples before and after MW pretreatment at 95, 135 and 175°C. As the MW pretreatment temperature increased, the brown color of the sample became darker, indicating the formation of more melanoidins. It is believed that melanoidins are refractory compounds that might cause a lag phase and methanogen inhibition and reduction in biogas production (Alqaralleh et al., 2016; Shahriari et al., 2012). In the current study, the biogas production for all pretreated samples did not show a significantly longer lag phase compared to the control (i.e., un-pretreated TWAS), as can be observed from Figures 4.5 (a), (b) and (c). It is believed that the presence of FOG in the pretreated samples provided soluble fatty acids that caused a quick anaerobic digestion start,

Table 4.6: Summary of BMP results for biogas production, CH_4 (%), and actual/predicted methane yield.

Sample	FOG	MW Temp	NaOH	VS Reduction	Cumulative Biogas	CH_4	Actual Methane yield	CH_4 Yield* Improvement	Predicted CH_4 yield	Residual
	(%)	(°C)		(%)	(ml)	(%)	(ml/ g VS)	(%)	(ml/ g VS)	$\lvert\Delta\%\rvert$[1]
TWAS(Control)	0	--	--	65.3±1.1	690.7±40.1	61.6±0.6	303.9±24.7	--	--[2]	--
20%FOG samples										
TWAS, 20%FOG, MW95	20	95	--	70.8±0.6	921.8±100.0	65.2±0.4	429.2±65.2	41.2	434.4	1.2
NaOH, TWAS, 20%FOG, MW95	20	95	NaOH	71.5±1.0	923.9±92.5	64.9±0.5	428.7±60.6	41.1	431.0	0.5
TWAS, 20%FOG, MW135	20	135	--	71.2±0.9	947.8±101.5	65.1±0.2	440.7±66.1	45.0	432.9	1.8
NaOH, TWAS, 20%FOG, MW135	20	135	NaOH	72.4±0.6	920.4±73.9	64.8±0.4	426.0±47.9	40.2	421.9	4.1
TWAS, 20%FOG, MW175	20	175	--	74.1±1.0	962.5±65.5	65.9±0.3	452.8±43.1	49.0	455.4	1.0
NaOH, TWAS, 20%FOG, MW175	20	175	NaOH	76.0±0.8	860.5±33.9	65.1±0.1	400.1±22.0	31.7	401.9	0.4
40%FOG samples	40									
TWAS, 40%FOG, MW95	40	95	--	74.8±0.7	971.2±66.5	66.4±0.3	460.3±44.1	51.5	455.6	1.0
NaOH, TWAS, 40%FOG, MW95	40	95	NaOH	74.9±1.1	919.7±115.5	66.2±0.4	433.5±76.2	42.8	429.8	0.9
TWAS, 40%FOG, MW135	40	135	--	75.1±0.6	982.1±83.4	67.0±0.3	469.9±55.9	54.6	471.3	0.3
NaOH, TWAS, 40%FOG, MW135	40	135	NaOH	77.0±0.9	837.8±68.6	65.9±0.5	394.4±45.2	29.8	396.5	0.5
TWAS, 40%FOG, MW175	40	175	--	76.2±0.4	1065.6±73.5	67.1±0.3	510.4±49.3	68.0	511.0	0.1
NaOH, TWAS, 40%FOG, MW175	40	175	NaOH	77.4±0.7	747.2±123.9	66.5±0.2	354.9±82.4	16.8	352.3	0.8
60%FOG samples										
TWAS, 60%FOG, MW95	60	95	--	77.1±1.2	1310.2±51.5	67.1±0.3	627.9±34.5	106.6	627.4	0.1
NaOH, TWAS, 60%FOG, MW95	60	95	NaOH	78.0±0.6	1109.2±119.8	66.5±0.1	527.0±79.7	73.4	528.4	0.3
TWAS, 60%FOG, MW135	60	135	--	78.6±0.9	1366.1±111.4	67.3±0.3	656.7±74.9	116.1	660.3	0.5
NaOH, TWAS, 60%FOG, MW135	60	135	NaOH	79.1±0.5	1000.6±41.9	66.9±0.4	473.1±27.7	55.7	470.9	0.5
TWAS, 60%FOG, MW175	60	175	--	79.4±1.0	1503.4±97.5	67.1±0.2	720.3±65.4	137.0	717.2	0.4
NaOH, TWAS, 60%FOG, MW175	60	175	NaOH	79.8±0.7	841.5±126.7	66.8±0.8	401.5±84.7	32.1	402.4	0.2

[1] The absolute error (%) between actual and predicted Methane yield values. [2] This sample (control) was not included in the regression analysis.

and this might have helped in overcoming the inhibitory effects of the melanoidins, especially at the beginning of the AD process.

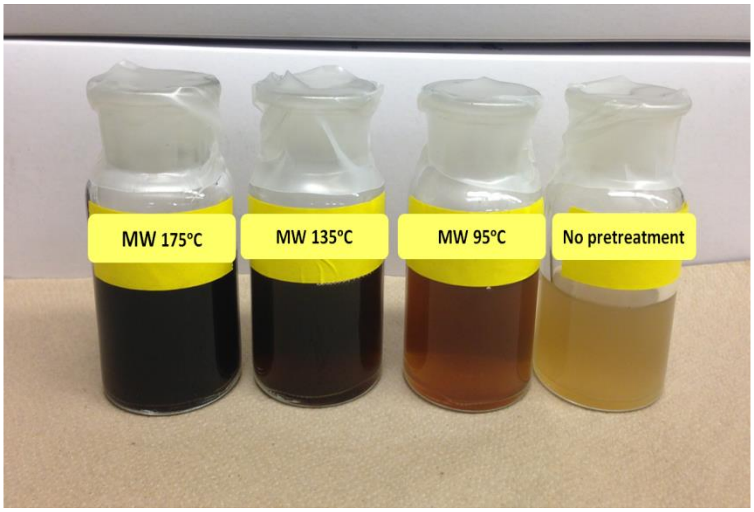


Figure 4.6: Difference in the color of the soluble part between the un-pretreated sample and MW-pretreated samples at 95, 135 and 175°C.

4.4.3.2 RSM analysis for methane yields of pretreated samples

The methane yield results were then used for ANOVA analysis for the predicted response surface quadratic model for both the MW- and NaOH-MW-pretreated samples. The results of this analysis are listed in Table 4.7.

The F-values and *p*-values of 689.6 (p <0.0001) and 320.9 (p <0.0001) of the methane yield regression models for the MW- and NaOH-MW-pretreated samples, respectively, show that both models are statistically significant. The *p*-values, shown in Table 4.7, indicate that there is less than 0.01% chance that the high F-values in both of the models are due to noise. The high R^2 and (adjusted R^2) of 0.9986 (0.9971) and 0.9969 (0.9938) for the MW- and NaOH-MW-pretreated samples, respectively, further indicate that the quadratic models adequately fit the experimental data and can be used to predict methane yields for MW- and NaOH-MW-pretreated samples with different FOG% and under a variety of MW irradiation temperatures.

Table 4.7: ANOVA for a full quadratic model for methane yield of MW- and NaOH-MW-pretreated samples.

Solubilization	**MW pretreatment**				**NaOH-MW pretreatment**			
Source	**df**	**Mean square**	***F*-value**	***p*-value**	**df**	**Mean square**	***F*-value**	***p*-value**
Model	5	20105.2	689.6	<0.0001	5	4286.5	320.9	< 0.0001
A-MW Temp.	1	4598.2	157.7	<0.0001	1	9024.9	675.6	< 0.0001
B-FOG	1	77566.1	2660.3	<0.0001	1	3591.7	268.9	< 0.0001
AB	1	1183.4	40.6	0.0014	1	2347.4	175.7	< 0.0001
A^2	1	363.4	12.5	0.0167	1	76.0	5.7	0.0628
B^2	1	14374.3	493.0	<0.0001	1	6301.4	471.7	< 0.0001
Residual	5	145.8			5	13.4		
Lack of Fit	3	48.6		insignificant	3	22.3		insignificant
Pure Error	2	0.0			2	0.0		
R^2	0.9986				0.9969			
Adjusted R^2	0.9971				0.9938			
Adeq.precision*	71.3				65.2			

* Adeq. Precision measures the signal to noise ratio; a ratio of > 4 is desirable for an adequate signal.

Moreover, the adequate precision ratios of 71.3 and 65.2 for the methane yields of the MW- and NaOH-MW-pretreated samples, respectively, indicate an adequate signal, as they are larger than the boundary value of 4 (Akindele and Sartaj, 2018). Lack of fit is insignificant for both models as well. The fitted regression models for the methane yields for the MW- and NaOH-MW-pretreated samples are represented by Equations (4.7) and (4.8), respectively.

$$\textbf{\textit{Methane yield}} = 471.29 + 27.68\,\boldsymbol{A} + 113.7\,\boldsymbol{B} + 17.2\,\boldsymbol{AB} + 11.98\,\boldsymbol{A}^{2} + 75.33\,\boldsymbol{B}^{2} \tag{4.7}$$

$$\textbf{\textit{Methane yield}} = 396.51 - 38.78\,\boldsymbol{A} + 24.47\,\boldsymbol{B} - 24.23\,\boldsymbol{AB} - 5.48\,\boldsymbol{A}^{2} + 49.87\,\boldsymbol{B}^{2} \tag{4.8}$$

Where: ***Methane yield*** is the cumulative volume of CH_4 produced per gram of VS added to the BMP serum bottle (ml CH_4/g VS), ***A*** is the MW temperature (°C), and ***B*** is the FOG (%) in the TWAS:FOG sample.

There is good agreement between the actual methane yields measured from the BMP assay and the predicted methane yields from the regression models for both the MW- and NaOH-MW-pretreated samples with an absolute error that does not exceed 4.1% for any of the samples, as shown in Table 4.6. The surface response of methane yield for the MW- and NaOH-MW-pretreated samples are shown in Figures 4.7 (a) and (b), respectively.

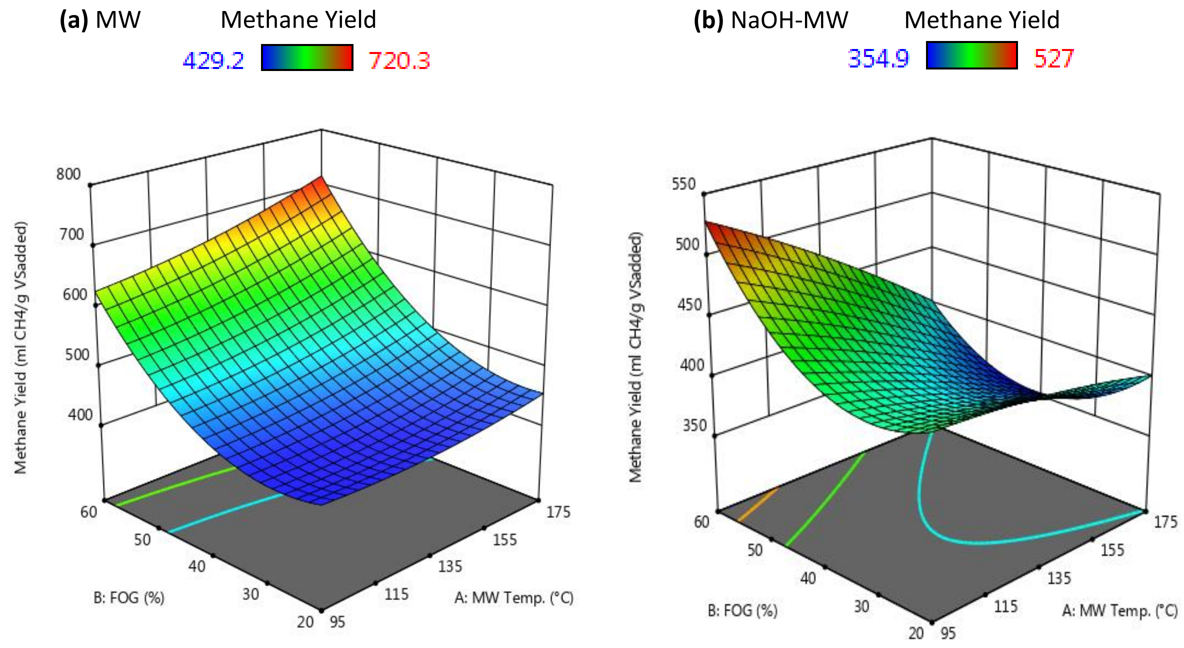


Figure 4.7: Surface response of methane yield for (a) MW-pretreated samples, and (b) NaOH-MW-pretreated samples.

It is evident from Figure 4.7(a) that the increase in methane yield occurred with increases in FOG% (due to FOG high methane yield), as well as increasing the MW irradiation temperature.

This resulted in better disintegration and solubilization of the TWAS:FOG samples, which led directly to higher biogas production and methane yield. Figure 4.7(b), on the other hand, clearly shows a negative correlation between the addition of NaOH and the methane yield. This is undoubtedly produced from the NaOH-MW-pretreated samples' degradation. It can be observed that the negative effect of alkaline conditions becomes more significant at higher FOG% (40% and 60%) and with higher MW irradiation temperatures. A possible reason for this behavior might be the saponification reaction between FOG and NaOH. As FOG% increased in the sample, more NaOH was added to it to reach the desired pH of 10. The high FOG% and the NaOH doses along with elevated MW temperatures might have helped in the creation of the saponification reaction (Ahmad et al., 2015; Bursali et al., 2006). The by-products of the saponification reaction could be compounds that are harder to digest or have lower biogas and methane yields compared to FOG. This would result in lower final cumulative biogas production and methane yield as compared to the MW-only pretreated samples. These results indicate that combining the alkaline with MW irradiation for TWAS:FOG pretreatment is not as effective as the use of the pure MW pretreatment option to maximize TWAS:FOG solubilization and anaerobic degradation.

4.4.4 Energy analysis for different pretreatment conditions

A simplified energy analysis based on the lab-scale experiments was performed to compare the different pretreatment options in terms of the net energy required or produced per tonne of VS treated (kWh/tonne VS). The specific input, output and net energy for the TWAS:FOG un-pretreated and pretreated samples under all the different conditions considered in this study are shown in Table 4.8, where Input energy = $E_{HR} + E_{MW}$, and output energy = $E_B + E_{HP}$.

As can be observed from the net energy results shown in Table 4.8, the only samples with positive net energy were the three un-pretreated samples with 20, 40 and 60% FOG. For these three samples, the energy resulting from methane production via anaerobic co-digestion exceeded the energy required to heat the samples to thermophilic temperatures. Higher FOG% had higher positive net energy value because of the FOG% contribution to the increase in biogas production and methane yield, as previously discussed. For example, the co-digestion of samples with 60%FOG had the highest positive net energy, of about 4.6 MWh/ tonne VS $_{\text{treated}}$.

All the MW- and NaOH-MW-pretreated samples had a negative net energy. Despite the significantly high methane yield results for the MW-pretreated samples compared to the un-pretreated samples, the net energy was negative due to the high electrical energy consumed by the lab-scale MW machine. This makes MW pretreatment a very energy intensive option. The higher MW irradiation temperature of 175°C was the best for obtaining the maximum possible solubilization before the AD, and also to obtain maximum biogas production and methane yield from the AD. However, at the same time, it unmistakably had the highest energy demand. The negative net energy made MW pretreatment infeasible from an economic point of view based on the energy analysis of this study. It is important to highlight here that the positive effects on TWAS:FOG samples due to MW pretreatment, especially at high MW temperature of 175°C, which were shown in the form of increase in samples solubilization, and significant improvement in biogas production, methane yield, and VS reduction should not be degraded just because of the negative net energy results. The results and energy calculations in the current study are based on lab-scale MW equipment, and therefore, energy transfer efficiency could be much lower than in a full-scale MW system. The low intensity used (3.5 °C/min) also might have caused an overestimation of the input energy required for the MW pretreatment. The dilemma of the negative net energy for MW pretreatment was reported before in the open literature and had often caused a general conclusion that MW pretreatment is not feasible from an economic point of view (Cella et al., 2016; Saha et al., 2011). Therefore, full-scale MW pretreatment studies and comprehensive cost-benefit analysis should be done as soon as possible in order to determine whether MW pretreatment is feasible or not in actual anaerobic digestion applications.

Table 4.8: Net energy analysis for pretreated samples.

Sample	**Energy In (kWh[1]/tonne VS)**	**ENERGY Out (kWh/tonne VS)**		**Net Energy (kWh/tonne VS)**	
		without NaOH	With NaOH	without NaOH	With NaOH[2]
TWAS, 20%FOG	927.4	3505.5	--	2578.0	--
TWAS, 20%FOG, MW95	2974.6	4551.0	4546.8	-25190.6	-25194.8
TWAS, 20%FOG, MW135	46736.8	5550.5	5425.4	-41186.3	-41311.4
TWAS, 20%FOG, MW175	63732.0	6554.8	6107.0	-57177.3	-57625.1
TWAS, 40%FOG	726.0	3847.8	--	3121.8	--
TWAS, 40%FOG, MW95	18910.1	4619.8	4395.9	-14290.3	-14514.2
TWAS, 40%FOG, MW135	29715.8	5406.8	4764.6	-24951.2	-24951.2
TWAS, 40%FOG, MW175	40521.6	6457.6	5135.0	-34064.0	-35386.6
TWAS, 60%FOG	524.5	5133	--	4609.0	--
TWAS, 60%FOG, MW95	13861.8	5848.3	4991.0	-8013.5	-8870.8
TWAS, 60%FOG, MW135	21782.8	6603.7	5042.0	-15179.1	-16740.8
TWAS, 60%FOG, MW175	29703.8	7654.6	4943.7	-22049.3	-24760.1

[1] kWh = 3600 kJ. [2] For NaOH-MW pretreatment the cost of alkaline added (NaOH cost) was not included in the energy calculation.

The combined NaOH-MW pretreatment results invalidated the hypothesis that the combination of alkaline pretreatment (at pH 10) with MW pretreatment will significantly improve solubilization and hence improve biogas and methane production, leading to higher net energy as compared to un-pretreated and MW-only pretreated samples. The low solubilization achieved, low biogas production and hence methane yield, as well as the extra cost needed for the alkali used (the cost of NaOH was not included in the net energy calculations) as compared to MW pretreatment leads to the conclusion that the combined NaOH-MW pretreatment is not suitable for TWAS:FOG samples prior to the AD process.

4.5 Conclusion

- MW pretreatment significantly improved TWAS:FOG solubilization and disintegration prior to the AD process. The high MW temperature of 175°C resulted in the maximum solubilization (%) of 68.2% for the 20%FOG samples.
- FOG% in the TWAS:FOG samples had a significant effect on biogas production and methane yield. Samples with 60%FOG pretreated at an MW temperature of 175°C had the maximum methane yield among all tested samples; the methane yield was 137% higher than the methane yield of the control (i.e., TWAS alone).
- Despite the benefits of MW pretreatment on TWAS:FOG samples, which include significant solubilization increase, dewaterability improvement, high VS reduction, and methane yield, the net energy analysis resulted in negative net energy values for all the MW-pretreated samples. The energy calculations based on the lab-scale MW device used in this study are believed to overestimate the input energy required for MW pretreatment.
- The combined NaOH-MW pretreatment was found to be an ineffective option for TWAS:FOG pretreatment before the AD process.

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CHAPTER 5

Microwave, Hyper-thermophilic, and Conventional Heat Pretreatment of Thickened Waste Activated Sludge and Fat, Oil, and Grease Mixture: A Comparison of Molecular Weight Distribution and Digestion of The Soluble Organic Matter

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5.1 Abstract

Thickened waste activated sludge (TWAS) and fat, oil and grease (FOG) mixed samples (80:20 based on total volatile solids) were subjected to three different pretreatments, i.e., microwave (MW) pretreatment at 175°C, hyper-thermophilic stage (Hyper) at 70°C and conventional heat (Heat) pretreatment at 70°C. The soluble matter extracted from the non-pretreated sample and the three pretreated samples were subjected to an ultrafiltration process in series using four different membrane sizes (300, 100, 10, and 1 kDa). The resulting different molecular weight fractions were used for solubility measurements and biodegradability tests. The results showed that each of the tested pretreatment methods studied (MW, Hyper, and Heat) had a different effect on the soluble SCOD and TVFA and resulted in unique molecular weight distributions. The MW and Hyper methods were much more effective in sample solubilization and biogas

production compared to the Heat pretreatment. The hyper-thermophilic sample had the maximum improvement in cumulative biogas production (CBP) from all the molecular weights among the three pretreated samples and achieved 86.5% higher CBP compared to the control.

5.2 Introduction

Anaerobic co-digestion of TWAS using the right dose of FOG has been shown to significantly increase biogas production and energy recovery from the anaerobic digestion process. This increase in biogas production is due to the high methane potential of FOG compared to TWAS and other organic substrates. Anaerobic digestion (AD) of fat has a theoretical yield of 1430 ml biogas/g compared to protein and carbohydrates, which have theoretical yields of 930 and 840 ml biogas/g, respectively (Long et al., 2012; Alves et al., 2009). TWAS:FOG co-digestion can be performed under mesophilic or thermophilic conditions (Long et al., 2012); however, recent studies strongly recommended the thermophilic conditions (at 55°C) for FOG co-digestion since thermophilic conditions increase the reactor ability to degrade long chain fatty acids resulted from FOG solubilization. This has been shown to reduce problems caused by the formation of scum layer in the reactor compared to mesophilic conditions (Alqaralleh et al., 2018; Long et al., 2012).

Hydrolysis is the rate-limiting step in the degradation of complex organic materials like TWAS that causes limitations in the anaerobic digestion process. TWAS consists mainly of extracellular polymeric substances (EPS) floc matrix, which is associated with the bacterial cells present in TWAS (Toreci et al., 2010). EPS is known to be the macromolecular organic compounds that give structural and functional integrity to the sludge, and it presents physical and chemical barriers to hydrolysis and exo-enzyme degradation (Toreci et al., 2010; Flemming & Wingender, 2001). Therefore, the disruption and disintegration of polymers and EPS is a crucial step before TWAS degradation (Toreci et al., 2010).

Many pretreatment methods, including thermal, chemical, physical, and their combinations, have been studied to improve the anaerobic degradation of complex wastes, especially TWAS; however, there has been much less focus on the effects of pretreatment on co-digestion mixtures such as TWAS and FOG (Li et al., 2013). Microwave (MW) pretreatment is an innovative method that represents an alternative to the conventional heating pretreatment of sludge. MW can heat the sludge uniformly in a shorter time and at less cost compared to other methods since the energy is selectively concentrated in materials that have high dielectric factors (Coelho et al., 2014). MW pretreatment, particularly at an elevated temperature of 175°C, has shown to be highly effective in solubilizing TWAS samples prior to AD (Toreci et al., 2009). MW pretreatment at 175ºC has shown as well to significantly improve the solubilization of TWAS:FOG samples with different FOG% in a previous study that investigated MW pretreatment of TWAS:FOG samples prior to anaerobic co-digestion (Alqaralleh et al., results of chapter 4 are not published yet).

Recently, a hyper-thermophilic stage at 70ºC for 2 days prior to the AD proved to be a potential alternative to the pretreatment of TWAS:FOG samples prior to anaerobic co-digestion (Alqaralleh et al., 2018; Alqaralleh et al., 2016). It is hypothesized that the hyper-thermophilic stage at 70ºC is more efficient in solubilizing and enhancing biogas production from the anaerobic co-digestion of TWAS:FOG samples compared to a conventional heating pretreatment at the same temperature and pretreatment duration, due to the extra anaerobic microbial and enzymatic activities that exist in the hyper-thermophilic reactor.

The molecular weight distribution of the soluble compounds resulting from intermediate and end products of sludge degradation and cell decomposition vary over a wide range, from 1 to 10,000 kDa (Toreci et al., 2009; Boero et al., 1996). Ultrafiltration (UF) is a commonly used method for determining the molecular weight distribution for sludge samples (Coelho et al., 2014; Toreci et al., 2009; Boero et al., 1996). However, to the best of our knowledge, there are no studies that have investigated the molecular weight distribution using UF for TWAS:FOG samples.

Taking the above points into consideration, the objective of this study is to investigate the effect of three different pretreatment methods, namely microwave (MW @175°C), hyper-thermophilic (Hyper @70°C) and conventional heat (Heat @70°C), on the solubilization of TWAS:FOG samples, as well as the molecular weight distribution of the soluble matter using UF. Furthermore, the biodegradation of the different molecular weight fractions will be evaluated. This study will evaluate the hypothesis suggesting that the hyper-thermophilic stage is more effective for solubilizing TWAS:FOG samples compared to conventional heat pretreatment at the same temperature and duration.

5.3 Materials and Methods

5.3.1 Materials

Thickened waste activated sludge (TWAS) was provided by City of Ottawa Robert O. Pickard Environmental Center (ROPEC), Gloucester, ON, Canada. ROPEC is a secondary wastewater treatment plant (WWTP) that performs preliminary and primary treatment followed by a conventional activated sludge process at a solids retention time (SRT) of 5 days. Ferric chloride is added to the sludge for phosphorous removal prior to thickening. TWAS sample contained 4.2±0.1% total solids (TS) with 68.7±1.2% VS.

The FOG was obtained from Organic Resources Management Inc. (ORMI), Ottawa, ON, Canada. ORMI provides grease-trap cleaning services for different wastewater treatment plants in Ontario, Canada. The FOG samples contained 28.3±1.2% TS with 98.0±0.1% VS. Both substrates (TWAS and FOG) were stored at 4°C before use. The substrates were allowed to reach room temperature before the experiments and tests. The TWAS:FOG sample used in this study contained 80% TWAS and 20% FOG based on the total volatile solids (VS).

5.3.2 Pretreatment Methods

Microwave pretreatment (MW): This pretreatment was carried out using a Mars X MW unit [Microwave Accelerated Reaction System, 1250W ±15% at full power, 2450MHz, temperature range 25-260°C, CEM Corporation, NC, USA]. The conditions used for the MW pretreatment were: temperature of 175°C, intensity of 3.5°C/ min, and minimum holding time of 1 min. These conditions were selected based on the results of a previous study that used similar materials for MW pretreatment at different temperatures, where MW pretreatment at 175°C resulted in maximum solubilization and biogas production for the TWAS:FOG mixture. The TWAS:FOG sample was MW irradiated until reaching the desired temperature (175°C) in 12 Teflon vessels (40 g sample in each vessel). The Teflon vessels were capped and dressed with special composite sleeves before being placed on the rotating turntable inside the MW machine. After reaching the target temperature, the samples were kept for a 1 min holding time before removing them from the MW machine, and let cool to room temperature before opening in order to avoid any mass loss via evaporation. Next, the pretreated samples were stored at 4ºC until used for the designated experiments and tests.

Hyper-thermophilic digestion stage (Hyper): For this stage the TWAS:FOG sample was fed into a lab-scale hyper-thermophilic reactor that is operating at 2 days HRT. The hyper-thermophilic reactor was a 2.5 L reactor with an 800 ml working volume. During the study, 400 ml effluent was withdrawn and 400 ml of the TWAS:FOG sample was fed into the reactor every 24 hours. The reactor was kept in a 70°C incubator. After running the hyper-thermophilic reactor for 6 HRTs (= 12 days) using the TWAS:FOG sample as feed, the effluents were collected, mixed well, and used for the experiments and sample analysis (TWAS:FOG sample after 2d hyper-thermophilic digestion).

Conventional heat pretreatment (Heat): This pretreatment was conducted in order to compare the effect on the TWAS:FOG sample of the hyper-thermophilic digestion stage at 70ºC and 2d HRT to the effect of conventional heat pretreatment at the same temperature and time (i.e., 70ºC and 48 hours duration). For the conventional heat pretreatment, flasks containing 400 ml of the TWAS:FOG mixture were sealed with aluminum foil and submerged in a New Brunswick digital water bath shaker at 70ºC for duration of 48h. After pretreatment, the samples were allowed to cool to room temperature before unsealing the flasks and saving the pretreated samples at a temperature of 4ºC until used for further analysis.

The effects of the different pretreatments were determined by measuring the increase in SCOD and TVFA for the samples before and after pretreatment and by analyzing the samples' supernatant (i.e., soluble sample part filtered through a 0.45 µm filter) distribution on a molecular level using ultrafiltration at 4 different cut-off membrane sizes. Testing the anaerobic biodegradability of the different molecular sizes using a biochemical methane potential test (BMP) was also used to determine the effects of the different pretreatments. Ultrafiltration and BMP assay methods are discussed next.

5.3.3 Ultrafiltration

The control sample (non-pretreated TWAS:FOG sample) and the three pretreated samples, namely MW (microwave), Hyper (hyper-thermophilic) and Heat (conventional heat), were centrifuged using a thermo-scientific centrifuge machine (Sorvall Legend™, T+/RT+ Thermo Scientific, Germany) at 9000 relative centrifugal force for 40 min to separate the supernatant. The separated supernatant was then filtered through a 0.45 µm pore size filter. To avoid the membranes clogging during UF, the filtered supernatant was diluted 6:1 using Milli-Q water, as recommended by other researchers (Coelho et al., 2014; Toreci et al., 2009). For the UF process, four 400 ml Amicon model 8400 stirred cells (Amicon Corp., Danvers, MA) were used along with flat (7.6 cm diameter), high-recovery, low-organic adsorption hydrophobic membranes (Millipore, Billerica, MA, USA). The membranes used were of four different cut-off sizes, namely 300 (PES300), 100 (YM100), 10 (YM10) and 1(YM1) kDa. The membranes were set in a cascade series, as shown in Figure 5.1, to eliminate the membranes clogging, especially membranes with a lower cut-off (Eskicioglu et al., 2006). The membranes used for the UF process were first rinsed with Milli-Q water for 1 hr with at least three water changes during this time. Next, the membranes were placed in the UF cells and the reservoirs filled with 300 ml of the supernatant to be filtered. Finally, the units were closed, and pressure applied using nitrogen gas in order to start the UF process. The applied pressure, as recommended by the manufacturer, was 10 psi for the PES 300 and 20 psi for the YM 100 to 1 kDa. The UF process was stopped when the liquid inside the UF reservoir was at 30 ml (10% of the original added volume). The units were then depressurized and the cells stirred for an additional 15 min to help improve the recovery of molecules adsorbed on the membrane surfaces. Afterward, the remaining 30 ml in the UF reservoirs were collected and labeled as retentates, and the filtered portions that passed through the filters were labeled as permeates (Coelho et al., 2014).

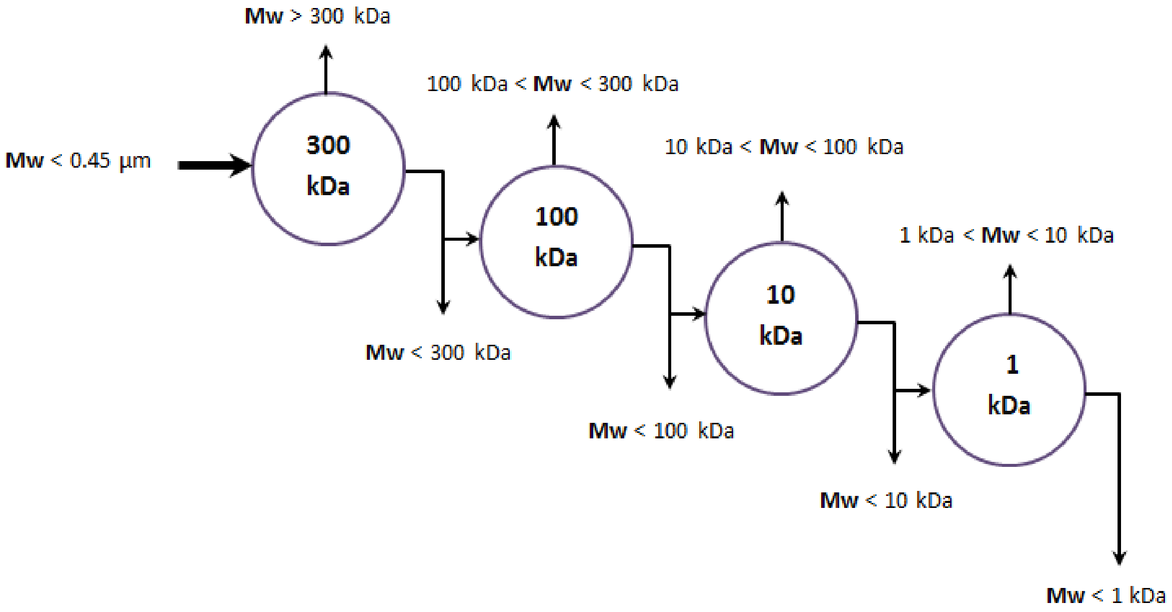


Figure 5.1: Cascade series set-up of ultrafiltration units used for the molecular weight (Mw) distribution experiments.

The UF cycles were repeated until enough volume of different retentates and permeate were obtained to perform the required experiments. At the end of each UF cycle, the membranes were soaked in a 0.1 M NaOH solution bath for about 1 hr and then rinsed with Milli-Q water before the membranes were used for a new cycle.

5.3.4 Biodegradability Assays

Biochemical methane potential assays (BMP) were used to measure the biodegradability of each UF fraction of the retentates and permeates. 15 ml of thermophilic inoculum and 65 ml of each sample were added to a 125 ml glass serum bottle to obtain a final volume of 80 ml in each bottle. Equal parts NaHCO3 and $KHCO_3$ were added to the mixture to obtain alkalinity of 4000-5000 mg/L as $CaCO_3$, and then the bottles were purged with nitrogen gas for 2-3 min to remove any oxygen gas from the headspace before sealing the bottles with rubber stoppers and aluminum caps. The BMP assays were done in duplicates, forming a total of 66 serum bottles that were kept at a 55±1°C controlled-temperature incubator shaker which was set at a speed of 90 rpm (New Brunswick Scientific Co. Inc., NB, Canada). The conditions tested in the BMP assays are given in Table 5.1.

5.3.5 Analytical Analysis

The soluble chemical oxygen demand (SCOD) and total chemical oxygen demand (COD) of the samples were measured using HACH TNT 822 (20-1500 mg/L) and TNT 823 (250-15000 mg/L) respectively, including spectrophotometer, heating block, and supplies. Total volatile fatty acids (TVFA) were measured using HACH TNT 872 (50-2500 mg/L CH_3COOH). During the BMP assays, volumetric biogas production was measured periodically using a gas manometer until the biogas production became negligible (33 days). The net biogas volume for each bottle was calculated by subtracting the average biogas produced from the inoculum bottles and then corrected to standard ambient temperature (25°C) and pressure (1 atm) conditions (STP).

Table 5.1: Test conditions for the BMP assays.

Test	Phase	Sample[1]	Membrane (kDa)	Inoculum (ml)	Final volume (ml)
1	Permeate	Control	1	15	80
2	Permeate	Control	10	15	80
3	Permeate	Control	100	15	80
4	Permeate	Control	300	15	80
5	Retentate	Control	1	15	80
6	Retentate	Control	10	15	80
7	Retentate	Control	100	15	80
8	Retentate	Control	300	15	80
9	Permeate	MW	1	15	80
10	Permeate	MW	10	15	80
11	Permeate	MW	100	15	80
12	Permeate	MW	300	15	80
13	Retentate	MW	1	15	80
14	Retentate	MW	10	15	80
15	Retentate	MW	100	15	80
16	Retentate	MW	300	15	80
17	Permeate	Hyper	1	15	80
18	Permeate	Hyper	10	15	80
19	Permeate	Hyper	100	15	80
20	Permeate	Hyper	300	15	80
21	Retentate	Hyper	1	15	80
22	Retentate	Hyper	10	15	80
23	Retentate	Hyper	100	15	80
24	Retentate	Hyper	300	15	80
25	Permeate	Heat	1	15	80
26	Permeate	Heat	10	15	80
27	Permeate	Heat	100	15	80
28	Permeate	Heat	300	15	80
29	Retentate	Heat	1	15	80
30	Retentate	Heat	10	15	80
31	Retentate	Heat	100	15	80
32	Retentate	Heat	300	15	80
33	Inoculum	--	--	15	15

[1] Control= no pretreatment, MW= Microwave, Hyper= hyper-thermophilic, and Heat= conventional heating.

5.3.6 Data Analysis

The AD kinetics for the different molecular weight fractions for the control and the samples with different pretreatment conditions were evaluated using the modified Gompertz (MG) equation (Equation 6.1) (Donoso-Bravo et al., 2010; Lo et al., 2010).

$$B = B_0 \,.\, \exp\left(-\exp\left(\frac{Rm\,.e}{B_0}(\lambda - t) + 1\right)\right) \qquad (6.1)$$

where: B_0 is the ultimate biogas yield (mL/g TVS), B is the cumulative biogas yield (mL/g TVS), t is incubation time (h), R_m is the maximum biogas production rate (mL/g TVS.h), and λ is the lag phase duration time (h). The MG equation was used in this study based on previous studies on MW pretreatment and hyper-thermophilic anaerobic digestion where the MG equation was shown to be more accurate than the first-order reaction equation for modeling the BMP assays biogas production. In the mentioned studies the Pearson coefficient of determination (R^2) was higher than 0.98, and there was a very good visual fit between observed data and model values (using MG equation) (Alqaralleh et al., 2016; Coelho et al., 2014).

5.4 Results and Discussion

5.4.1 Effect of Pretreatments on Solubilization

Figure 5.2 below shows the results of SCOD and TVFA for the TWAS:FOG samples before pretreatment (Control) and after the three different pretreatments considered in this study. The data in the figure represent the mean for two measurements, and the error bars are the absolute difference between the measured values and the calculated mean.

As expected, the effect of the three different pretreatments on the samples' solubilization resulted in a distinct increase in SCOD and TVFA for each pretreated sample compared to the un-pretreated control. However, each pretreatment method produced a different level of solubilization. It can be observed from Figure 5.2 that the MW pretreatment at 175°C had the greatest effect on solubilizing the TWAS:FOG sample compared to the control, increasing the

SCOD and TVFA by 109.6 and 120 %, respectively, On the other hand, compared to the control, hyper-thermophilic hydrolysis resulted in an 87.4 and 97.3 % increase in SCOD and TVFA, respectively. It was observed that the conventional heat had the smallest effect on sample solubilization, as it resulted in a 45.2 and 52.4 % increase in SCOD and TVFA, respectively, compared to the control.

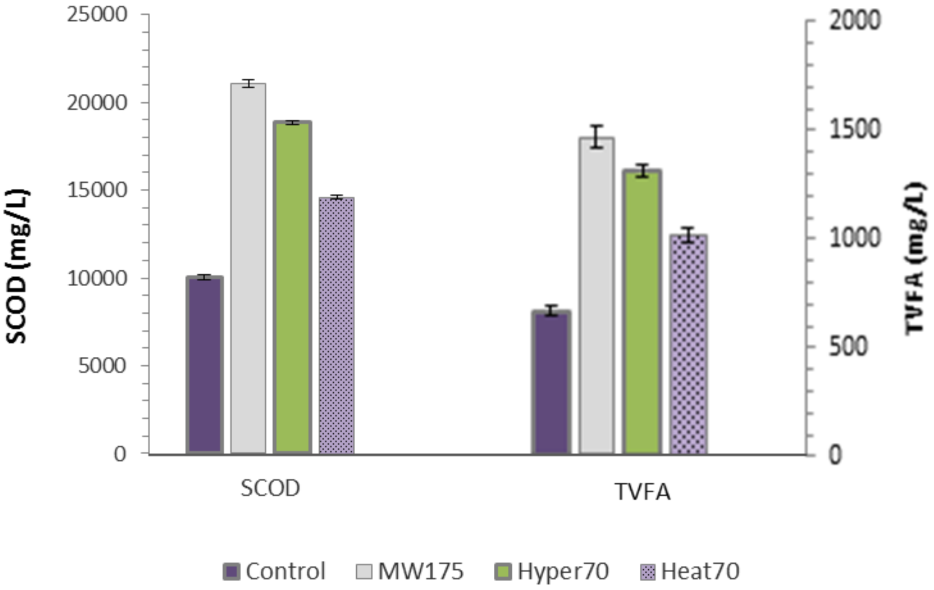


Figure 5.2: SCOD and TVFA concentrations for the soluble fraction (<0.45 µm) of non-pretreated control and the pretreated samples.

It has been shown in previous studies that different types of thermal pretreatment cause a breakdown of the extracellular polymeric substrates (EPS) matrix, resulting in a release of the soluble compounds from the cells within the TWAS (Coelho et al., 2014, Coelho et al., 2011, Toreci et al., 2009). Hence, a similar effect is expected with the TWAS:FOG sample considered in this study for thermal pretreatments using MW, Hyper (hyper-thermophilic) and Heat (conventional heat). For the TWAS part (80% of the mixture based on TVS), thermal pretreatment is believed to cause a breakdown of cell walls and release the intercellular matter to the aqueous phase (Pilli et al., 2015). Thermal pretreatment will also solubilize the FOG part (20% of the mixture based on TVS) and release the long-chain fatty acids (LCFA) that will degrade anaerobically via β-oxidation pathway (Equation 5.2) to acetate and H_2, which will subsequently convert to methane (Long et al., 2012; Kim et al., 2004).

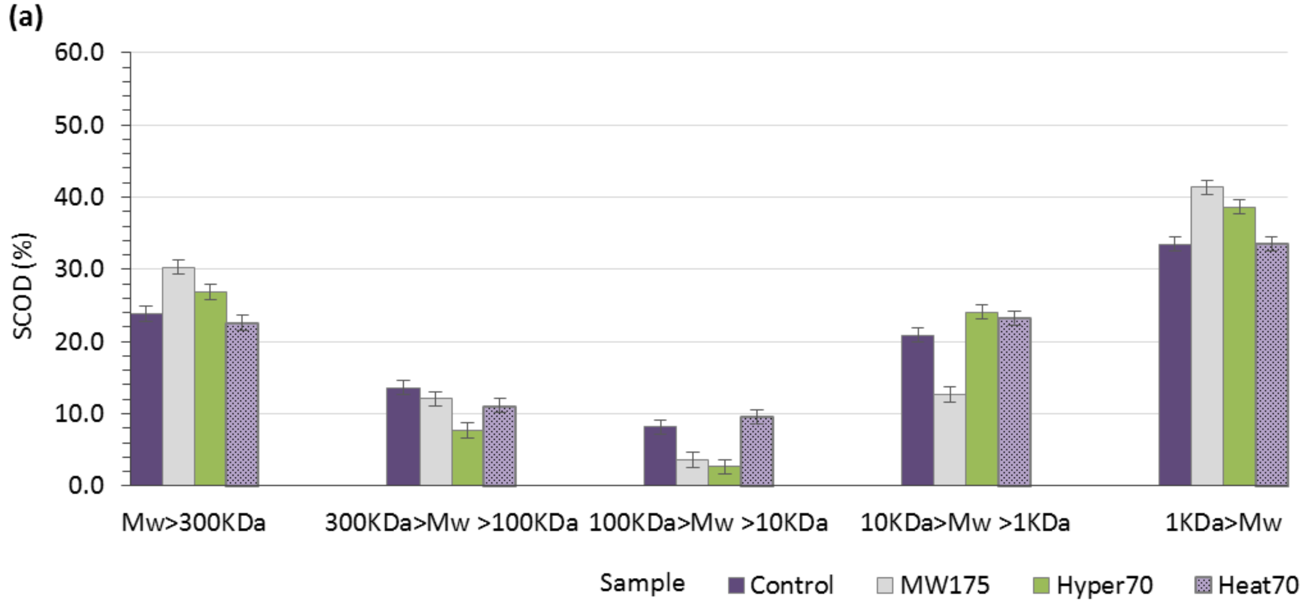
$$CH_3(CH_2)_nCOOH + 2H_2O \rightarrow CH_3(CH_2)_{n-2}COOH + CH_3COOH + 2H_2 \quad (5.2)$$

The solubilization effect on the TWAS:FOG mixture depends on the type of pretreatment used to deliver the thermal effects, and depends on the temperature as well. It is not surprising that the MW pretreatment resulted in the highest solubilization of SCOD and TVFA since the MW pretreatment was performed at an elevated temperature of 175°C. MW pretreatment works to solubilize the mixture via a combination of MW thermal and athermal effects (Coelho et al., 2014). This particular MW temperature has been proved in some other studies to cause maximum solubilization when used for pretreating TWAS alone (Saha et al., 2011; Toreci et al., 2010). However, pretreatment temperatures that exceed 175°C are not recommended, as they cause the formation of refractory compounds (melanoidin compounds) that inhibit the biodegradation process (Pilli et al., 2015). On the other hand, the low-temperature thermal pretreatments using the hyper-thermophilic step, and the conventional heat pretreatment, both at 70°C, did result in a reasonable increase in SCOD and TVFA. Taking into account the large difference in the temperature applied, hyper-thermophilic pretreatment caused much higher SCOD and TVFA solubilization compared to the conventional heating at the same temperature (70ºC) and for the same contact time (2 days). This validates the hypothesis that pretreating the mixture in a hyper-thermophilic anaerobic reactor does have different effects compared to conventional heating. It is believed that in addition to the thermal effects of the 70ºC temperature, the microbial and enzymatic activities in the hyper-thermophilic reactor help in solubilizing the mixture to a higher level compared to conventional heating.

5.4.2 Molecular Weight Distribution of Soluble Matter after UF

Figures 5.3 (a) and (b) show the results obtained after the UF of the soluble supernatant of the TWAS:FOG mixture (supernatant <0.45 µm) before pretreatment (Control) and after the three different pretreatments, i.e., MW175, Hyper70, and Heat70.

It can be observed from Figures 5.3 (a) and (b) that the three types of pretreatment used caused a noticeable change in the molecular weight distribution of SCOD and TVFA compared to the non-pretreated sample. The control sample (non-pretreated TWAS:FOG) had more than the half of its SCOD (54.4%) and 42.5% of its TVFA in the smaller molecular weight range (Mw <10 kDa), and 37.5% of its total SCOD as well as 43.0% of the TVFA in the larger molecular weight range (Mw> 300 kDa and 300> Mw >100 kDa). These results are consistent with other researchers' results, which showed that low-molecular-weight compounds were predominant in the soluble phase for young sludge (SRT 5d) (Coelho et al., 2014; Toreci et al., 2009). In this study, 80% of the control sample was TWAS (based on total VS), and TWAS used for the experiments was also young sludge (SRT =5d). This helped in comparing the results of this study with the previous work of Coelho et al. (2014) and Toreci et al. (2009).



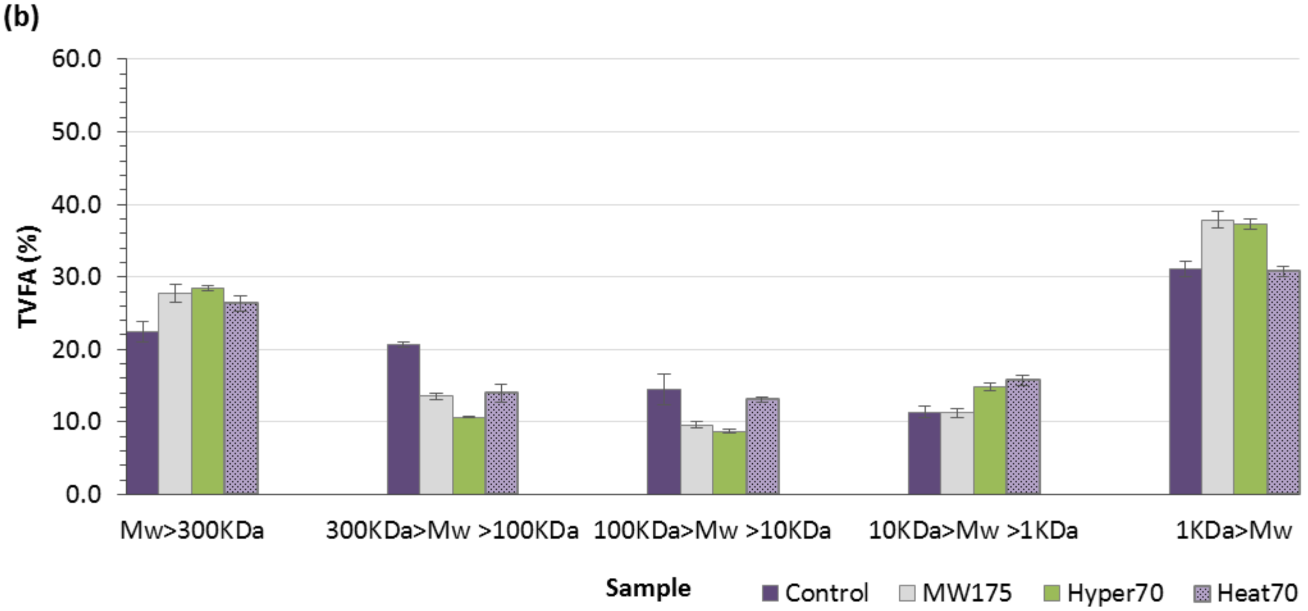


Figure 5.3: Molecular weight (Mw) of the soluble dissolved matter after UF for (a) SCOD and (b) TVFA.

The molecular weight distribution of the sludge was significantly affected by the operating and treatment conditions of the treating system. For example, young sludges (low SRT) have high amounts of soluble microbial products of low molecular weight (Coelho et al., 2014; Duncan and Stuckey., 1999; Boero et al., 1996). As shown in Figures 5.3 (a) and (b), microwave pretreatment (MW175°C) caused a noticeable increase in the SCOD and TVFA at the lowest molecular weight (Mw<1 kDa). The SCOD and TVFA increased at this molecular weight from 33.5% and 31.2% before pretreatment to 41.4% and 37.9% after MW pretreatment, respectively. The same observation was noticed for the highest molecular weight (Mw >300 kDa), as there was a noticeable increase in SCOD and TVFA, from 23.9% and 22.4% before pretreatment to 30.3% and 27.7% after MW pretreatment, respectively. The soluble matter within the large molecular weight size of Mw > 300kDa most likely contains cell wall fragments and exopolymers such as exopolysaccharides, humic and nucleic acids and protein (Eskicioglu et al., 2006; Duncan and Stuckey., 1999). Therefore, the increase in SCOD at this molecular size after the MW pretreatment is expected, as it indicates that the MW pretreatment helped in disintegrating and rupturing the sludge flocs and microbial cell walls. As a result, the MW pretreatment released a significant amount of cell wall fragments as well as intra- and extra-cellular biopolymers from the activated sludge. The increase in SCOD and TVFA in the smaller molecular weight ranges (10kDa >Mw >1kDa and Mw<1 kDa) is believed to be attributed to the breakage of bacterial cell walls and sludge flocs releasing low molecular weight compounds from the TWAS (Coelho et al., 2014) and solubilizing the FOG part by converting long-chain compounds to shorter-chain compounds and fatty acids (Long et al., 2012). On the other hand, the SCOD and TVFAs of the intermediate molecular weight sizes showed a decrease compared to the control, as shown in Figures 5.3 (a) and (b), except for the TVFA of molecular weight 10 >Mw> 1 kDa, as it did not change compared to the control.

The hyper-thermophilic step (Hyper70°C) also caused a change in the SCOD and TVFA distribution compared to the control. The changes caused by the Hyper step are somehow different compared to the changes caused by the MW pretreatment on the SCOD and TVFA distribution. The Hyper step caused an increase in SCOD and TVFA for the large molecular weight (Mw > 300 kDa) from 23.9% and 22.4% before pretreatment to 26.9% and 28.5% after pretreatment, respectively. This increase in SCOD after the Hyper step is significantly smaller than the increase observed after the MW pretreatment. This indicates that the MW pretreatment at 175°C was more powerful in disintegrating the sludge flocs and microbial cell walls. The Hyper sample showed another increase in SCOD and TVFA for the smaller molecular weight sizes (10 kDa > Mw >1 kDa and Mw <1 kDa) and a decrease in SCOD and TVFA for the two intermediate molecular weight sizes of 300> Mw >100 kDa and 100>Mw>10 kDa. After the Hyper step it was observed that the majority of the SCOD (62.7% of total SCOD) was concentrated in the smallest molecular range (10 kDa > Mw). These changes in soluble matter distribution indicate that the mechanism for solubilizing the TWAS:FOG sample might be different for the Hyper step compared to MW pretreatment. It seems that the effect of the Hyper step is a gradual hydrolysis process that solubilizes the sample by decreasing large and medium molecular weight matter to smaller molecular weight compounds. The relatively long time of 2 days for the Hyper step might have contributed to this gradual process, which ended with high solubilization for the small molecular weight sizes. This is different than the effect of MW pretreatment, which is an intense pretreatment at a high temperature of 175°C that occurs within a short time (intensity was 3.5°C/min, which means around 43 min of pretreatment time), which caused a sudden rupturing effect for the microbial cell walls and sludge flocs.

As for the heat sample, the SCOD also changed compared to the control, but the changes were different than the changes observed for the previous two pretreated samples (i.e., MW and Hyper), as can be observed from Figure 5.3 (a) and Table 5.2. The SCOD for the Heat sample showed a slight decrease in the large molecular weight sizes (Mw> 300 kDa and 300> Mw >100 kDa) from 23.9% and 13.6% for the control to 22.6% and 11.1% after pretreatment, respectively. An increase in the SCOD for the following two molecular sizes was observed, and no change in the smallest molecular size occurred, as shown in Figure 5.3 (a). The TVFA showed an increase in the molecular weight sizes Mw > 300 kDa and 10 kDa> Mw > 1 kDa and a decrease in the other molecular weight sizes. These results show clearly that Heat pretreatment using conventional heat (for 2 days) has its special way in solubilizing and distributing the soluble matter within the different molecular weight sizes. It does not follow any of the trends observed for the MW and Hyper pretreatments, yet the TVFA values for the Heat sample indicate that Heat pretreatment does help in sample solubilization by weakening and breaking the bonds and dividing the large compounds into smaller units, causing a release of TVFA. However, these results show that the effect of Heat pretreatment on SCOD and TVFA, especially for smaller molecular sizes, is noticeably less than the effect produced by the Hyper step which had the same temperature and duration (70°C for 2 days). This confirms that the effect of the hyper-thermophilic step goes beyond just the thermal effect. It is believed that microbial and enzymatic activities in the hyper-thermophilic stage are incorporated in the solubilization process, resulting in better sample solubilization.

5.4.3 Biodegradability of Different Molecular Weight Fractions

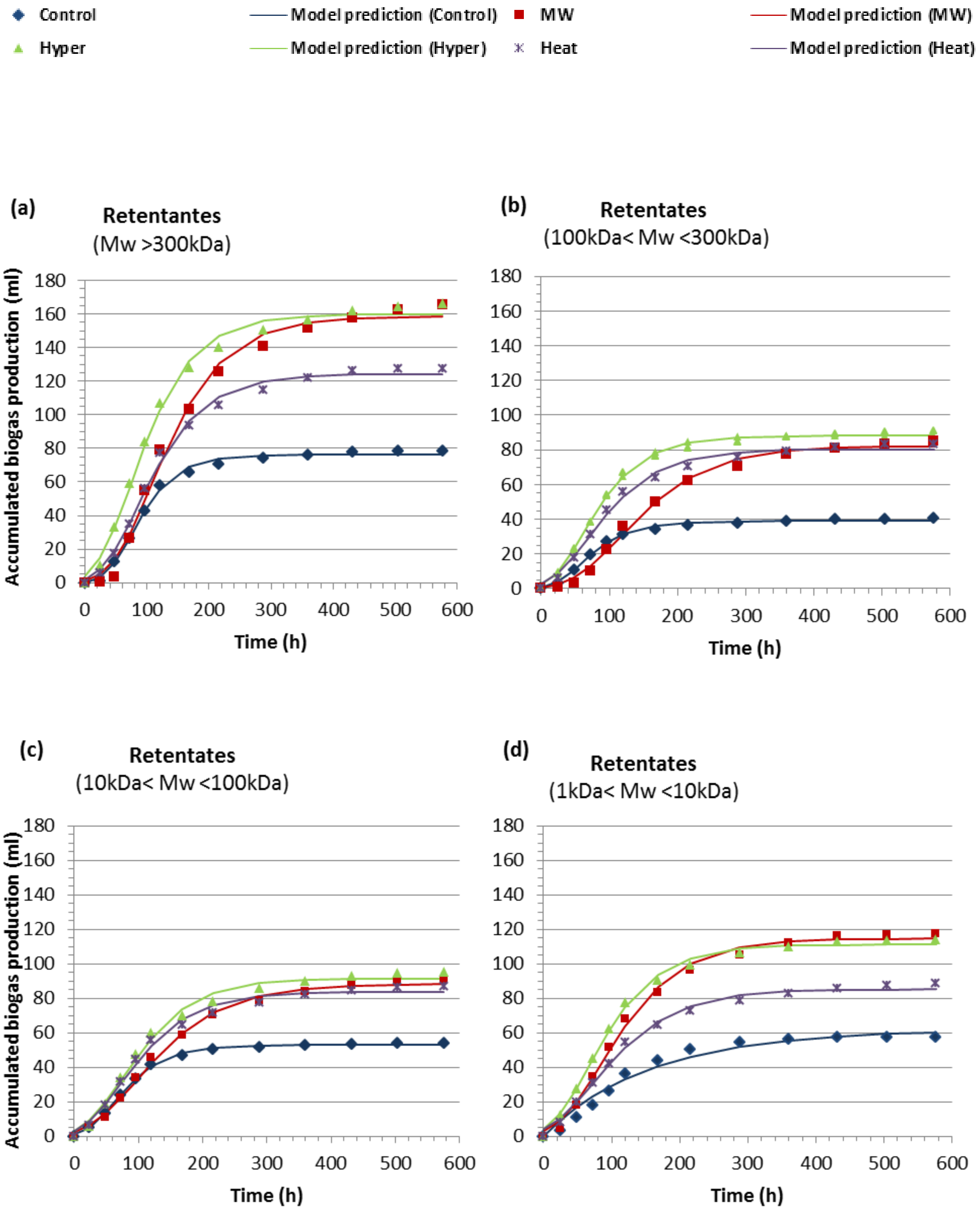
All the soluble fractions produced from the UF as permeates (i.e., fraction that passed the filter) and retentates (fraction retained by the filter) were used for a biodegradability assays (BMP). The BMP assays were run in duplicate under thermophilic conditions. The cumulative biogas BMP results were used for non-linear regression using the MG equation (Equation 5.1). Figure 5.4 shows the experimental cumulative biogas production from the BMP assays (the discrete data points) and the regression model using the MG equation (i.e., the continuous line). The average measured cumulative biogas production (CBP), the estimated lag-phase (λ, h^{-1}), the maximum biogas production rate (Rm, mL/gTVS.h), and the Pearson coefficient of determination (R^2) are presented in Table 5.2. The Pearson coefficient of determination R^2 was greater than 0.993 for all the cases tested, except for one case that had R^2=0.971, as shown in Table 5.2. The high R^2 for all the cases indicates that there is a very good match between the observed (BMP results) and modeled values (using the MG equation).

From analyzing the curves in Figure 4 and the Lag-phase values in Table 5.2, it can be seen that the control's and all the pretreated samples' retentates and permeates had a slight delay in biogas production with a measured lag-phase that does not exceed 46.6 h. The control sample had a lag-phase that ranged from 11.8 to 32.0 h. This kind of lag-phase in the initial stage of digestion is common in TWAS digestion; it is a temporary lag period before the substrate degradation activity starts (Coelho et al., 2014; Duncan and Stuckey., 1999). However, the lag-phase periods for the control samples in the current study are much lower compared to the lag-phases recorded for the control in Coelho et al. (2014), with their control sample being solely TWAS. The reason behind that is most likely because of the FOG portion that is included as a part of this study's control sample (control = 80%TWAS:20%FOG). The FOG portion provides a quick dose of volatile fatty acids in the soluble matter, which helps in starting the digestion process faster compared to TWAS alone. It is noticeable that the MW pretreatment caused an increase in the lag-phase for all retentate and permeate sizes except for retentate 1< Mw <10, which had a slightly lower lag-phase compared to the control. The increase in lag-phase after MW pretreatment is believed to be related to the formation of some inhibitory compounds which occurred in some of the retentates and permeates at the high MW temperature (175°C). Another possible reason for the longer lag-phase might be due to the inoculum which was not acclimated to the MW-pretreated samples (i.e., the inoculum used for the BMP assay was not acclimated for any of the pretreatments used for this study; it was the effluent from a thermophilic reactor that digested TWAS alone (non-pretreated) at 20-24 d HRT). Regardless of the relatively longer lag-phases for the MW-pretreated retentates and the permeates of different molecular weight sizes, the MW-pretreated samples had higher maximum biogas production rates for most of the molecular sizes (for 5 samples out of 8) compared to the control samples, and produced much more biogas compared to the control samples, as shown in Figure 5.4(a) to (h).

On the other hand, all the Hyper samples (retentates and permeates) had lower lag-phases compared to both the control and MW samples. The Hyper samples had a higher R_m for all samples compared to the corresponding control and MW samples except for the permeate sample (Mw< 1 kDa), which had a lower R_m compared to the corresponding MW sample. These results (low lag-phases and high R_m values) plus the higher biogas production for all the Hyper samples compared to the control indicate that the hyper-thermophilic step not only increased the sample solubilization, as pointed out previously (Figures 5.2 and 5.3) but also improved biogas production without having inhibitory effects.

Heat samples had lower lag-phases and higher R_m compared to the control samples, except for two permeate samples that had similar or lower R_m values compared to the control samples (Mw<300 and Mw<10 kDa). The cumulative biogas production of all the Heat samples was higher than that of the control; however, the cumulative biogas productions of all Heat samples were noticeably lower than the MW and Hyper samples. The Heat pretreatment results indicate that thermal pretreatment alone (Heat at 70°C) is effective in solubilizing the sample and in increasing biogas production from each of the different molecular weight distribution samples. However, the Heat effects were significantly less potent compared to the MW and Hyper pretreatments.

Table 5.3 shows the sum of the CBP for all the permeates and retentates of the control and the three pretreated samples, and also the improvement (%) in total CBP for each pretreated sample compared to the control. The Hyper sample had a total CBP for all the soluble fractions that was 86.5% higher than that of the control. As well, the MW-pretreated sample had an 80.1% higher total CBP compared to the control; this improvement in CBP is very close to the improvement achieved by the Hyper sample, indicating that Hyper-thermophilic and MW pretreatment were the most effective pretreatment methods among the three methods tested. The Heat sample had the least improvement (46.0%) in total CBP compared to the control, indicating that Heat pretreatment (conventional heat) had the least effect on improving biogas production from the different fractions of the soluble sample.


Control
Model prediction (Control)
MW
Model prediction (MW)
Hyper
Model prediction (Hyper)
Heat
Model prediction (Heat)
(a)
Retentantes
(Mw >300kDa)
(b)
Retentates
(100kDa< Mw <300kDa)
(c)
Retentates
(10kDa< Mw <100kDa)
(d)
Retentates
(1kDa< Mw <10kDa)
Accumulated biogas production (ml)
0
20
40
60
80
100
120
140
160
180
Time (h)
0
100
200
300
400
500
600

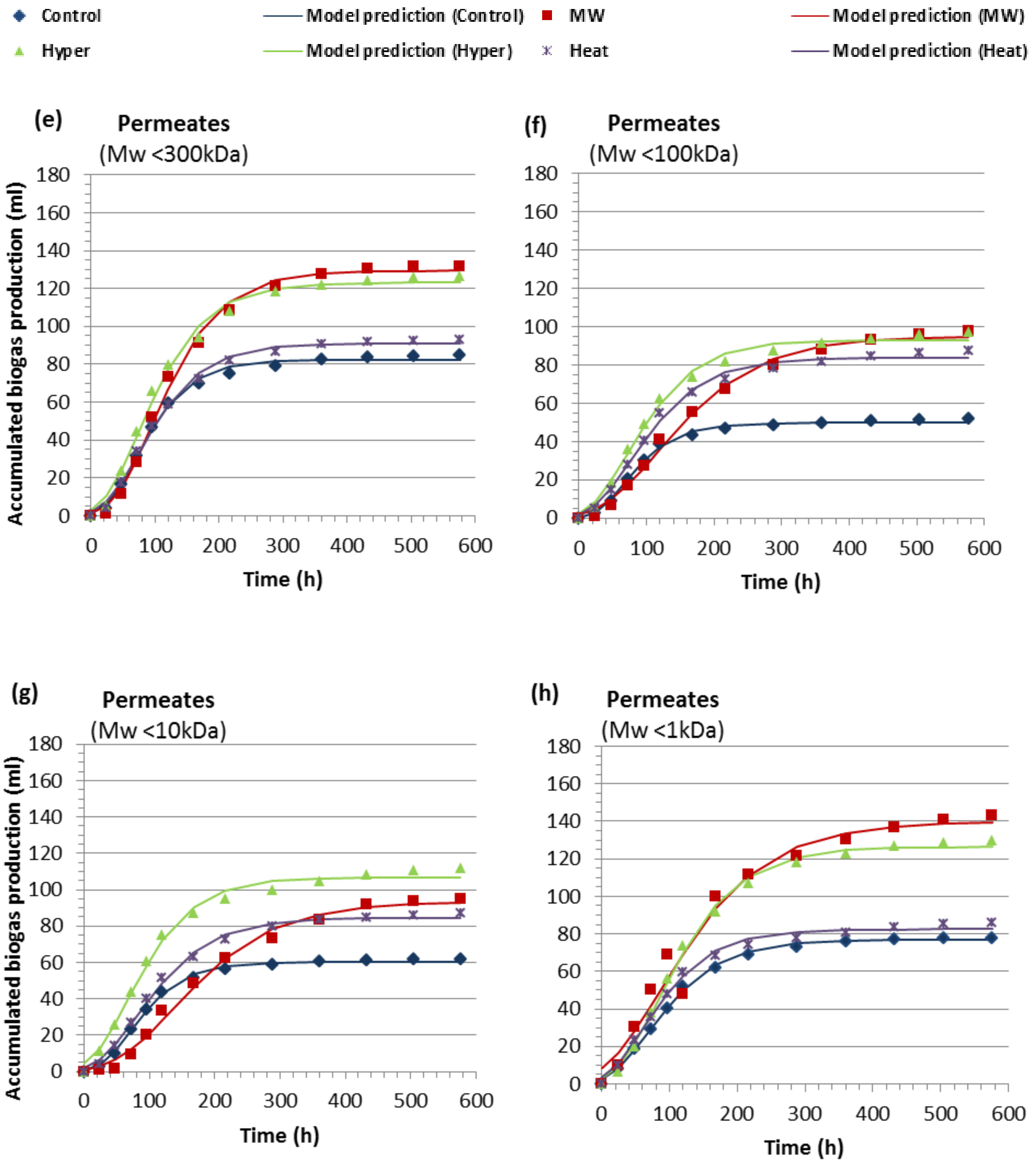


Figure 5.4: Observed and predicted cumulative biogas production results for: Retentates (a) Mw>300, (b) 100< Mw <300, (c) 10< Mw <100, (d) 1< Mw <10, and Permeates (e) Mw <300, (f) Mw <100, (g) Mw <10, (h) Mw <1 kDa. Control = No pretreatment, MW = microwave pretreatment, Hyper = hyper-thermophilic digestion step, and Heat = conventional heat.

Table 5.2: Anaerobic biodegradability results for all the molecular weight fractions.

Mw (kDa)	Cumulative biogas production (ml)				Ultimate biogas production (mL) [Lag-phase (h)]^2				Rm (mL/gTVS.h) [R^2]^3			
	Control	**MW**	**Hyper**	**Heat**	**Control**	**MW**	**Hyper**	**Heat**	**Control**	**MW**	**Hyper**	**Heat**
Retentates												
Mw >300	78.6±2.7	165.7±5.7	166.3±2.8	127.6±0.7	76.4[32.0]	158.7[40.7]	159.9[16.3]	124.3[24.6]	0.67 [0.996]	0.87 [0.992]	1.03 [0.995]	0.76 [0.995]
100< Mw <300	40.4±0.7	84.9±1.1	90.9±0.7	83.4±1.7	38.9[17.6]	82.1[44.6]	88.0[14.5]	80.3[13.2]	0.35 [0.994]	0.42 [0.995]	0.67 [0.996]	0.52 [0.993]
10< Mw <100	53.9±1.0	90.6±1.5	95.1±2.3	87.1±1.1	52.8[17.9]	88.3[21.7]	91.5[13.8]	83.6[11.9]	0.44 [0.998]	0.43 [0.997]	0.55 [0.993]	0.50 [0.992]
1< Mw <10	57.6±1.1	117.3±1.1	113.9±3.0	88.5±0.8	56.8[20.8]	114.6[20.2]	111.1[10.3]	85.2[8.1]	0.35 [0.998]	0.64 [0.996]	0.72 [0.996]	0.47 [0.994]
Permeates												
Mw <300	84.6±0.7	131.6±2.1	126.5±2.6	92.8±2.6	82.4[22.3]	129.4[34.2]	123.1[17.8]	90.9[19.3]	0.63 [0.996]	0.78 [0.996]	0.79 [0.995]	0.60 [0.997]
Mw <100	51.7±1.2	97.5±1.0	97.3±1.7	87.5±2.0	49.9[26.0]	95.3[32.4]	93.1[16.4]	83.8[19.6]	0.44 [0.996]	0.41 [0.995]	0.60 [0.994]	0.52 [0.995]
Mw <10	61.9±1.4	95.1±5.3	112.0±2.1	87.0±1.8	60.5[25.9]	93.8[46.6]	106.8[10.5]	84.7[19.2]	0.49 [0.998]	0.39 [0.994]	0.70 [0.993]	0.49 [0.996]
Mw <1	78.0±3.2	129.8±2.4	143.1±4.6	86.0±1.6	76.7[11.8]	126.4[20.3]	139.8[2.7]	82.4[7.5]	0.48 [0.998]	0.71 [0.997]	0.59 [0.971]	0.55 [0.994]

[1] Cumulative biogas production results obtained from the BMP assays. Data are the average ± the absolute difference between the mean and the duplicate measured values. [2] The ultimate biogas production (mL) and lag phase (h) as determined from the GM equation. [3] The maximum biogas production rate (ml/g TVS.h) and [Pearson coefficient of determination R^2] as determined from GM equation.

Table 5.3: The sum of CBP (mL) for all fractions of the control and pretreated samples.

Sample	Fraction	300 kDa	100 kDa	10 kDa	1 kDa	Sum of CBP (mL)	Total CBP (mL)	Relative[1] Improvement (%)
Control	Retentate	78.6	40.4	53.9	57.6	230.5	506.7	
	Permeate	84.6	51.7	61.9	78.0	276.2		
MW	Retentate	165.7	84.9	90.6	117.3	458.5	912.5	80.1
	Permeate	131.6	97.5	95.1	129.8	454.0		
Hyper	Retentate	166.3	90.9	95.1	113.9	466.2	945.1	86.5
	Permeate	126.5	97.3	112.0	143.1	478.9		
Heat	Retentate	127.6	83.4	87.1	88.5	386.6	739.9	46.0
	Permeate	92.8	87.5	87.0	86.0	353.3		

[1] Improvement in CBP was calculated related to the control.

5.5 Conclusions

Each of the three tested pretreatment methods in this study (MW, Hyper, and Heat) had a different effect on soluble matter (SCOD and TVFA) molecular weight distribution.

MW pretreatment substantially increased the SCOD for the larger molecular weight (Mw >300 kDa) and the smaller molecular weight (Mw <1 kDa), whereas the Hyper stage had more of an effect on increasing SCOD in the small molecular weight range, mainly 1 kDa >Mw> 10 kDa, and Mw< 1 kDa.

The effect of Heat pretreatment on SCOD and TVFA, especially on the smaller molecular sizes, is noticeably less than the effect produced by the Hyper step, which had the same temperature and duration (i.e., 70°C for 2days).

The results of this study confirm that the effect of the hyper-thermophilic step goes beyond just the thermal effect. The microbial and enzymatic activities in the hyper-thermophilic stage have a significant effect in achieving high solubilization for the TWAS:FOG sample and changing the molecular weight distribution of the soluble matter.

Gompertz equation provided an accurate description of the biogas production from the different molecular weight fractions of the different samples.

The Hyper-thermophilic sample had the maximum improvement in the sum of the cumulative biogas production from all the molecular weights among the three pretreated samples, achieving 86.5% higher CBP compared to the control.

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CHAPTER 6

6.1 Thermophilic Anaerobic Co-digestion of Thickened Waste Activated Sludge and Liquid Part of Fat, Oil, and Grease

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6.1.1 Abstract

Anaerobic Digestion (AD) became one of the leading methods used in converting organic waste into renewable energy (biogas). AD low cost, energy production, and limited environmental impact make it a reliable technology for stabilizing organic waste, specifically sewage waste. This study aims to investigate the feasibility of using the liquid part separated from Fat, Oil, and Grease (L-FOG) as a co-substrate in the AD of thickened waste activated sludge (TWAS) for biogas production. Two biochemical methane potential (BMP) tests were conducted. The first BMP test aimed to determine the ideal substrate to inoculum ratio for the digestion of the L-FOG whereas the second BMP test investigated the anaerobic co-digestion of L-FOG and TWAS, and the effect of using different percentage (based on total volatile solids) of L-FOG on the methane production. The results showed a significant increase in the methane production with the addition of L-FOG compared to TWAS alone digestion.

6.1.2 Introduction

Due to the dramatic depletion of fossil fuels, which was the main energy source for decades, the energy sector has become the current most important interest in the world (Divya et al., 2015), and renewable energy became the buzzword of today. Renewable energy promises to become one of the main power sources of the future (Zhou et al., 2012). Biogas produced from the anaerobic digestion (AD) process contains mainly methane gas (CH_4) which is considered an economical and environmentally friendly biofuel (Alqaralleh et al., 2015; Hosseini et al., 2014). Methane gas can be used directly to produce heat and electricity, or it can be injected into the natural gas distribution network.

AD Anaerobic digestion can be defined as the process of oxidizing and decomposing the biodegradable organic material by microorganisms in the absence of oxygen. The final results of the AD process are mainly two useful final products: biogas (mainly methane gas) and residual digestate with a concomitant reduction in the mass of substrate (soluble or insoluble) in the liquid digestate. The AD of complex organic polymers is commonly divided into four main steps: hydrolysis, fermentation (also known as acidogenesis), β-oxidation (acetogenesis) and methanogenesis (Nayono, 2010). AD has many advantages such as providing organic mass stabilization and volume reduction, reducing pathogens and odor problems, small space requirements compared to other treatment options in addition to the production of methane gas that can be used as an energy source. These advantages make AD treatment of solid organic wastes a very attractive trend for sludge/waste disposal and bioenergy production (Martinez et al., 2012).

Sludge, particularly waste activated sludge (WAS) has low biodegradability which makes it hard to digest under the traditional mesophilic conditions (37°C) (Mann et al., 2013; Coelho et al., 2011). Anaerobic co-digestion of sludge by adding a co-substrate has been proven to enhance the AD process, and as a result, it increases biogas production and concomitant organic matter degradation. Adding a co-substrate to sludge prior to AD offers some additional potential benefits for both substrates such as dilution of inhibitory compounds, increase buffer capacity, a more balanced carbon to nitrogen ratio as well as access to essential micro and macro nutrients (Li et al., 2013).

Lipid-rich wastes usually known as fat, oil, and grease (FOG) are an attractive co-substrate to be used in anaerobic co-digestion of sludge due to its high methane potential (Wan et al., 2011). Several studies reported the beneficial effect of anaerobic co-digestion of sewage sludge and FOG (Alqaralleh et al., 2013; Luostarinen et al., 2009). However the use of FOG for AD is facing a significant challenges due to its inhibitory effects on the anaerobic microbial consortia particularly methanogens, as well as causing several operational problems related to clogging, scum formulation and sludge flotation as a result of the adsorption of FOG around the biomass surface (Kim et al., 2004). This study aims to investigate the viability of using the liquid part of FOG (L-FOG) for the anaerobic co-digestion of TWAS, as it is believed that the L-FOG should be less problematic for the AD process but still having a high methane potential to boost the biogas (methane) production during the AD process.

6.1.3 Materials and Methods

6.1.3.1 Substrates and inoculum

Thickened waste activated sludge (TWAS) was obtained from the thickener centrifuge at the Robert O. Pickard Environmental Center (ROPEC), Ottawa, ON, Canada and contained 5% TS of which about 71% were volatile solids. FOG sample was provided from the Organic Resources Management Inc. (ORMI), Ottawa, ON, Canada. ORMI provides grease trap cleaning services for different wastewater treatment plants in Ontario. Both substrates (TWAS and FOG) were stored at 4^{o}C prior to use. The liquid part of FOG was extracted by passing the homogenized FOG sample through an 850 µm sieve; the passing part of sample through the sieve was collected and used in the experiments as L-FOG. The thermophilic anaerobic inoculum (55^{o}C) was obtained from the effluent of a 10L thermophilic anaerobic digester acclimated to TWAS and operated at a 20 days hydraulic retention time (HRT) in our research lab. The characteristics of substrates and inoculums used in this study are listed in Table 6.1.1.

Table 6.1.1: Characteristics of substrates and inoculum.

Parameter	TWAS	L-FOG	Thermophilic inoculum
pH	6.9±0.1	3.2±0.0	7.7±0.1
TS (g/Kg)	50.1± 2.0	35.7±1.3	32.7±1.0
VS (g/Kg)	35.6±1.9	30.7± 1.3	16.0±0.3
VS/TS	0.71±0.02	0.86±0.01	0.49±0.02

Data represents the arithmetic mean ± standard deviation. (n=4).

6.1.3.2 Biochemical methane potential assays (BMP)

This study included two BMP tests; the first BMP test was used to investigate the ideal substrate to inoculum ratio (S/I) ($gTVS_{substrate}/gTVS_{inoculum}$) for the AD of the L-FOG. Six different S/I ratios were used based on the total volatile solids (TVS) 0.5, 0.75, 1.0, 1.25, 1.5 and 2.0. The ideal S/I ratio provided from this BMP test was used to design the second BMP test that investigated the co-digestion of TWAS and L-FOG using 20, 40, 60 and 80% L-FOG (based on TVS) (Table 6.1.2).

To mitigate the pH effects, supplemental alkalinity was added to all the 160 ml BMP serum bottles using equal amounts of $NaHCO_3$ and $KHCO_3$ to provide approximately 4000 mg/L alkalinity as $CaCO_3$. The pH of all BMP bottles was within the ideal range for anaerobic digestion system (6.5-7.5) (Droste, 1996). The serum bottles were next purged with N_2 gas for 5 minutes to maintain anaerobic conditions. After sealing with butyl rubber stoppers and screw caps, bottles were kept in a temperature-controlled incubator shaker (New Brunswick Scientific Co. Inc., NB, Canada) at $55\pm1^{o}C$ and 100 rpm for 40 days for both BMP1 and BMP2.

Table 6.1.2: Digestion mixtures used for BMP1 and BMP2.

BMP1				BMP2			
Sample	**Inoculum (ml)**	**L-FOG (g)**	**S/I* (ratio)**	**Sample****	**Inoculum (ml)**	**L-FOG (%)**	**(TWAS) (%)**
Blank 55°C	65	0	0	**Blank 55°C**	65	0	0
L-FOG (0.5)	65	16.3	0.5	**0.0% L-FOG**	65	0	100
L-FOG (0.75)	65	24.5	0.75	**20% L-FOG**	65	20	80
L-FOG (1.0)	65	32.6	1.0	**40% L-FOG**	65	40	60
L-FOG (1.25)	65	40.8	1.25	**60% L-FOG**	65	60	40
L-FOG (1.5)	65	48.9	1.5	**80%L- FOG**	65	80	20
L-FOG (2.0)	65	62.2	2.0				

* S/I ratio based on total volatile solids. ** For BMP 2 all samples had S/I ratio =1.0 (the ideal S/I ratio as resulted from BMP1).

6.1.3.3 Analytical methods

All analysis was done in replicates, following the standard methods for the Examination of Water and Wastewater (APHA, 1998). Total and volatile solids were determined according to Standard methods 2540G. Alkalinity analysis was done based on Standard methods 2320B using a Fisher Accument® XL25 pH meter. Biogas was measured using a manometer every 24 hours the first 7 days of the experiment, and it was measured occasionally after the biogas production slowed down until the end of the BMP experiments (40 days for both BMP assays). The volume of the biogas was corrected to the standard ambient temperature and pressure conditions (STP, 25°C and 1 atm). The biogas composition was monitored weekly using a Hewlett Packard 5710A gas chromatograph equipped with a 3380A model integrator, a 5705A thermal conductivity detector and using Helium as the carrier gas.

6.1.4 Results and Discussion

6.1.4.1 The ideal (S/I) ratio for L-FOG anaerobic digestion (BMP1)

The net cumulative biogas production during the first BMP test (BMP1) for all samples are shown in Figure 6.1.1, where 0.5, 0.75, 1.0, 1.25, 1.5 and 2.0 represent the S/I ratio of the samples based on total VS.

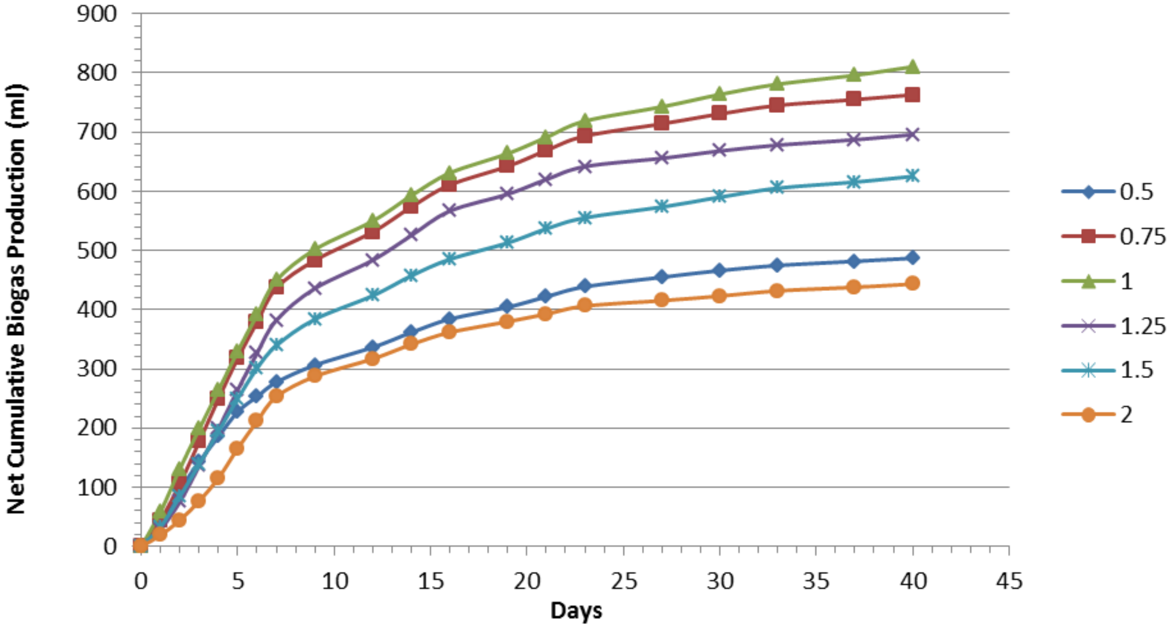

Figure 6.1.1: Net cumulative biogas production during BMP1 (with different S/I ratios).

Data in the figure represents the arithmetic mean of triplicates. Samples with S/I ratios of 0.75 and 1.0 start the degradation faster than the other samples as demonstrated in Figure 6.1.1. These two samples had the highest biogas production at the end of the BMP test compared to all other S/I ratios because the S/I ratio in this range provides the right amount of microorganisms required for the AD of L-FOG. Hence, we can conclude from the net cumulated biogas results that the S/I ratio in the range of 0.75-1.0 is preferable for a quick start degradation process of L-FOG. However, since the sample with S/I ratio of 1.0 resulted in the maximum biogas production with the highest methane content which amounts to 810.5±11.8 ml biogas/g TVS $_{added}$ with 79±1.1% methane, we further conclude that S/I ratio of 1.0 represents the ideal ratio for L-FOG AD. On the other hand, the sample with S/I ratio of 2.0 exhibited the slowest degradation start and resulted in a significant lower biogas production compared to all other samples. This sample (S/I ratio =2.0) produced a net cumulative biogas production of 443.4±42.2 ml biogas/g TVS $_{added}$ with 52.7±0.9% methane content, which represents 63.4% less methane production per g of TVS added compared to the sample with the ideal S/I ratio (sample with S/I ratio =1.0).

6.1.4.2 Effect of different L-FOG % in the co-digestion mixture on the biogas production (BMP2)

BMP2 was designed using the ideal S/I ratio (S/I =1.0) resulted from BMP1. The 0.0, 20.0, 40.0, 60.0 and 80.0% of L-FOG were used to prepare the co-digestion mixtures (TWAS and L-FOG), and the L-FOG% was calculated based on TVS. The net cumulative biogas production for all samples are represented in Figure 6.1.2, where 00L, 20L, 40L, 60L and 80L represent samples with 0.0, 20.0, 40.0, 60.0 and 80.0% of L-FOG (based on TVS), respectively. The sample with 0.0% L-FOG (00L) is the control sample used in this BMP test.

The experimental results show that all samples with different L-FOG% (based on TVS) resulted in a significant increase (t-test, $p<0.05$) in biogas production compared to the control sample 00L (TWAS with no L-FOG added). It is noticeable that as the L-FOG% increases, the biogas production increases as well with no exceptions for all samples (Figure 6.1.2). The control sample produced 437.4±31.4 ml biogas/g TVS$_{added}$, and adding 20.0, 40.0, 60 and 80% of L-FOG resulted in 496.4±27.0, 624.4±30.3, 685.8±25.7 and 800.3±22.5 ml biogas/g TVS $_{\text{added}}$, respectively. This is equivalent to 13.5, 42.8, 56.8 and 83.0% increase in biogas production per g of TVS added compared to the control sample (00L). The results mentioned above confirm the validity of using the ideal S/I ratio obtained using the BMP1.

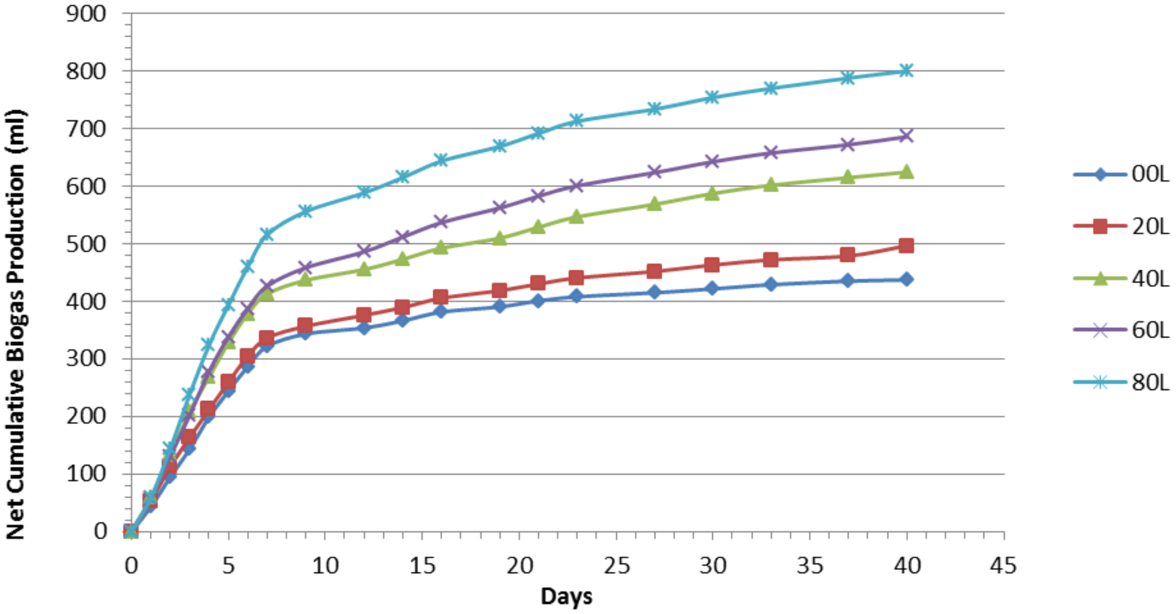

Figure 6.1.2: Net cumulative biogas production during BMP2 assay (with different L-FOG % in the co-digestion mixture).

6.1.5 Conclusion

The feasibility of determining the ideal S/I ratio required for the AD of the L-FOG prior to conducting the BMP test has proven. The first BMP test in this study showed the important role of S/I ratio on the degradation and biogas production from the AD of the L-FOG. The BMP1 test indicated that the S/I ratio of 1.0 is the ideal ratio to be used for L-FOG AD. This ratio resulted in a maximum biogas production and methane content. The second BMP showed the positive effect of L-FOG on TWAS co-digestion when the S/I of 1.0 was used; the results of this test showed that as L-FOG % increases in the anaerobic co-digestion mixture (based on TVS), the biogas production increased significantly. The maximum biogas production was recorded for the sample with 80% L-FOG which produced 83.0% more biogas than the control sample. It is noteworthy that for all samples in BMP2, no inhibition occurred during the test which indicates that the L-FOG can be used as a safe co-substrate option for TWAS co-digestion to boost the degradation and biogas production without inhibition or negative effects on the anaerobic digester.

6.1.6 References

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6.2 Biogas Recovery from Hyper-thermophilic Anaerobic Co-digestion of Thickened Waste Activated Sludge, Organic Fraction of Municipal Solid Waste and Fat, Oil and Grease

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6.2.1 Abstract

The use of organic fraction of municipal solid waste and fat oil and grease (FOG) as co-substrates for thickened waste activated sludge anaerobic digestion has the potential to improve the biodegradation process and significantly enhance biogas production and methane yields. This will not only help convert these potential waste streams from landfills increasing the longevity of existing landfills but also provide a sustainable waste to energy waste management method. In this study, the anaerobic co-digestion of organic fraction of municipal solid waste, with thickened waste activated sludge (50:50, w/w based on total volatile solids) was investigated using anaerobic digestion thermophilic and hyper-thermophilic biochemical methane potential (BMP) assays. The hyper-thermophilic BMP assays outperformed the thermophilic BMP assays by providing faster biogas production rates, higher cumulative biogas productions, and methane yields. Additionally, 10, 20 and 30 % FOG (based on total volatile solids) were added to the co-digestion mixtures in order to boost the biogas production and methane yield in three hyper-thermophilic assays. 30% FOG in the co-digestion mixture

enhanced the biogas methane content for sample TWAS:OFMSW:30%FOG(H) to 66.4% compared to 60.1% for the control sample TWAS(T), and accordingly improved the methane yield to be 84.4% higher than the methane yield of the control.

6.2.2 Introduction

Renewable energy industries have been developed incredibly fast in the recent years (Divya et al., 2015). Biogas harvested from organic wastes is a potential renewable source of energy alternative to fossil fuels. Anaerobic digestion (AD) is a widely-applied method for generating biogas from organic waste. However, the biogas utilization in wastewater treatment plants (WWTPs) is still limited; for example, in the US less than 10% of the WWTPs produce biogas for beneficial use such as heat and power generation (Shen et al., 2015). The slow biogas production rate during the AD process, biogas low energy content (low methane %), and the high cost to upgrade the produced biogas are some of the critical challenges that WWTPs industry is facing to deploy biogas production and utilization (Gianico et al., 2015; Shen et al., 2015). Sewage sludge in the form of primary sludge (PS), waste activated sludge (WAS) and thickened waste activated sludge (TWAS) is the most popular waste that is treated using AD. However, sludge low (C/N) ratio that generally ranges from 6 to 9 negatively impacts the efficiency of AD process especially under the traditional mesophilic conditions (Alqaralleh et al., 2016; Coelho et al., 2014). The organic fraction of municipal solid waste (OFMSW) has high C/N ratios, when co-digested with TWAS, OFMSW will balance the overall C/N ratio in the mixture for more stable AD process. Additionally, adding FOG to the co-digestion mixture proved to be an effective way to boost the biogas production and increase methane yields resulting from the

AD process. This is due to FOG high theoretical methane potential (0.70-1.43 m^3CH_4/ kg volatile solids) (Xu et al., 2015; Mata-Alvarez et al., 2014).

Hyper-thermophilic digestion at 70±1°C temperature is a relatively new approach in the sludge/ TWAS AD. Alqaralleh et al., 2016 used the hyper-thermophilic method for the co-digestion of TWAS and FOG. The results showed that hyper-thermophilic digestion improved the overall anaerobic co-digestion process which led to higher biogas production, higher methane yield, as well as increasing the ability of the digester to digest mixtures with high FOG contents (up to 60% based on TVS) without causing adverse effects on the AD process (Alqaralleh et al., 2016).

Therefore, the present study aimed to investigate the hyper-thermophilic AD of a binary mixture of TWAS:OFMSW (50:50, w/w) and trinary mixtures of TWAS:OFMSW and FOG with different FOG % (10, 20 and 30%). The performance of the binary and trinary mixtures hyper-thermophilic co-digestion were investigated in comparison with the thermophilic mono-digestion of TWAS. Linear and non-linear regression models were used to fit the experimental results from different co-digestion samples and conditions to better understand and compare the co-digestion results.

6.2.3 Materials and Methods

6.2.3.1 Substrates and inoculum

Thickened waste activated sludge (TWAS) was obtained from Robert O. Pickard Environmental Center (ROPEC), Ottawa, ON, Canada. It contained 5% TS of which about 72% were VS.

FOG sample was provided from the Organic Resources Management Inc. (ORMI), Ottawa, ON, Canada. ORMI provides grease trap cleaning services for different wastewater treatment plants in Ontario.

Organic fraction of municipal solid waste (OFMSW) to minimize the day-to-day variation in the organic waste, a simulated organic fraction of municipal solid waste was used in the current study to provide a homogeneous waste composition that is representative for the kitchen waste in Canada and North America based on the Canadian Food Guide and the USDA Food Patterns (USDA, 2016; Health Canada, 2016; Alqaralleh et al., 2015). The simulated OFMSW used in this study consists of bread (6 wt%), cooked rice (12 wt%), cooked pasta (12 wt%), apples (11 wt%), bananas (11 wt%), cabbage (11 wt%), carrots (11 wt%), ground beef (10 wt%), fish (10 wt%), and boiled eggs with shells (6 wt%). All waste components were prepared fresh and mixed in a food processor to generate a particle size ranges from 1 to 5 mm prior to use. The prepared OFMSW had a total solids concentration of 17.3±0.2% (w/w).

All three substrates TWAS, FOG and OFMSW, were stored at 4^{o}C prior to use.

Thermophilic anaerobic inoculum (55^{o}C) was obtained from the effluent of a 10L thermophilic anaerobic digester fed with TWAS at a 20 days hydraulic retention time (HRT) in our research lab.

Hyper-thermophilic anaerobic inoculum (70^{o}C) was the effluent from a 2L hyper-thermophilic anaerobic digester digesting TWAS at HRT of 2days.

The characteristics of substrates and inoculums used in this study are listed in Table 6.2.1.

Table 6.2.1: Characteristics of substrates and inoculum.

Parameter	**TWAS**	**OFMSW**	**FOG**	**Thermophilic inoculum**	**Hyper-thermophilic inoculum**
pH	6.8±0.1	4.7±0.1	4.1±0.1	7.7±0.1	6.8± 0.1
TS (%)	4.9±0.1	17.3±0.2	29.2±0.2	3.2±0.1	3.3±0.3
VS (%)	3.6±0.1	10.7±0.1	28.3± 0.2	1.6±0.3	1.8±0.2
VS/TS	0.72±0.01	0.61±0.2	0.97±0.03	0.49±0.02	0.55±0.02

Data represents the arithmetic mean ± standard deviation. (n=4).

6.2.3.2 BMP assays

The BMP batch tests were performed in 250 ml Kimax glass bottles with 160ml working volume. All BMP assays were run in triplicates except for inoculum assays were run in duplicates. The inoculums and substrates were added to the bottles to obtain approximately 1:1 substrate to inoculum ratio (S:I) based on the total volatile solids (TVS). Equal amounts of $NaHCO_3$ and $KHCO_3$ were added to each BMP bottle to maintain the alkalinity of 4000-6000 mg/L as $CaCO_3$. Before finally sealing the BMP bottles, each bottle was purged with N_2 gas for 3 minutes to remove the O_2 gas from the headspace and maintain anaerobic condition. The BMP bottles were kept in a temperature-controlled shaker at 55 ± 1^oC and 100 rpm for the thermophilic BMP assays. For the hyper-thermophilic BMP assays BMP bottles were first kept in a 70 ± 1^oC incubator for 2 days and then moved to the 55 ± 1^oC and 100 rpm shaker for the BMP test duration. The composition of BMP bottles are represented in Table 6.2.2 below.

Table 6.2.2: Inoculum and digestion mixtures used for the BMP assays.

Sample	Test Condition	Inoculum 55°C (ml)	Inoculum 70°C (ml)	TWAS (%)[a]	OFMSW (%)	FOG (%)
Blank 55°C	Thermophilic, 55°C	70	0	0	0	0
Blank 70°C	Thermophilic, 55°C	0	60	0	0	0
TWAS (T)	Thermophilic, 55°C	70	0	100	0	0
TWAS:OFMSW (T)	Thermophilic, 55°C	70	0	50	50	0
TWAS:OFMSW (H)	Hyper-thermophilic, 70°C/ Thermophilic, 55°C [b]	35	30	50	50	0
TWAS:OFMSW: 10%FOG (H)	Hyper-thermophilic, 70°C/ Thermophilic, 55°C	35	30	45	45	10
TWAS:OFMSW: 20%FOG (H)	Hyper-thermophilic, 70°C/ Thermophilic, 55°C	35	30	40	40	20
TWAS:OFMSW: 30%FOG (H)	Hyper-thermophilic, 70°C/ Thermophilic, 55°C	35	30	35	35	30

[a] The % is the weight percentage based on the total volatile solids (TVS) in the co-digestion mixture.[b] The BMP assay bottles were kept for 2 days in the hyper-thermophilic conditions (70°C) then moved to the thermophilic conditions (55°C) for the rest of the assay duration.

The 50:50 ratio (based on TVS) for the TWAS:OFMSW mixture was used in our experiments as recommended by Liu et al., 2016 in order to get the best synergetic effect of OFMSW in the co-digestion process and avoid ammonia accumulation that may occur when high OFMSW concentrations are used in the co-digestion mixture (Liu et al., 2016).

6.2.3.3 Analytical methods

The analysis for the BMP assays was done in triplicates, using standard methods (APHA, 1998). Total and volatile solids were determined according to standard method 2540G. Alkalinity analysis was measured based on standard method 2320B using a Fisher Accument® XL25 pH meter. Biogas was measured using a manometer every 24 hours for the first 7 days of the experiment, and it was measured occasionally after the biogas production slowed down until the end of the BMP assay (40 days). The volume of the biogas was corrected to the standard ambient temperature and pressure conditions (STP, 25^{o}C and 1 atm). The net biogas volumes for samples were calculated by subtracting the biogas produced in the inoculum bottles from the biogas produced in each of the samples bottles. The biogas composition (methane %) was monitored weekly using a Hewlett Packard 5710A gas chromatograph.

6.2.3.4 Data analysis

The reaction curve model (RC) was used for the non-linear regression to evaluate the anaerobic digestion performance and estimate the lag phase time (λ, h), predict the ultimate cumulative biogas yield (B_0, mL/g TVS), and find the maximum biogas production rate (R_m,mL/gTVS.h) for each of the BMP conditions tested in this study. The RC model represented by Equation 6.2.1 has been used successfully in literature for anaerobic digestion evaluation (Alqaralleh et al., 2016; Donoso-Bravo et al., 2010).

$$B = B_0\,(1 - exp\,(-\frac{Rm\,(t-\lambda)}{B_0}\,)) \qquad (6.2.1)$$

Where: B_0 is the ultimate biogas yield (mL/g TVS), B is the cumulative biogas yield (mL/g TVS), t is incubation time (h), R_m is the maximum biogas production rate (mL/g TVS.h), and λ is the lag phase duration time (h).

The first-order equation (Equation 6.2.2) was used to estimate the apparent hydrolysis rate coefficient (first-order biogas production rate) (k, h^{-1}) for each of the BMP conditions (Ghatak et al., 2014).

$$B = B_0\,(1 - e^{-kt}\,) \qquad (6.2.22)$$

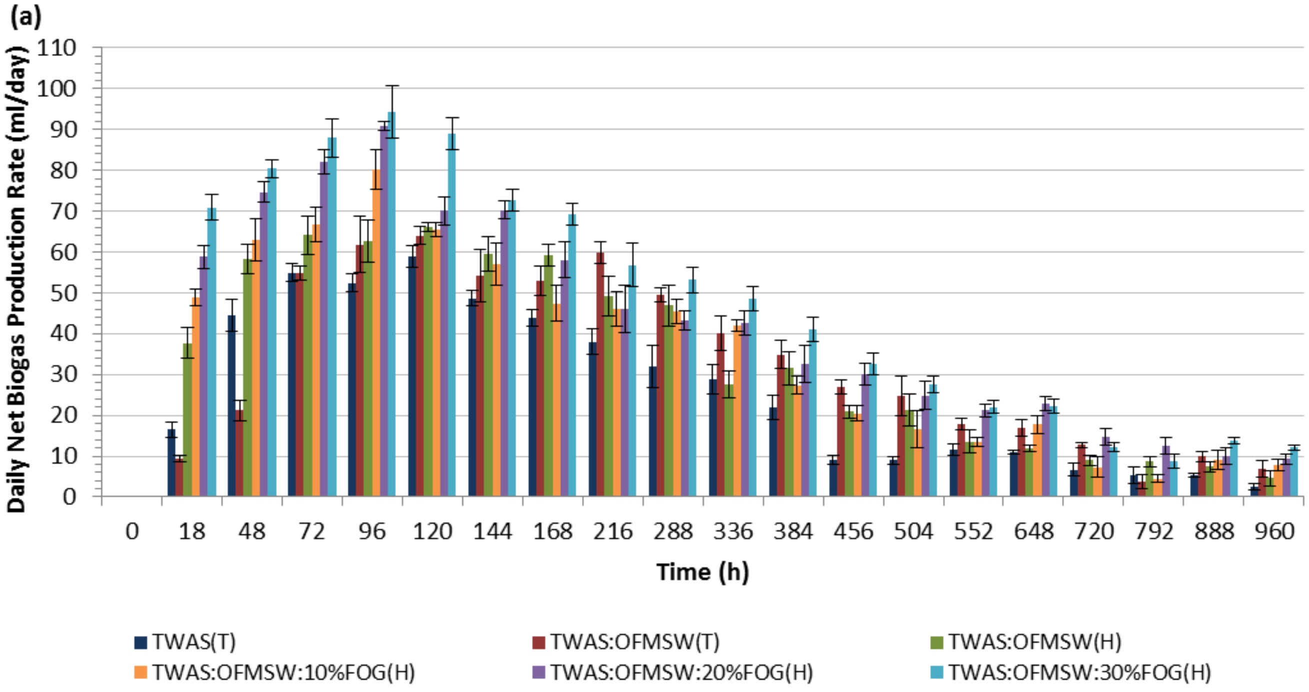
Where *k is the first-order apparent hydrolysis rate* (h^{-1}), B_0, B and t have the same definition as defined in the RC model above (Equation 6.2.1).

Statistical analysis of the collected data included t-test (*p*-value = 0.05), one-way analysis of variance (ANOVA), Pearson coefficient of determination (R^2) and degree of freedom.

6.2.4 Results and Discussion

6.2.4.1 Biogas production and methane yields

The average biogas production rates and the accumulative biogas production results for all BMP assays are illustrated in Figures 6.2.1 (a) and (b), respectively. In addition to Table 6.2.3 that represents the average biogas productions, methane contents, methane yields and the improvement in methane yields compared to the control sample TWAS(T).



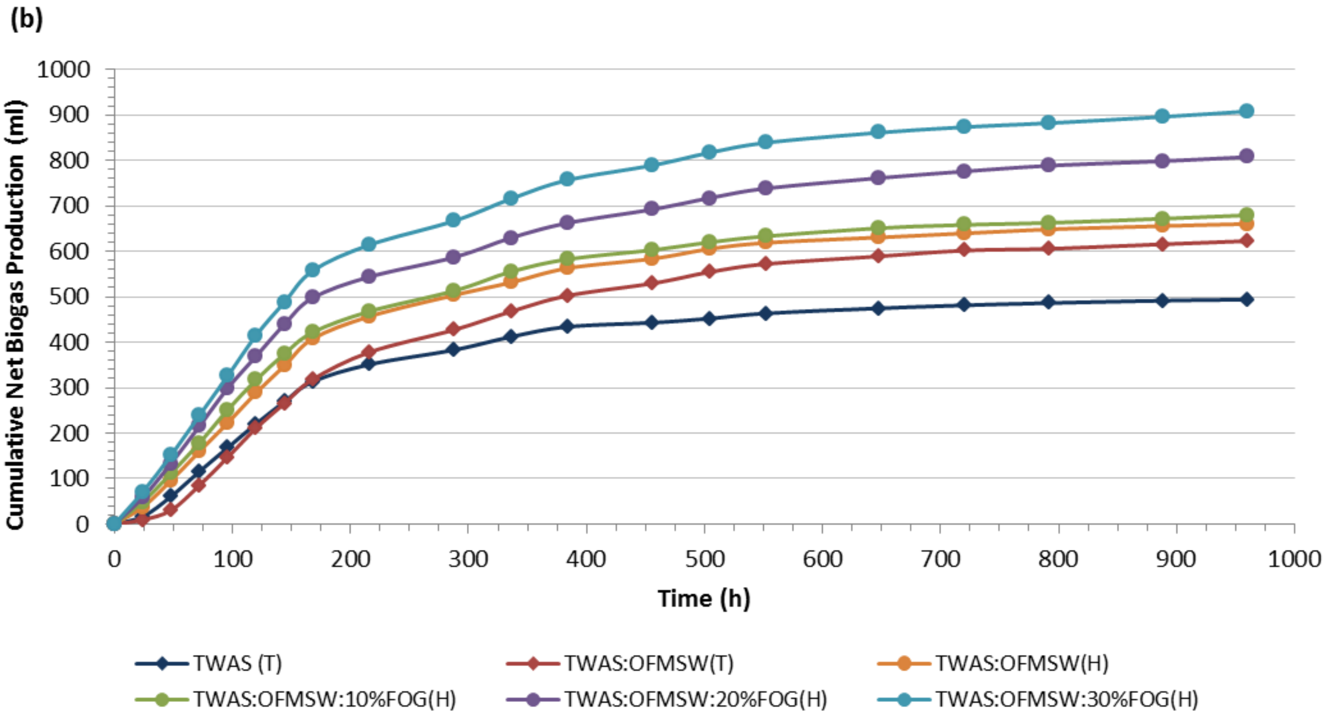


Figure 6.2.1: The BMP biogas production results, (a) the average net biogas production rate and (b) the average cumulative net biogas production.

Comparing TWAS:OFMSW(T) sample with the control sample TWAS(T) in order to evaluate the effect of 50% (w/w, based on TVS) OFMSW as a co-substrate in the co-digestion mixture. First, from Figure 6.2.1a, we notice that TWAS:OFMSW(T) sample had lower biogas production rate at the beginning of the BMP assay compared to TWAS(T) sample. This can be related to the fact that the inoculum used in the BMP assays was acclimated to TWAS digestion, but not to OFMSW. Hence the microorganisms were not adapted to OFMSW that probably caused a delay in starting the digestion process for the sample with OFMSW. However, TWAS:OFMSW(T) sample biogas production rate recovered around the day 3 (72 h) and outperformed the TWAS(T) sample until the end of the test resulting in a cumulative biogas production of

622.6±37.4 ml compared to 494.0±15.9 ml the cumulative biogas production of the control sample TWAS(T). Additionally from Table 6.2.3 below, we can observe that the addition of OFMSW to the digestion mixture did not cause any change in the biogas methane content since both samples TWAS:OFMSW(T) and TWAS(T) had similar (with no statistically significant difference) methane content in the rage of (60.1- 60.6) %. By the end of the BMP assay, the net methane yield for TWAS:OFMSW(T) sample was 13.3 % higher than the methane yield of the control TWAS(T). This increase in biogas production and methane yield confirms the positive effect of OFMSW as a co-substrate for TWAS AD. However, due to the high solids nature of OFMSW it is often suggested in literature the use of a pre-treatment method that will help to solubilize the co-digestion mixture before AD to help to get the maximum benefits from the co-digestion process (Koch et al., 2015; Ara et al., 2014).

As from sample TWAS:OFMSW(H) results, we can notice clearly that this hyper-thermophilic digested sample started the BMP with much higher biogas production rate compared to TWAS(T) and TWAS:OFMSW(T). Thanks to the 2 days hyper-thermophilic digestion of TWAS:OFMSW(H) sample at a temperature of 70±1°C prior to the following thermophilic digestion. The 2 days hyper-thermophilic step is believed to help to solubilize the co-digestion mixture and increase the soluble organic matter that becomes available to be utilized by the microorganisms and converted to biogas. TWAS:OFMSW(H) sample ended the BMP assay producing 660.3±20.2 ml biogas with 61.8±0.7 % methane. This methane content (%) is slightly higher than the methane content for both samples digested under the regular thermophilic conditions; TWAS(T) and TWAS:OFMSW(T). The methane yield of TWAS:OFMSW(H) sample was

370.7±11.3 ml CH_4/g TVS at the end of the BMP assay, and this is a 24.8% increase in the methane yield compared to the control sample TWAS(T).

The next three samples are samples that included 10, 20 and 30 % FOG (as a percentage of TVS of the co-digestion mixture). It is evident from Figures 6.2.1 a and b, which these three samples had the highest biogas production rates during the first 5 days of digestion (120 h). Higher FOG % contributed to higher biogas production rate and higher accumulative biogas production. The addition of 10% FOG (w/w) to the TWAS:OFMSW co-digestion mixture increased the biogas methane content to 63.3±0.8% which is a statistically significant increase in the biogas methane content compared to samples that did not contain FOG. Increasing FOG % in the co-digestion mixture to 20 and 30% resulted in a further increase in the biogas methane content to 65.9±0.5% and 66.4±0.6%, respectively. This noticed a boost in methane content caused by FOG addition to the co-digestion mixtures led to a significant increase in methane yields to reach as high as 547.9±16.8 ml CH_4/g VS_{added} for the TWAS:OFMSW:30%FOG sample and this represents 84.4% higher methane yield compared to the control sample TWAS(T).

6.2.4.2 Linear and non-linear regression

To accurately evaluate the co-digestion performance parameters for the AD digestion process the non-linear regression was utilized using the RC equation (Equation 6.2.1). Also, linear regression using the first-order equation (Equation 6.2.2) was used to estimate the hydrolysis rate coefficient k (h^{-1}) for the biogas production results. Table 4 represents the linear and non-linear regression results.

Table 6.2.3: The BMP results (accumulative net biogas production, methane (%), methane yield and methane yield improvement).

Parameter	Unit	TWAS(T)/ Control	TWAS-OFMSW(T)	TWAS-OFMSW(H)	TWAS-OFMSW-10%FOG	TWAS-OFMSW-20%FOG	TWAS-OFMSW-30%FOG
Cumulative biogas*	**(ml)**	494.0±15.9	622.6±37.4	660.3±20.2	679.5±48.7	807.7±30.9	908.1±27.8
CH_4	**(%)**	60.1±0.8	60.6±0.4	61.8±0.7	63.3±0.8	65.9±0.5	66.4±0.6
CH_4 Yield	**(ml/g VS_{added})**	297.0±9.6	342.7±20.6	370.7±11.3	430.3±30.8	484.1±18.5	547.9±16.8
CH_4 Yield Improvement[a]	**(%)**	-----	13.3	24.8	44.9	63.0	84.4

* Net biogas and methane production in (ml). Data represents the arithmetic mean±standard deviation of replicate samples. [a] methane yield improvement calculated compared to the control sample (TWAS(T)).

Table 6.2.4: Parameters estimated from linear and non-linear regression for the BMP assays.

Sample	**Measured biogas Yield**[a]	**RC model**					**FO eq.**
	(ml/g VS)	**B_0 (ml/gVS)**	**λ (h)**	**R_m (ml/gVS.h)**	**R^2**	**Δ**[b] **(%)**	**K (h^{-1})**
TWAS (T)	494.0	492.9	11.0	2.7	0.992	0.2	4.97 E-3
TWAS-OFMSW(T)	622.6	639.8	17.9	2.6	0.991	2.8	3.49 E-3
TWASOFMSW(H)	660.3	658.8	7.5	3.4	0.995	0.2	4.84 E-3
TWAS-OFMSW-10%FOG(H)	679.5	672.9	4.8	3.6	0.996	1.0	5.13 E-3
TWAS-OFMSW-20%FOG(H)	807.7	793.1	2.5	4.1	0.994	1.8	4.99 E-3
TWAS-OFMSW-30%FOG	908.1	895.8	3.1	4.6	0.996	1.4	4.98 E-3

[a] Average net biogas yield as ml biogas/ gVS_{added} calculated from the BMP assays results. [b] Δ% is the calculated difference between the experimental and the predicted biogas yields.

From Table 6.2.4 and specifically from RC model results, we can observe that RC model fitted accurately all the BMP assay results with high R^2 values that ranged from 0.991 to 0.996. Also, the difference between the experimental and predicted biogas yields (Δ%) ranged from 0.2 to 2.8. It worth to mention here that RC model was preferred over other different linear and non-linear models used to represents the biogas production results of BMP tests by Alqaralleh et al., 2016 and Donoso-Bravo et al., 2010. In both studies, RC model fitted accurately the biogas production results with Δ% ≤10 (Alqaralleh et al., 2016; Donoso-Bravo et al., 2010).

It could be noticed from Table 4 that the control sample TWAS(T) had 11 h estimated lag phase time before starting the biogas production. However, the thermophilic co-digestion sample TWAS:OFMSW(T) had 17.9h estimated lag phase time. This increase in the lag phase of TWAS:OFMSW(T) sample compared to the control sample TWAS(T) is probably because the inoculum used in the BMP assays was not acclimated to OFMSW as mentioned previously, so microorganisms required longer time to start the AD process. As regards the hyper-thermophilic co-digested samples, it is evident that all hyper-thermophilic digested samples had shorter lag phase time compared to the thermophilic digested samples. Comparing TWAS:OFMSW(H) sample with TWAS:OFMSW(T) sample we can notice the effect of the 2 days hyper-thermophilic digestion step in shortening the lag phase time from 17.9 h in the thermophilic digestion of TWAS:OFMSW(T) sample to 7.5h in the hyper-thermophilic digestion of the TWAS:OFMSW(H) sample. This is believed to be due to the solubilization of the co-digestion mixture that occur during the 2 days hyper-thermophilic digestion providing more soluble organic matter that is ready to be utilized by the methanogens in the followed thermophilic part of the experiment.

From the results of the maximum methane production rate (R_m, ml/g TVS.h) it can be observed that sample TWAS:OFMSW(T) had the lowest R_m value among all other samples. On the other hand, the hyper-thermophilic digested sample TWAS:OFMSW(H) had significantly higher R_m compared to TWAS:OFMSW(T) sample. FOG addition clearly boosted the R_m of the three samples that contained FOG. The maximum R_m was for sample TWAS:OFMSW:30%FOG and it was 4.6 (ml biogas/g VS .h).

The hydrolysis rate coefficients (k, h^{-1}) resulted from the linear regression using the first-order equation and presented in Table 6.2.4, show the same trend as R_m discussed in RC equation results above. Adding OFMSW to TWAS thermophilic digestion caused a drop in the hydrolysis coefficient (k) from 4.97 E-3 to 3.49 E-3 h^{-1}. This drop in (k) is expected, and it is related to the dense nature of the OFMSW (high TS and VS) compared to TWAS. In fact, this is the main reason why pre-treatment is always suggested before the AD of OFMSW, to help to solubilize the OFMSW and make it easier for digestion (Ara et al., 2014). Solubilizing the waste for easier digestion is exactly what can be offered by the hyper-thermophilic step in the hyper-thermophilic digestion. This is evident by the increase in k-value for TWAS:OFMSW(H) sample to 4.84 E-3 h^{-1} compared to 3.49 E-3 h^{-1} for TWAS:OFMSW(T) sample. The rest three hyper-thermophilic samples that contained FOG (TWAS:OFMSW:10%FOG, TWAS:OFMSW:20%FOG and TWAS:OFMSW:30%FOG) showed higher k values compared to both thermophilic samples and the hyper-thermophilic sample without FOG. The discussed results show that FOG addition up to 30% (w/w, based on TVS) to the co-digestion mixture helped to increase the hydrolysis rate for the co-digestion mixture and boosted the anaerobic co-digestion process resulting in obtaining higher methane productions and higher methane yields.

6.2.5 Conclusion

Based on the results of this study the thermophilic co-digestion of 50:50 (w/w based on TVS) mixture of TWAS and OFMSW resulted in higher biogas production and 13.3% higher methane yield compared to the thermophilic digestion of TWAS alone. However, the presence of OFMSW in the co-digestion mixture increased the lag-phase time before the AD system started the biogas production. Hyper-thermophilic anaerobic digestion proved to be a reliable alternative for the pre-treatment methods usually needed prior to AD of high solid wastes like OFMSW. The 2-days hyper-thermophilic anaerobic digestion step helped to solubilize the co-digestion mixture, providing more easy to digest materials. This solubilization led to faster biogas production rate, higher accumulative biogas production, and methane yield. Adding 10-30% FOG to the hyper-thermophilic TWAS:OFMSW mixtures significantly boosted the biogas methane content, increased the cumulative biogas production, and the methane yields up to 84.4% higher than the methane yield resulted from the thermophilic digestion of TWAS alone. Linear and non-linear regression models were used to represent the entire anaerobic digestion process. The estimated parameters resulted from the regression provided valuable information for better understanding the effect of OFMSW, hyper-thermophilic and FOG addition on the AD process.

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CHAPTER7

Synthesis, Integration and General Discussion of the Research Results

Anaerobic co-digestion was used in this research study as a sustainable method for converting thickened waste activated sludge (TWAS) and fat oil and grease (FOG) into biogas rich in methane as a source of renewable energy. The co-digestion was tested using regular single-stage thermophilic anaerobic digestion (AD) and an innovative dual-stage hyper-thermophilic/ thermophilic AD. This research included several experimental phases that were designed to bridge the gap found in the literature regarding TWAS:FOG anaerobic co-digestion; namely, the optimum FOG concentration as a percentage that gives the best AD performance and maximum methane yield, the feasibility of the dual-stage hyper-thermophilic/ thermophilic AD for TWAS:FOG co-digestion, the effect of microwave pretreatment with and without NaOH on TWAS:FOG solubilization and co-digestion, and the effect of three different pretreatment methods on the molecular weight distribution and digestion of the soluble organic portion of the samples. As well, other uses of FOG for increasing biogas and methane production from TWAS AD were also investigated, such as the use of just the liquid part of FOG with TWAS for the co-digestion process, and the co-digestion of FOG with TWAS and an organic fraction of municipal solid waste. The main research results and findings are presented in the following.

7.1 Phase 1

Thermophilic and hyper-thermophilic anaerobic co-digestion of TWAS and FOG: Batch experiments

The main questions to be answered through this phase are:

- The feasibility of the innovative dual-stage hyper-thermophilic/ thermophilic anaerobic digestion for TWAS:FOG anaerobic digestion for the first time.
- The optimum FOG% in the TWAS:FOG mixture to get the maximum biogas production from the AD process. In the open literature, there is no accurate value determined.

During this phase, thermophilic and hyper-thermophilic anaerobic co-digestion of TWAS and FOG were investigated, tested, and compared. The effects of using 20, 40, 60, and 80% FOG in the TWAS:FOG mixtures (based on total volatile solids (TVS)) were tested using two sets of biochemical methane potential (BMP) assays. The hyper-thermophilic co-digestion of TWAS with up to 60% FOG was shown to significantly increase the biogas and methane production as well as reduce the VS as compared to the ordinary single-stage thermophilic co-digestion of the same TWAS:FOG mixture. The hyper-thermophilic co-digestion TWAS:FOG mixture with 60% FOG resulted in 112.7% higher methane yield compared to the control (TWAS thermophilic digestion). 80% FOG in the TWAS:FOG mixture (based on TVS) was found to inhibit the AD process; this percentage caused a reduction in the biogas production due to the inhibitory effects of the cumulating long-chain fatty acids (LCFAs) on the methanogenic activity. Both linear and non-linear regression models were used to accurately represent the co-digestion results.

7.2 Phase 2

Thermophilic and hyper-thermophilic anaerobic co-digestion of TWAS and FOG: Semi-continuous reactor experiments

After finding hyper-thermophilic anaerobic digestion effective for TWAS:FOG co-digestion in batch test experiments, it was time to examine the hyper-thermophilic digestion performance using a semi-continuous reactor mode in the next experimental phase. In this phase, a more accurate determination of the optimum FOG% for the hyper-thermophilic and thermophilic digestion was determined by using a gradual increase in the FOG% used, from 20% FOG to 60% FOG, followed by a 5% FOG increase after the 60% FOG.

The questions to be answered by this phase's results:

- The applicability of the dual-stage hyper-thermophilic digestion for a long-term semi-continuous reactor experiments. A semi-continuous dual-stage hyper-thermophilic/ thermophilic reactor was not tested before in the open literature for TWAS:FOG co-digestion.
- The optimum FOG% to the closest 5% (based on TVS) for both dual-stage hyper-thermophilic/ thermophilic co-digestion and single-stage thermophilic co-digestion.

A dual-stage hyper-thermophilic co-digestion system and a single-stage thermophilic co-digestion reactor were both tested and compared to a control (single-stage thermophilic reactor digesting TWAS alone). The dual-stage hyper-thermophilic reactor was shown to be very effective in the anaerobic co-digestion of TWAS:FOG mixtures, outperforming the single-stage thermophilic co-digestion reactor for all three HRTs tested by providing higher VS reduction, higher methane yields, and more stable reactor performance. At 15d HRT, the dual-stage hyper-thermophilic/ thermophilic system successfully digested TWAS:FOG mixtures with up to 70% FOG, and achieved 148.2% higher methane yield compared to the control, which digested solely TWAS at the same HRT. At lower HRTs of 12 and 9d, the dual-stage hyper-thermophilic/thermophilic system efficiently co-digested 40% FOG and produced high methane yields and effluents that met Class A biosolids standards. From the results of this semi-continuous reactor study, the optimum FOG% in the TWAS:FOG mixture was determined to be 70% FOG for the dual-stage hyper-thermophilic/ thermophilic system and 65% FOG for the single-stage thermophilic co-digestion reactor. At those optimum FOG percentages, both reactors showed stable performance and produced the highest methane yields.

The energy balance achieved by the dual-stage hyper-thermophilic/ thermophilic system proved the feasibility of this system, as it was consistently able to produce positive net energy at HRTs of 15 and 12d. The increase in FOG% in the TWAS:FOG mixtures led to higher net energy values.

The first two research stages clearly showed the high efficiency of the dual-stage hyper-thermophilic/ thermophilic system for TWAS:FOG co-digestion, especially at high FOG percentages, in the batch and semi-continuous experiments. The dual-stage hyper-thermophilic system proved to be a possible AD approach worth being considered for pilot and full-scale application. The 2d hyper-thermophilic AD step offers significant solubilization and hydrolysis effects that prepare the TWAS:FOG mixture for easier digestion in the next thermophilic step.

7.3 Phase 3

MW and alkaline-MW pretreatment experiments

Another way to enhance the anaerobic co-digestion of TWAS:FOG mixtures is by using pretreatment before the ordinary thermophilic AD step. The third experimental phase was designed to examine two different pretreatment methods, which were microwave (MW) pretreatment at three different temperatures 95, 135, and 175ºC, with and without combined alkaline pretreatment, using NaOH at pH10. MW and alkaline-MW pretreatments were chosen because those particular methods have been tested in the literature for TWAS-alone pretreatment and have shown to be very effective in solubilizing and enhancing the AD of TWAS.

Question to be answered by the results of this phase:

- The feasibility of those two pretreatment options for TWAS:FOG mixture solubilization and thermophilic AD. Pretreatment methods were rarely tested on TWAS:FOG mixtures prior to the AD, and particularly, MW and alkaline-MW pretreatments were not tested before on TWAS:FOG mixtures prior to AD.

MW pretreatment at a temperature of 175ºC proved to significantly increase the TWAS:FOG mixture solubilization and therefore enhance the mixtures' biodegradability and increase methane production and VS reduction. This high MW temperature (175ºC) resulted in 106.6, 116.1, and 137.0% increases in the methane yields for the samples with 20, 40, and 60% FOG as compared to the control. MW pretreatment proved to be very effective for TWAS:FOG co-digestion; however, brief net energy calculations based on the lab-scale MW device used in this study showed that MW pretreatment, especially at elevated temperatures (e.g., 175°C), is an energy-intensive option that resulted in negative net energy values for all the tested MW pretreatment options.

Further, combining alkaline (pH=10 using NaOH) and MW pretreatment was found to be ineffective for TWAS:FOG solubilization and AD. It is believed that the combination of NaOH addition and the heat from the MW pretreatment in the TWAS:FOG samples caused a saponification reaction to occur, which caused a reduction in the biogas and methane yields from the anaerobic co-digestion of the pretreated TWAS:FOG samples as compared to the un-pretreated samples.

7.4 Phase 4

Comparison of the effect of three different pretreatment methods on the molecular weight distribution and digestion of the soluble organic matter

Since the results of Phases 1 and 2 have shown the effectiveness of the 2d hyper-thermophilic step at 70°C in solubilizing the TWAS:FOG mixture before the AD process, the hyper-thermophilic step was chosen to be one of the three pretreatment methods to be compared in this phase. The second method of pretreatment was MW pretreatment at 175°C because based on the Phase 3 results, this high-temperature pretreatment was a powerful option that resulted in the highest degree of solubilization for TWAS:FOG mixtures. The third pretreatment option was conventional heating at 70°C for a 2d contact time. The conventional heating pretreatment had the same temperature and duration as the hyper-thermophilic step in order to better compare those two methods in solubilizing the TWAS:FOG mixtures on a molecular scale. The results from this phase showed that each of the three pretreatment options uniquely changed the soluble molecular distribution. For example, the MW pretreatment caused the highest increase in solubilization due to the sudden intense thermal and athermal effects of the high-temperature pretreatment (175°C) on the TWAS:FOG sample; however, MW pretreatment was responsible for the longest lag-phases during the anaerobic digestion BMP tests for the different molecular weight substrates. The lag-phase observed at the beginning of the BMP tests indicates that the methanogens needed a longer time to start degrading the MW pretreated TWAS:FOG mixtures. Inoculum acclimation for MW pretreated TWAS:FOG samples might help in reducing the lag-phase during the AD process.

On the other hand, the hyper-thermophilic step had the second-most significant effect in increasing the TWAS:FOG sample solubilization, whereas the BMP test for all the different molecular weights for the hyper-thermophilic samples had the maximum biogas production, at 86.5% higher than the control. The hyper-thermophilic had gradual hydrolysis and solubilization effects on the samples, which resulted in minimal lag phases during the BMP tests. Finally, conventional heating was the least effective pretreatment option in this study compared to the MW and hyper-thermophilic methods. Conventionally heated pretreated samples showed the smallest increase in solubilization and biogas production compared to the control. The final results of this phase confirmed that the effect of the hyper-thermophilic step represented more than just the thermal effect. The combination of thermal, enzymatic, and microbial activity effects in the 2d hyper-thermophilic step had a significant role in achieving high solubilization for the TWAS:FOG samples, and changed the molecular weight distribution of the soluble matter.

By the end of the fourth phase, the advantages of the hyper-thermophilic AD on TWAS:FOG co-digestion were obvious, as it had much more powerful solubilizing effects compared to regular conventional heating. As well, when compared to the intensive high-temperature MW pretreatment, the hyper-thermophilic step offers milder solubilization effects that do not adversely affect the methanogen activity and do not lead to long lag-phases.

In Phases 1 and 2, the dual-stage hyper-thermophilic step followed by thermophilic anaerobic digestion showed to be a very promising effective approach for converting TWAS:FOG to high-quality biogas, with as high as 71.7% CH_4 content. This dual-stage hyper-thermophilic/ thermophilic system is worth being considered for full-scale applications. The numerous benefits that can be gained from this dual-stage system, especially the high methane yields, can quickly repay the extra capital cost required for adding the small hyper-thermophilic reactor before the main thermophilic reactor to create the dual-stage system. The high waste reduction and conversion to biogas, plus the Class A biosolid end products that can be used as soil fertilizer, will close the loop on TWAS waste, providing an effective, sustainable way for TWAS:FOG treatment and management by converting TWAS and FOG to a renewable source of energy (biogas) and nutrients.

7.5 Phase 5

Testing two other options for using FOG in TWAS AD

During Phase 5, two other methods to improve TWAS treatment using FOG via anaerobic co-digestion were explored. First, the use of just the liquid part of FOG (L-FOG) as a co-substrate for TWAS co-digestion was investigated. The results of this experimental phase showed that L-

FOG provided a significant improvement in the co-digestion of TWAS. The TWAS:L-FOG sample containing 80% L-FOG (based on TVS) achieved the maximum biogas production, i.e., 83.0% higher than the control sample (TWAS alone). No inhibitions occurred during the anaerobic co-digestion of samples with 20% to 80% L-FOG, which indicates that L-FOG is a potential co-substrate with a low inhibition potential in an AD reactor. Therefore, the use of only the liquid FOG part for the co-digestion of TWAS might be an alternative way of getting the benefits of FOG in the anaerobic co-digestion of TWAS. By separating the FOG into L-FOG and concentrated (dewatered) FOG, each part of the FOG can be used in a different application. L-FOG can be used as a co-substrate in the anaerobic co-digestion, providing benefits in boosting biogas production and increasing methane yield. In addition, the concentrated FOG can be used for biodiesel production, as a co-substrate for anaerobic co-digestion, or other uses.

The second part of Phase 5 investigated the dual-stage hyper-thermophilic co-digestion of a tri-substrate mixture including TWAS, organic fraction of municipal solid waste (OFMSW), and FOG. Dual-stage hyper-thermophilic co-digestion showed as being effective for the tri-substrate mixture tested. The presence of OFMSW with TWAS balanced the C/N ratio for the mixture and therefore enhanced the anaerobic degradation of the TWAS:OFMSW sample, resulting in a 13.3% higher methane yield compared to the TWAS alone sample (the control). The addition of 30%FOG (based on TVS) to the TWAS:OFMSW mixture further boosted biogas production and increased the methane yield to 84.4% compared to the control.

The results of the 5 different research phases presented in this thesis have provided the following answers:

- FOG is a potential co-substrate for TWAS co-digestion, and use of up to 70% FOG (based on TVS) significantly boosted biogas and methane production without causing adverse effects in the AD reactor. FOG addition was also shown to enhance biogas production and methane yields from the anaerobic co-digestion of other organic waste combinations such as TWAS:OFMSW mixtures.

- The dual-stage hyper-thermophilic/ thermophilic anaerobic digestion system is a potential approach for the co-digestion of TWAS and FOG on batch- and semi-continuous scales.

- 70% and 75% FOG were shown to be the inhibition threshold FOG percentages for single-stage thermophilic and dual-stage hyper-thermophilic digestion, respectively.

- The hyper-thermophilic step at 70°C for 2d has been shown to be a more efficient method for enhancing TWAS:FOG mixture co-digestion and methane production compared to the high-temperature MW pretreatment at 175°C.

- The use of L-FOG only as a co-substrate for TWAS AD is an effective way of obtaining boosting effects on biogas production from the co-digestion process. The remaining concentrated FOG also has other potential uses such as biodiesel production.

CHAPTER 8

Overall Conclusions and Recommendations

8.1 Conclusions

The results of this research work have proved the efficiency of using FOG, as a co-substrate, and hyper-thermophilic digestion for enhancing the anaerobic co-digestion of TWAS. TWAS:FOG co-digestion was shown to increase the efficiency of organic solids removal, and to boost the biogas production from the process with high methane content. These results may help in introducing the use of FOG as a co-substrate for anaerobic co-digestion at wastewater treatment plants. The main conclusions of this research work can be summarized as the following:

- TWAS co-digestion with FOG proved to significantly improve methane production during the thermophilic and hyper-thermophilic BMP assays. The hyper-thermophilic co-digestion of TWAS with 60% FOG resulted in 112.7% higher methane production compared to the thermophilic mono-digestion of TWAS alone. 80% FOG in the TWAS-FOG mixture (based on TVS) was found to be the threshold of FOG since this percentage of FOG limited the co-digestion process and caused a severe inhibition in the methanogens activity.
- The non-linear regression, using GM equation and RC model, has shown to be an effective tool for providing an accurate estimation of important co-digestion parameters including lag-phase, maximum methane production rate, and ultimate methane

production. This helped much in establishing a better understanding of the co-digestion process and helped in making a more reliable comparison between the different conditions tested during the BMP assays.

- The dual-stage hyper-thermophilic digestion, proposed and tested in this study, has been shown very useful in the anaerobic co-digestion of TWAS:FOG mixtures containing up to 70%FOG at an HRT of 15d. This was apparent from the significant improvement in biogas production quantity, and quality (with higher CH_4%) achieved using the dual-stage hyper-thermophilic system as compared to the single-stage thermophilic reactor.
- It was also shown that the dual-stage hyper-thermophilic/thermophilic system can co-digest 40%FOG mixtures at lower HRTs of 12 and 9d, yet produce high methane yields and effluents that meet Class A biosolid standards. This has a real potential to reduce waste volume, as less time is needed for an efficient, stable co-digestion process.
- The gradual increase of FOG dosage in the TWAS:FOG mixture was found to be the key for the reactor to adapt and successfully co-digest TWAS:FOG mixtures with up to 70% FOG at 15d HRT in the hyper-thermophilic/thermophilic system.
- The superiority AD performance of the dual-stage hyper-thermophilic/thermophilic system compared to the single-stage thermophilic co-digestion reactor in generating methane became more evident at higher FOG%'s (60%-75%). For 70% FOG, a 148.2% higher methane yield was achieved as compared to the control. In comparison, the highest methane yield improvement reached by the conventional thermophilic was only 88.3% compared to the control, and this was reached at 65% FOG.

- The inhibition occurred in the hyper-thermophilic reactor and the thermophilic reactor due to the high FOG dosage (FOG >70% for the hyper-thermophilic reactor and >65% for the thermophilic reactor) was found to be reversible. Both reactors recovered to at least 92% of their daily methane productivity after running a recovery phase in which the reactors feed was switched to a lower FOG% (40%FOG).
- The energy balance achieved by the dual-stage hyper-thermophilic/thermophilic system, proves the feasibility of this system, as it was consistently able to produce positive net energy at HRTs of 15 and 12d, with higher net energy values for greater FOG%.
- MW pretreatment significantly improved TWAS:FOG mixtures solubilization prior to AD. The maximum solubilization was measured for the sample with 20% FOG pretreated at MW temperature of 175°C.
- FOG% in the TWAS:FOG sample had a direct effect on increasing biogas production and methane yield, as the FOG% increased the biogas production and methane yield increased as well. Hence the sample with 60%FOG and pretreated at MW temperature of 175°C produced the maximum methane yield during the BMP test.
- MW pretreatment proved to have several benefits on the TWAS:FOG samples including, significant solubilization increase, dewaterability improvement, high VS reduction, and high methane yields. However, the net energy analysis resulted in negative net energy values for all MW pretreated samples, concluding that MW pretreatment is an energy-intensive pretreatment option that seems to be not feasible from an economic point of view.

- The combined NaOH-MW pretreatment was found to be an ineffective pretreatment option for TWAS:FOG mixture prior to the AD.

The observed effects of MW pretreatment, hyper-thermophilic digestion, and conventional heat pretreatment methods on the TWAS:FOG soluble matter distribution over a range of molecular weight cut-off are:

- Each of the three pretreatment methods changed the soluble substrate molecular distribution differently. They also affected the biodegradability of the different molecular weight substrates.
- MW samples had most of the time longer lag phase at the beginning of the degradation compared to the control sample.
- Hyper-thermophilic digestion samples produced the highest total biogas from all the soluble substrate fractions degradation.

And last but not least,

- The liquid portion of FOG (L-FOG) was shown to be very effective as a co-substrate option to be used for TWAS AD. The addition of up to 80% L-FOG (based on total VS), improved the biogas production to 83% higher than the control, with no inhibition during the thermophilic AD process.
- The addition of 30% FOG to the TWAS:OFMSW mixture prior to the hyper-thermophilic digestion has significantly increased biogas production and boosted the methane

content in the biogas resulting in higher methane yield compared to thermophilic digestion of TWAS alone and TWAS:OFMSW.

- Hyper-thermophilic digestion proved to be a reliable alternative for pre-treatment methods usually needed prior to AD of high solid wastes like TWAS and OFMSW.

8.2 Recommendations

In order to further improve the understanding of the phenomena associated with the research work presented in this thesis, the following recommendations are suggested:

- It is recommended to use FOG as a potential co-substrate in full-scale anaerobic digesters.
- It is recommended to study the dual-stage hyper-thermophilic anaerobic digestion process with more details. For example, changing the second stage temperature to be hyper-thermophilic followed by mesophilic stage instead of the thermophilic stage.
- It is strongly recommended to have a pilot scale study for the dual-stage hyper-thermophilic anaerobic digestion system.
- Study the microbial community in the hyper-thermophilic and thermophilic stages.
- Investigate other pretreatment alternatives for TWAS:FOG pretreatment prior to anaerobic digestion, such as ultrasound pretreatment.
- MW pretreatment proved to be very effective for TWAS:FOG solubilization especially at high temperatures (175°C), however, a full-scale MW pretreatment studies and comprehensive cost-benefit analysis is required to determine the feasibility of MW pretreatment in actual AD applications.

Appendix A

A.1 Batch anaerobic digestion

BMP tests were performed using suitable size serum bottles (Figure A.1). Procedure for thermophilic and hyper-thermophilic/thermophilic BMP tests were done as described in the studies.

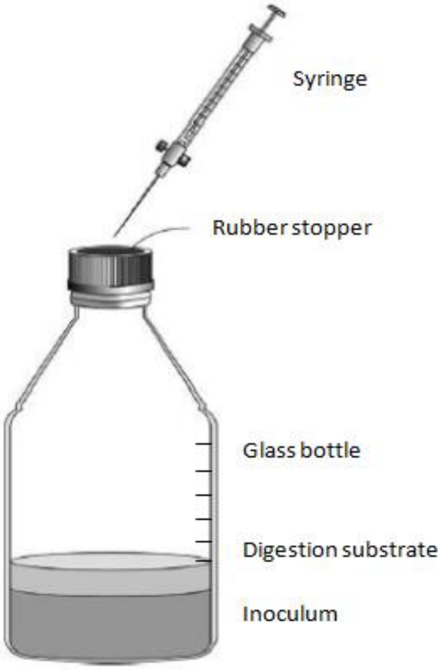

Figure A.1: BMP bottle set-up (updated from Li, 2012).

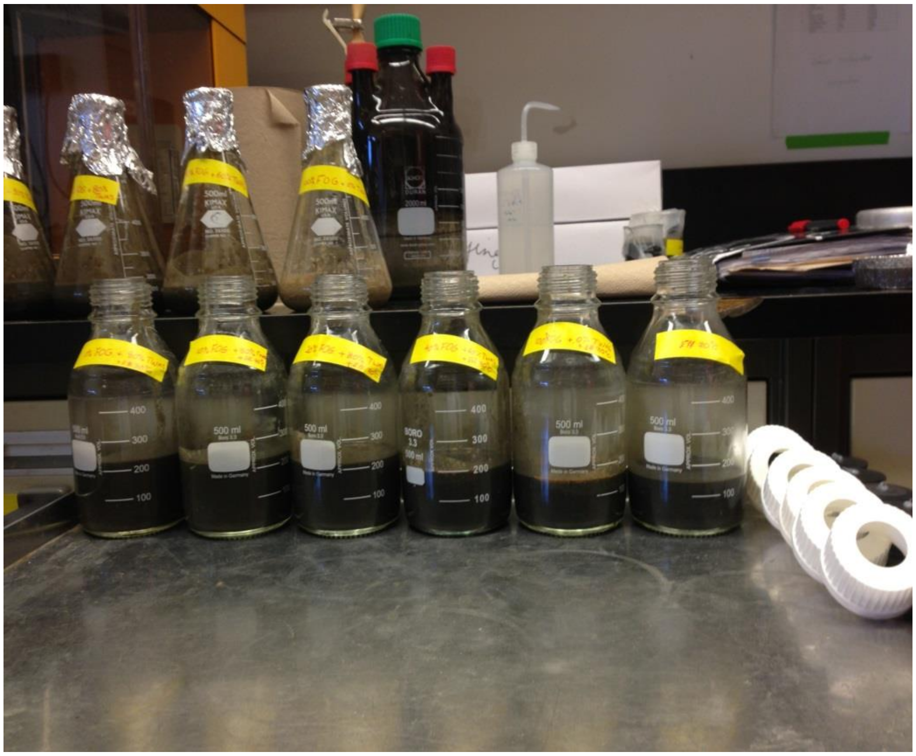

Figure A.2: BMP serum bottles: Preparation.

Figure A.3: BMP serum bottles: In the temperature controlled incubator.

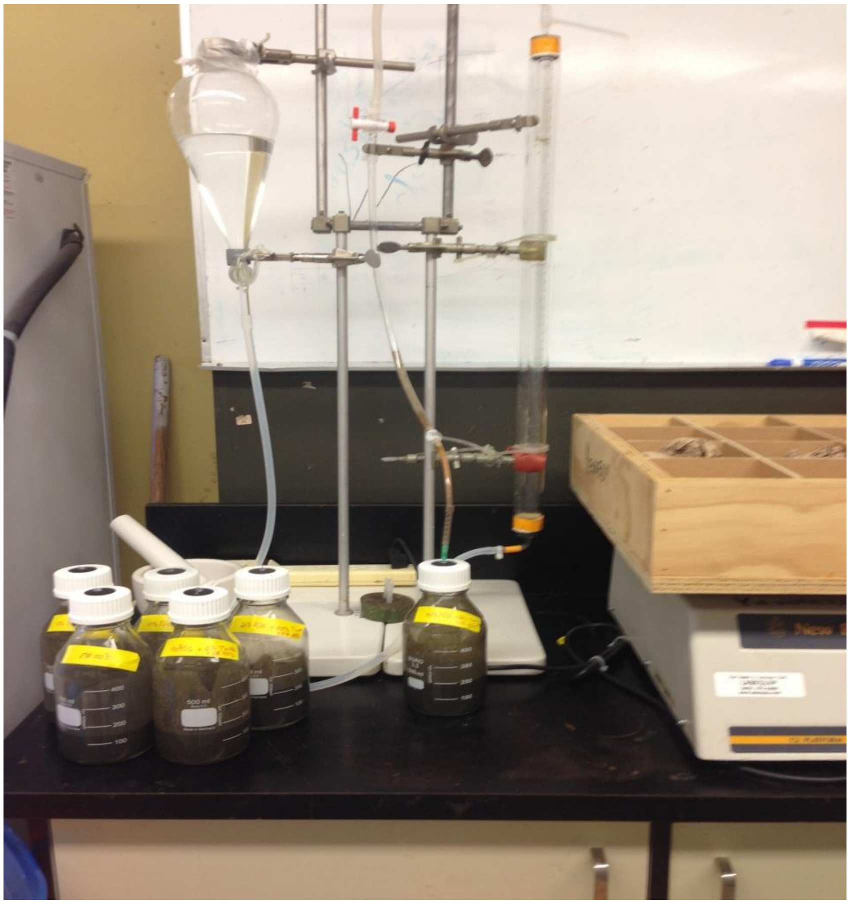

Figure A.4: BMP serum bottles: Biogas measuring using a small manometer.

A.2 Semi-continuous reactors

Semi-continuous digestion tests were performed for understanding the effects of FOG%, and the hyper-thermophilic step (before the regular thermophilic anaerobic digestion) on the anaerobic digestion performance and kinetics of TWAS:FOG co-digestion.

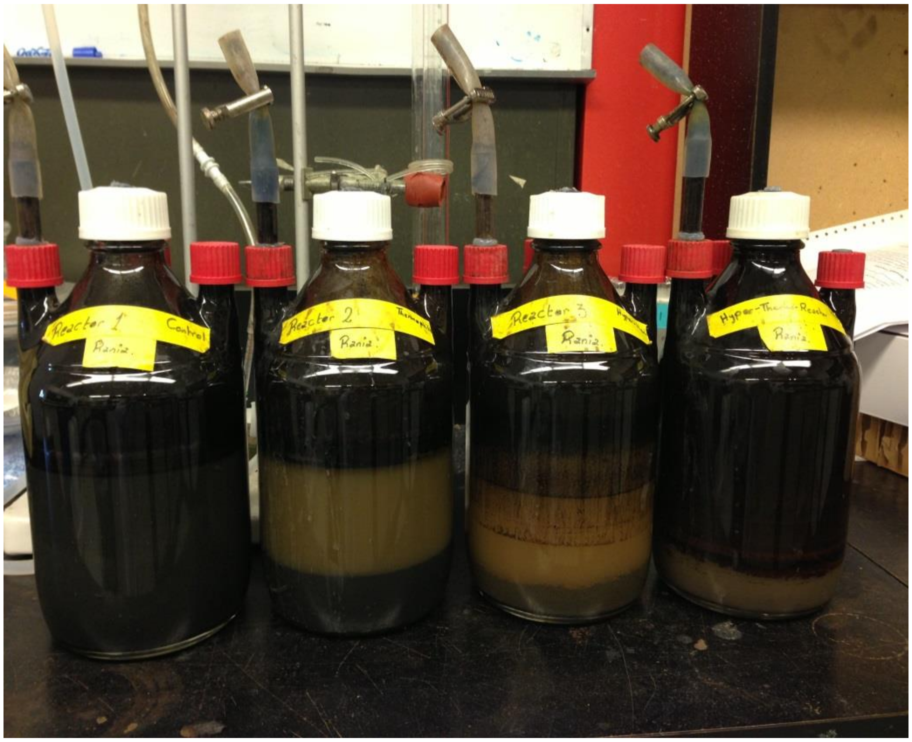

Figure A.5: Semi-continuous reactors, R1, R2, R3, and H (as defined in Chapter 3).

A.3 Materials used during the study

Some of the materials used in this study include but not limited to TWAS, FOG, inoculums, liquid part of FOG (L-FOG).

Figure A.6: FOG, TWAS, and inoculum (70°C).

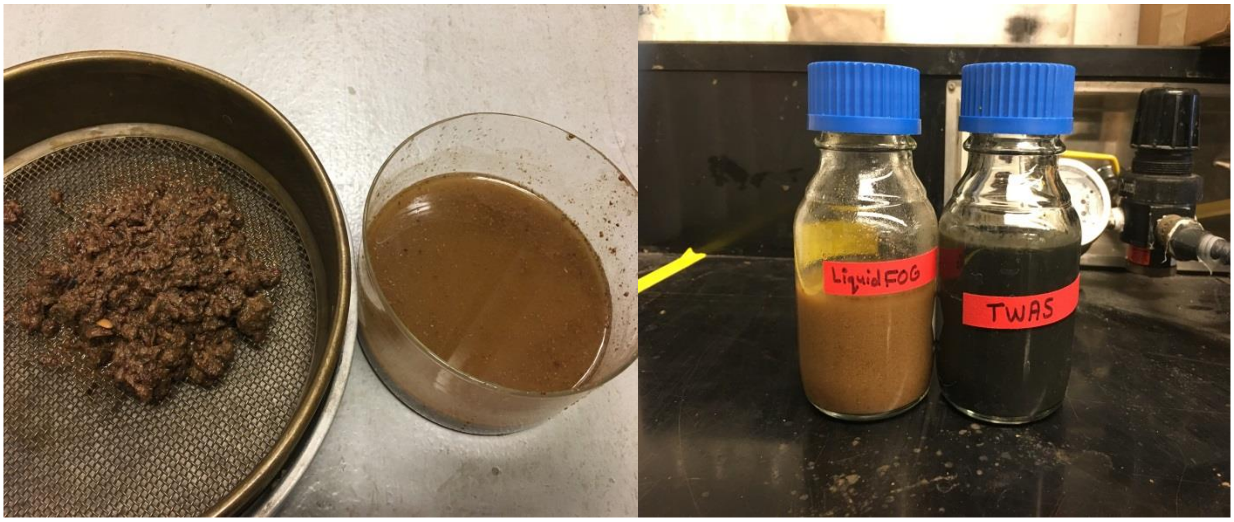

Figure A.7: Liquid part of FOG.

A.4 Microwave pretreatment:

Figure A.8: Teflon-vessels filled and sealed before the MW pretreatment (left), and the Mars X Microwave machine (right).

A.5 Ultrafiltration units

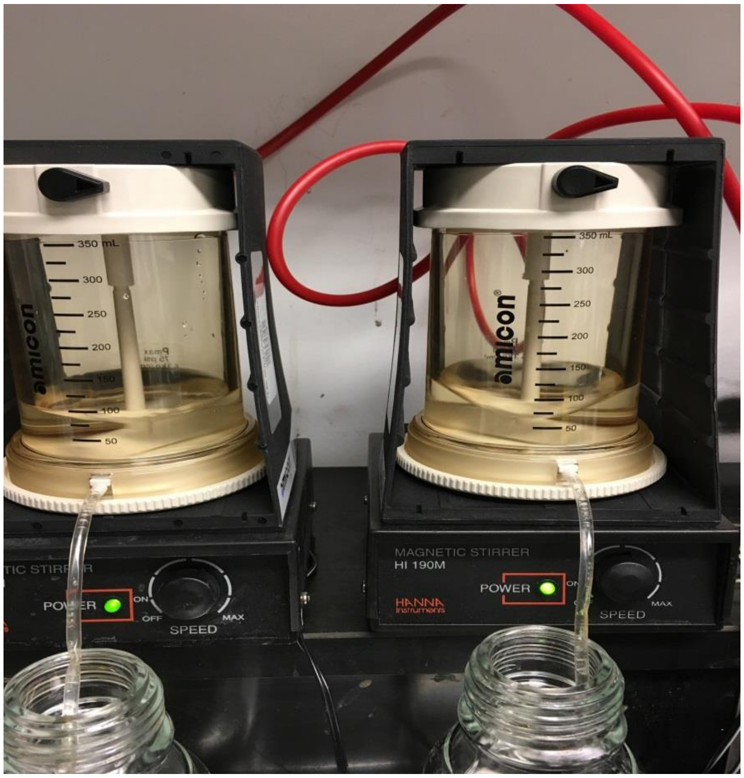

Figure A.9: Ultrafiltration cells.

A.6 References

Li, C. (2012). Using Anaerobic Co-digestion with Addition of Municipal Organic Waste and Pre-treatment to Enhance Biogas Production from Wastewater (Doctoral dissertation). Queen’s University. https://qspace.library.queensu.ca/bitstream/handle/1974/7487/Li_Chenxi_201209_PHD.pdf;jsessionid=976C14E9B3BD45A944C263E3394AF956?sequence=1