INFLUENCE OF TEMPERATURE PHASED ANAEROBIC DIGESTION ON STABILIZATION OF MUNICIPAL WASTEWATER SLUDGE

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Abstract

Wastewater sludge has the most significant volume among all the constituents that are removed through wastewater treatment, and the annual cost of treating and disposing it accounts for more than half of the overall operation cost of a wastewater treatment plant (WWTP). Among all methods for treating the sludge, anaerobic digestion is the most popular process for its ability to reduce the pathogens and odor potential, save energy by producing methane-rich biogas, as well as reduce the biosolids volume for disposal. Although anaerobic digestion is the most favorable option for the treatment of sludge, it still has some aspects that can be improved. Emerging sludge treatment technologies are currently being studied to reduce the digester volume required and enhance the biogas production in anaerobic digestion. This study investigated the effect of temperature phased anaerobic digestion (TPAD) on treatment of municipal sludge generated by Lulu Island WWTP (BC, Canada) and compared it to conventional single-stage mesophilic anaerobic digestion (AD) currently implemented at the Lulu Island plant.

A total number of five lab-scale digesters were operated according to the following scenarios: single-stage mesophilic AD (control), TPAD1 (acid/methane phase temperatures of 55/38°C), TPAD2 (acid/methane phase temperatures of 70/38°C). The systems were operated at three overall sludge retention times (SRTs) including 30, 20, and 15 days. The acid-phase of TPAD systems were able to improve the hydrolysis of sludge significantly and the acid phase of TPAD2 (70°C/2-d SRT) achieved the highest soluble to total chemical oxygen demand (COD), protein, humic acid and sugar ratios.

Overall, anaerobic digestion benefited considerably from TPAD in terms of methane yield, pathogen removal and dewatering rate of biosolids. Relative improvements (over control) in solids

removal and methane yield increased considerably by gradually decreasing the SRT from 30 to 20 and 15 days. TPAD1 system achieved the maximum methane production and pathogen destruction and it generated Class A biosolids according to Organic Matter Recycling Regulation of British Columbia at all operating SRTs, while the biosolids produced from the other digestion systems (control and TPAD2) could not meet the criteria for Class A and was classified as Class B.

Lay summary

Municipal wastewater sludge treatment or stabilization aims to reduce the potential health hazards associated with sludge disposal, to remove pathogens and odors, and to reduce biosolids volume for final disposal. The most commonly implemented process for stabilizing sludge is anaerobic digestion. This study investigated the effect of an advanced anaerobic digestion method, named temperature-phased anaerobic digestion (TPAD), on treatment of municipal sludge. The digestion temperature and sludge retention time (SRT) in the digesters were the variable parameters in this research. Overall, anaerobic digestion benefited considerably from TPAD in terms of methane production, pathogen destruction and dewatering rate of the treated sludge, and higher relative improvements in solids removal and methane production was observed in shorter SRTs.

Preface

The research presented in this thesis is the original work performed by the author. This thesis was supervised by Dr. Cigdem Eskicioglu at the Bioreactor Technology Group in the School of Engineering, University of British Columbia.

An abstract summarizing the results of this research has been accepted as podium presentation to the 2019 Value of Biogas West Conference to be held in Vancouver in January 15-16, 2019. Furthermore, an article is being prepared for submission to a peer-reviewed journal in the field.

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List of abbreviations

ANOVA	Analysis of variance
AP	Acid phase
CFU	Colony forming unit
COD	Chemical oxygen demand
CST	Capillary suction time
DAFT	Dissolved air flotation tank
EPA	Environmental Protection Agency
EPS	Extracellular polymeric substances
FPS	Fermented primary sludge
HA	Humic acid
ML	Mega Liter
MP	Methane phase
MPN	Most probable number
OLR	Organic loading rate
OMRR	Organic matter recycling regulation
ppm	Part Per Million
SCOD	Soluble chemical oxygen demand

SRT	Sludge retention time
STP	Standard temperature and pressure
TS	Total solids
TWAS	Thickened waste activated sludge
VFA	Volatile fatty acids
VOC	Volatile Organic Compounds
VS	Volatile solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

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To my family

Chapter 1: Introduction

The day by day growth of the human population and urbanization has led to an increase in waste production, including wastewater. Wastewater treatment plants (WWTP) process the wastewater and treat it before releasing it back to the water cycle. The constituents removed or produced as side-stream residuals in a WWTP include grit, scum, screenings, sludge, and biosolids. The sludge and biosolids produced from wastewater treatment processes are usually a mixture of solids and liquids or it can be considered as semisolids. The semisolids contain 0.25% to 12% solids (by weight), depending on the treatment process that they have undergone. In the United States (U.S.) the term biosolids, as defined by the Water Environment Federation (WEF 2012), refers to any sludge that has been stabilized to meet the criteria in the U.S. Environmental Protection Agency's (U.S. EPA) regulations and therefore, can be used beneficially. The term sludge is only used before achieving the beneficial use criteria.

Sludge has the most significant volume among all the constituents that are removed through wastewater treatment, and its processing, reuse and disposition present the most complicated issue in wastewater treatment (Metcalf & Eddy 2014). The process of sludge treatment includes converting the complex organic material in the sludge into simpler and inert compounds. This process is used for the stabilization of sludge. Sludge stabilization aims to remove pathogens and odors from the sludge. The most commonly implemented method for stabilizing sludge is anaerobic digestion. In this process, microorganisms break down the biodegradable compounds in the sludge in an oxygen-free environment. Final products of anaerobic digestion are carbon dioxide and methane along with trace amount of other gases, i.e., H_2S.

There are two general categories of anaerobic digestion in terms of process temperature: mesophilic digestion and thermophilic digestion. Mesophilic digestion is operated in the temperature range of 30°C to 40°C. This type of anaerobic digestion has a lower operating cost, higher effluent quality, and process stability linked to higher microbial diversity. However, it requires a longer retention time and is unable to remove sufficient amounts of pathogens (Song et al. 2004). Thermophilic digestion is conducted in the range of 50°C to 70°C. It tolerates higher organic loading, achieves higher volatile solids (VS) removal, and higher pathogen destruction. However, the drawbacks of this type of digestion include a higher energy requirement and low process stability due to the accumulation of volatile fatty acids (VFA). Another drawback is the decrease in effluent quality due to poor dewaterability, high soluble chemical oxygen demand (SCOD), and high ammonia content of the effluent (Song et al. 2004).

Advanced digestion systems are novel forms of anaerobic digestion. They are able to take the advantages of mesophilic and thermophilic digestion at the same time and omit the drawbacks of them. Temperature-Phased Anaerobic Digestion (TPAD) is an advanced digestion method which contains two stages in series. The first stage is a thermophilic digester, and it is followed by the second stage at the mesophilic condition. TPAD process improves the VS removal, as well as gas production and pathogen removal (Ge et al. 2011b; Carrère et al. 2010).

1.1 Motivation of research

Metro Vancouver operates five wastewater treatment plants across the Greater Vancouver area and the Lulu Island WWTP located in Richmond, BC, is one of them. This plant is one of the three plants that provide both primary and secondary treatments to the wastewater and its treatment capacity is 155 ML per day. The Lulu Island WWTP receives wastewater primarily from

residential sources but also a portion from industrial sources. Also, some storm water likely goes to the plant through inflow and infiltration. This plant provides pretreatment to the wastewater through a series of screens and pre-aeration tanks. In this stage, materials with a diameter larger than 1.25 mm are removed along with non-putrescible materials. Then the wastewater goes through primary treatment in which three large rectangular tanks with scum baffles are used to mechanically remove solids from it. The following step involves a primary clarifier in which most of the suspended solids settle out. Then the wastewater goes through secondary treatment using a solid's contact tank followed by a secondary clarifier. The final step is the disinfection of the effluent water from the secondary clarifier and then releasing the treated wastewater into the environment. The sludge produced from primary and secondary clarifiers is sent to the sludge treatment unit. The first step of sludge treatment is the thickening process using dissolved air flotation tanks (DAFT). The thickened sludge is then treated in two parallel single stage mesophilic anaerobic digesters operated at 38°C at a retention time of 30 days. The biosolids produced from sludge treatment are used within Metro Vancouver and throughout the Province of British Columbia to restore and reclaim landfills, mine sites and gravel pits; serve as a basis for topsoil used in landscaping projects, parks and green spaces; and, to fertilize rangeland, hayfields and forests.

In the past recent years, the population in the Greater Vancouver area has grown significantly and quickly and has resulted in an increased demand for a more efficient wastewater system which is able to treat the larger quantities of waste production. The main issue with the current sludge treatment system is that it has a long solids retention time in the anaerobic digesters. Therefore, it is a slow process. Furthermore, the current digestion system is not capable of producing Class A biosolids for safer land application. Lastly, anaerobic digesters work non-stop and do not allow for

periodic shutdowns to repair and maintain the facility. Therefore, Metro Vancouver is interested in improving the efficiency of the sludge treatment unit at the Lulu Island WWTP and has collaborated with UBC's Bioreactor Technology Group to upgrade the existing sludge digesters.

1.2 Objectives

The proposed research was intended to improve the efficiency of the single-stage mesophilic anaerobic digestion process for sludge treatment. The specific objectives of this study are as follows:

- Improving the quality of the biosolids resulted from anaerobic digestion in terms of pathogen destruction and the production Class A biosolids which are safer for land application,
- Decreasing the volume required for the digesters by decreasing the sludge retention time,
- Increasing the biogas (methane) production by enhancing organic solids degradation.

1.3 Novelty of research

The main factor that limits the efficiency of the anaerobic digestion and results in the requirement of long sludge retention times is the hydrolysis process. There are various solutions for improving the hydrolysis process, such as: pretreating the incoming sludge to the digester by exposing it to microwave or ultrasound, adjusting the temperature of digestion, and recycling the anaerobic sludge. Temperature-phased anaerobic digestion (TPAD) is another solution for improving the efficiency of sludge treatment at the Lulu Island WWTP.

Among different sludge treatment options, TPAD has shown to be more energy efficient due to additional methane production, as well as being more effective in terms of VS and pathogen

removal. Also, TPAD has a relatively low capital cost due to less volume required for the digesters in this system (Riau et al. 2010; Coelho et al. 2011). The two important parameters affecting the performance of a TPAD system are the temperature of the thermophilic stage and sludge retention time in the system (Lv et al. 2016; Riau et al. 2012; Ge et al. 2011b). There are only a few studies which investigated the effect of different temperatures and sludge retention times on the same system. This research intends to investigate the effect of various thermophilic temperatures and overall SRTs on a TPAD system treating Lulu Island WWTP mixed sludge. The research will also compare the TPAD systems with conventional single stage mesophilic anaerobic digestion which is currently being used at the plant. Figure *1.1* shows the proposed scenario for this study. Finding an optimum digestion temperature and SRT combination to enhance anaerobic digestion was the ultimate goal.

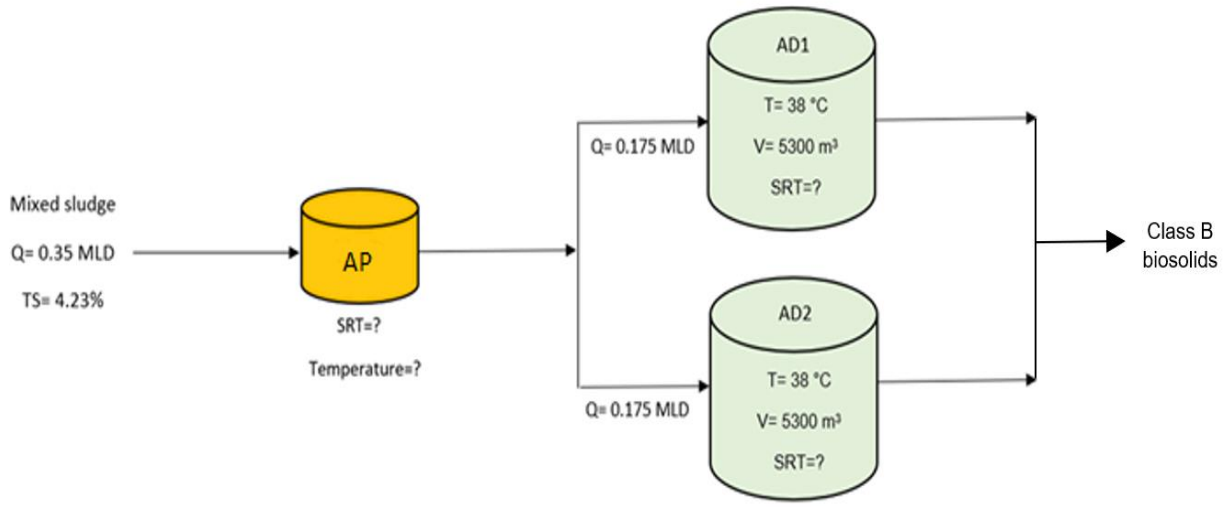


Figure 1.1 *Proposed scenario for Lulu Island WWTP sludge digestion unit (Q=flowrate, MLD=mega litre per day, TS=total solids, SRT=sludge retention time, AP= acid phase digester, AD1 and AD2=existing anaerobic digesters in parallel)*

1.4 Thesis organization

The first chapter, chapter 1: Introduction, describes the main topic of the research. Chapter 2: Literature review provides a general knowledge of wastewater treatment and explains the theory of anaerobic digestion and TPAD in detail. Chapter 3: Materials and methods describes the experiments performed, the materials and equipment used in the experiments, and the testing procedures for sample characterization. Chapter 4: Results and discussion provides the results of the experiments and discusses the results and their practical application. The final chapter, chapter 5: Conclusion, summarizes the major findings of this study.

Chapter 2: Literature review

2.1 Wastewater treatment

Wastewater treatment aims to dispose municipal and industrial wastewater without any impact on human health or damage to the environment. This process includes converting the wastewater to an outflow of treated water that can go back to the water cycle. The process of wastewater treatment consists of several levels as follows: primary treatment, secondary treatment and tertiary treatment. Figure 2.1 shows the process flow diagram of a treatment plant which applies all three levels of treatment on the wastewater. Table 2.1 indicates the characteristics of municipal wastewater.

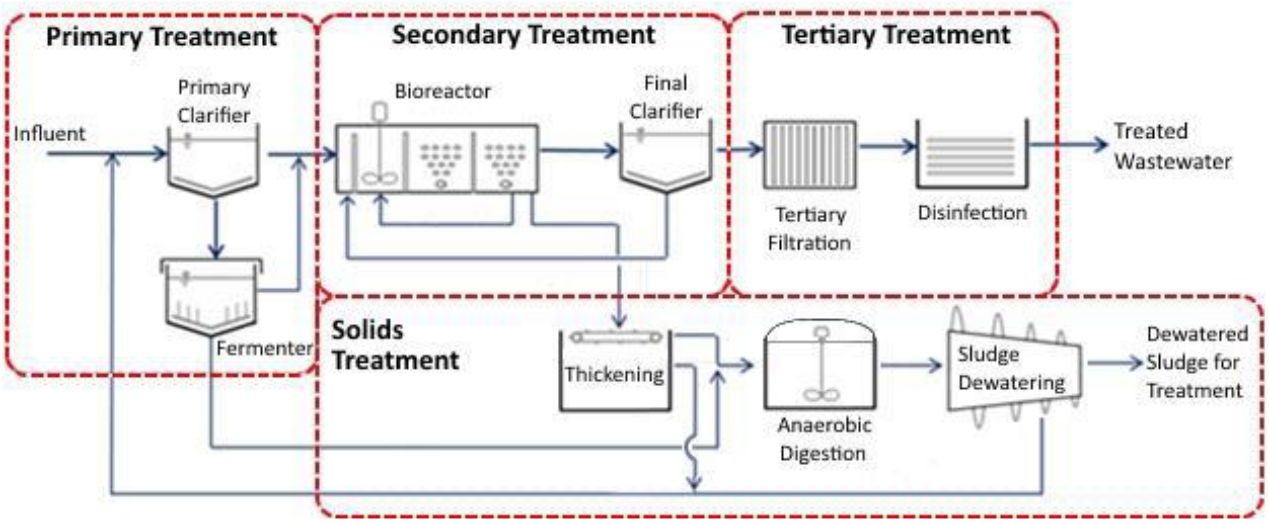


Figure 2.1. Process flow diagram of a wastewater treatment plant (from (Abel-denee 2017) *with permission from the author)*

Table 2.1. The typical characteristics of municipal wastewater (the data is adapted from (Metcalf & Eddy 2014)*)*

Description	Concentration in municipal wastewater		
	Low (750 L/capita/d)	**Medium (460 L/capita/d)**	**High (240 L/capita/d)**
Biochemical oxygen demand (mg/L)	110	190	350
Chemical oxygen demand (mg/L)	250	430	800
Total solids (mg/L)	390	720	1230
Dissolved solids (mg/L)	270	500	860
Suspended solids (mg/L)	120	210	400
Volatile organic compounds (mg/L)	<0.1	0.1-0.4	>400
Oil and grease (mg/L)	50	90	100
Sulfate (mg/L)	20	30	50
Total coliform (MPN*/100 mL)	$10^6 - 10^8$	$10^7 - 10^9$	$10^7 - 10^{10}$
Fecal coliform (MPN*/100 mL)	$10^3 - 10^5$	$10^4 - 10^6$	$10^6 - 10^8$

*****Most probable number

2.1.1 Primary treatment

In primary treatment, wastewater is screened to remove larger bulk materials. The screened wastewater then flows through primary sedimentation tanks or primary clarifiers in which heavy inorganics settle. Primary treatment consists of physical treatment processes, but depending on the requirements of a treatment plant, chemical and biological processes may be included as well.

An example of enhancing primary treatment through chemical treatment is the addition of metal salts such as ferric chloride. The addition of ferric chloride helps to flocculate solids into large particles that settle better in the primary clarifier. Chemically enhanced primary treatment can also result in the removal of trace constituents such as lead (Johnson et al. 2008).

Biological processes may also be needed in primary treatment since readily degradable organics may be necessary for downstream treatment processes. In this case, biological fermentation is included in the primary treatment. Biological fermentation leads to the production of VFAs (Chanona et al. 2006).

A byproduct of primary treatment of wastewater is primary sludge (PS). The primary sludge also needs to be treated before being released to the environment. Sludge treatment is discussed further in the upcoming sections of this thesis.

2.1.2 Secondary treatment

Secondary wastewater treatment consists of physical, chemical, and biological processes. Basically, it is biological oxidation that occurs by using aeration. The oxygen transfer through aeration provides an electron acceptor for the microorganisms. This process results in the flocculation of dissolved and suspended material as well as new microbial (biological) cell growth. The flocculated biomass is easier to separate through physical methods.

Biological growth has different methods and is divided into various categories based on the growth pattern of microorganisms. Suspended growth and attached growth are among the most common growth systems. In the suspended growth system, microorganisms are suspended in a mixture of wastewater and dissolved air. On the other hand, in attached growth systems microorganisms are attached to a solid medium and are exposed to a mixture of wastewater and dissolved air (Alleman & Prakasam 1983).

Similar to the primary treatment of wastewater, secondary treatment results in sludge production as a byproduct. This sludge is called secondary sludge, and its characteristics are different from primary sludge. The secondary sludge needs to be treated before disposing it to the environment.

The treatment and disposal of secondary sludge are further discussed in the upcoming sections of this thesis.

2.1.3 Tertiary treatment

After the secondary treatment of wastewater, there are residual constituents containing total suspended solids and colloidal solids present in the wastewater. These solids may adversely affect the extent of inactivation in the final disinfection process. Also, in some cases, it is necessary for the WWTPs to do further nutrient removal based on the regional disposal regulations. Therefore, tertiary wastewater treatment is necessary for some wastewater treatment facilities (Metcalf & Eddy 2014).

Tertiary treatment may consist of physical, chemical, and biological methods. However, the most commonly used processes in this treatment are physical and chemical ones. Some examples of tertiary treatments are filtration, ion exchange, carbon adsorption, and distillation. Filtration is the most commonly used method for tertiary treatment. In this process, particulate constituents in the wastewater are separated after passing through a membrane (Metcalf & Eddy 2014).

All of these advanced treatment methods result in a concentrated waste stream. In the filtration process, backwash cycles are required to clean the membrane and in the ion exchange process, wash water is necessary to recharge the resin. The wastewater from filter backwash and resin wash water are then recycled to the headworks of the wastewater treatment facility (Metcalf & Eddy 2014).

As discussed in the previous sections, the process of wastewater treatment results in the production of semi-solid residuals called “sludge”. As shown in Figure 2.2, there are three sources of sludge

resulting from the three levels of treatment as follows: Primary sludge, secondary sludge, and tertiary sludge.

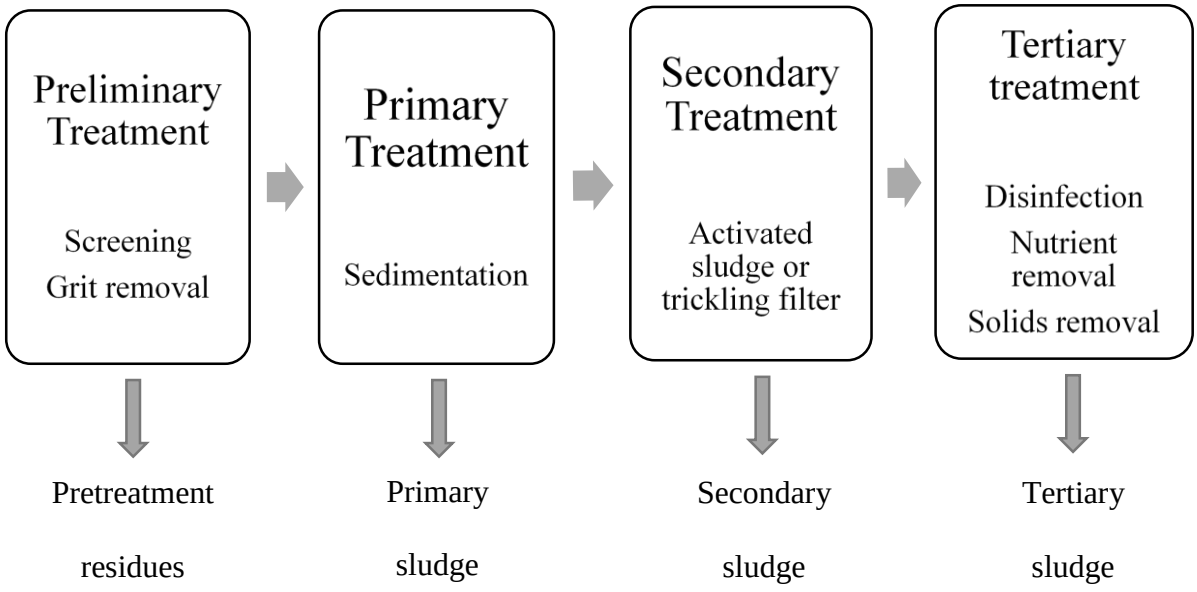


Figure 2.2. Flow diagram of the wastewater treatment process

Primary sludge and thickened waste activated sludge (TWAS) are the sludge streams from primary and secondary treatments, respectively. PS and TWAS have a putrescible nature which results in the contamination of air and water resources when untreated versions are disposed into the environment. Therefore, management of sludge is of a big concern for every wastewater treatment facility and accounts for the biggest portion of operational costs (Riffat 2012; Appels et al. 2008).

2.2 Wastewater treatment sludge

The annual cost of treating the sludge and then disposing it accounts for more than half of the overall cost of operating a WWTP (Bryden & Langman 2009). Managing the sludge is becoming critical nowadays since the amount of sludge being produced every year is increasing significantly

due to population growth. However, sludge disposal options are decreasing due to environmental concerns and strict regulations for disposal (Pérez-Elvira et al. 2006).

Biological processes are the most commonly used methods in modern WWTP for treating the wastewater. By using the continuously modified activated sludge system, progressed in the late 1800's (Alleman & Prakasam 1983), wastewater treatment process can produce effluents of very high quality.

Among all methods for treating the sludge, anaerobic digestion is the most popular one and is commonly used in WWTP. Anaerobic digestion causes volume reduction and stabilization of sludge, as well as pathogen removal and odor potential reduction. It also saves more energy than aerobic methods, since it does not require oxygen and produces methane which is a source of energy itself.

Although anaerobic digestion is the most favorable option for the treatment of sludge, it still has some aspects that can be improved. The process consists of four stages including hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis controls the rate of the whole process as is the slowest part of the process, especially for complex organic waste such as sludge (Eastman & Ferguson 1981). Therefore, hydrolysis causes a longer retention time and as a result higher volume required for the digesters.

By improving the hydrolysis step, efficiency of the process can be improved significantly. Pre-treatment techniques are being used for this purpose (Zhang et al. 2009). The primary aim of these techniques is to decrease size of the organics in sludge, which will then enhance the hydrolysis of the sludge. This will result in an increase in solids removal and methane production per unit mass of sludge that is fed to the digester (Ariunbaatar et al. 2014; Pilli et al. 2015; Carrère et al. 2010).

Pre-treatment methods can be categorized into: thermal, chemical, biological, and mechanical pre-treatment. These methods along with various combinations of them have been implemented in anaerobic digestion (Uma Rani et al. 2014). TPAD is a pre-treatment method under the biological category. This pre-treatment is found to be very beneficial as it leads to more solids removal, methane production, and pathogen destruction compared to conventional single-stage anaerobic digestion at mesophilic conditions (Han & Dague 1997; Schafer & Farrell 2000b; Fernández-Rodríguez et al. 2016; Akgul et al. 2017).

2.2.1 Sludge sources

In a WWTP, sludge is produced from different sources depending on the plant type and the method of operation. As mentioned earlier, the principal sources of sludge in WWTP include pre-aeration, primary sedimentation, and biological treatment (Metcalf & Eddy 2014).

2.2.2 Sludge characteristics

Characteristics of sludge depend highly on its origin, the treatment process that it has been subjected to at the treatment plant, and the sludge age (Metcalf & Eddy 2014). Primary sludge contains settleable organic and inorganic coarse solids and pathogenic organisms from animal and human feces. Primary sludge can be readily digested, and the biogas production and the dewaterability followed by it's treatment is not problematic (Ahmad et al. 2016).

Secondary sludge is composed of finer particles of organic and inorganic materials, encased in extracellular polymeric substances (EPS). EPS contains biopolymers, humic substances, and nucleic acids (Wilén et al. 2003). Biodegradability and dewaterability of secondary sludge are not as good as PS and depend heavily on the sludge age, operational method of upstream biological

treatment process and temperature (Carrère et al. 2008). Sludge characteristics can be categorized into three separate groups: physical properties, chemical properties, and biological properties.

2.2.2.1 Physical properties of sludge

To predict the performance of a treatment plant on the sludge, parameters like dewatering, conditioning, and settling are investigated. These parameters are influenced by physical characteristics, including density, viscosity, rheology, and water content.

Sludge dewaterability shows the ease of separating water from sludge and is measured by capillary suction time (CST) (Yin et al. 2004) which is a rapid and inexpensive method for it. The CST apparatus works based on filtration of water. A lower value for CST shows better dewaterability (Yin et al. 2004). Dewatering makes the handling of sludge easier and cheaper. It also makes the sludge more suitable for land application and improves the calorific value for incineration (Soller et al. 2003)

The water content of the sludge is categorized into four forms: free water, interstitial water, vicinal water, and water of hydration (Yin et al. 2004). Free water can be easily removed by filtration or gravity settling as it is not enclosed with sludge particles. Interstitial water is the water trapped in the flocs and it needs a mechanical force like centrifugation to be separated. Vicinal water and water of hydration have chemical bonds with the sludge's solid constituents. The only way to remove these two types of water is the thermo-chemical treatment of sludge.

2.2.2.2 Chemical properties of sludge

Controlling the sludge treatment process is based on parameters like chemical oxygen demand (COD), alkalinity, pH, and VFAs which are chemical characteristics of sludge. For instance, pH

values in a digester can indicate the performance of that digester. COD removal in a digester shows the extent of biodegradability of the sludge.

Some important parameters in sludge treatment, especially when considering the land application of sludge, are the nutrient content and toxicity level. The nutrient content includes potassium, nitrogen, and phosphorous. Phosphorous and nitrogen are usually present in high amounts in sewage sludge. Toxicity level depends on the heavy metals and toxic organics (McLaughlin 1984; Antoniadis et al. 2015).

2.2.2.3 Biological properties of sludge

Biological characteristics of sludge define its biological stability and pathogenic properties. These characteristics are highly dependent on the type of sludge and its age. Biological stability shows the potential of sludge for biological activity and it depends on chemical and biochemical oxygen demand as well as the organic fraction of sludge. Stabilization of sludge is done through processes like alkaline stabilization, composting, and aerobic and anaerobic digestion. Anaerobic digestion produces stable sludge with minimal production of post-biogas (Metcalf & Eddy 2014; Appels et al. 2008). Soluble COD or SCOD is an important parameter which is used for assessing how pre-treatments can improve the degree of solubilization before anaerobic digestion. Anaerobic digestion also leads to pathogen destruction. The extent of this pathogen reduction depends on the sludge retention time and operational temperature. Ideally, the coliform content of treated sludge should be below 1000 most probable number (MPN) per gram of total solids (TS) in order to be considered as Class A sludge (biosolids) (Walton & White 2015). Pathogen reduction usually leads to a reduction in odors and potential for putrefaction. Composting also reduces the pathogens because of high temperature and microbial competition that occurs in its process (Msunar & Stentiford 2009; Riffat 2012).

2.2.2.4 Typical characteristics of primary and secondary municipal sludge

Before choosing the best method for treatment or disposal of the sludge, it is important to know its characteristics and composition. In a typical WWTP, primary and secondary sludge streams are the main sludge products. Table 2.2 indicates the typical characteristics of municipal waste sludge.

Table 2.2. The typical characteristics of municipal sludge
(the data is adapted from (Metcalf & Eddy 2014)*)*

Description	Primary sludge (PS)	Waste activated sludge (WAS)
Specific gravity (-)	1.02	1.005
pH	5.0 - 8.0	6.5-8.0
Total solids (%)	5 - 9	0.8-1.2
Volatile solids (VS)/Total solids (TS) (%)	60 - 80	60-90
Total chemical oxygen demand (TCOD) (g-TCOD/g-VS)	2.0	1.4
Alkalinity (mg/L as $CaCO_3$)	500 - 1500	600 - 1200
Nitrogen (N, % of TS)	1.5 – 4	2.4 – 5.0
Energy content (kJ/kg TS)	23,000 - 29,000	19,000 - 23,000

Primary sludge typically consists of large readily biodegradable organics which can be removed easily by primary clarifiers. The amounts of total solids and COD in primary sludge is higher than secondary sludge which results in more energy content in primary sludge. Secondary sludge or WAS which is produced from biological treatment of wastewater consists of inorganic material, extracellular polymeric material (including proteins, lipids, polysaccharides, etc.) and microbial

cells, and it contains less amount of total solids, COD and pathogens compared to primary sludge (Metcalf & Eddy 2014).

2.2.3 Sludge stabilization

Sludge stabilization aims to remove pathogens and offensive odors from the sludge as well as reducing the putrefaction potential. These objectives can be achieved through biological reduction of the VS in the sludge and by adding some chemicals to the sludge to inhibit the microorganism's growth. Sludge stabilization not only prevents health and aesthetic issues, but also results in reducing the sludge volume, producing biogas rich in methane which can be used beneficially, and improving the dewaterability of sludge.

There are three commonly used methods for sludge stabilization: (1) alkaline stabilization (using lime), (2) aerobic digestion, (3) anaerobic digestion, and (4) composting. These methods are briefly explained in the following sections.

2.2.3.1 Alkaline stabilization

The stabilization strategy of alkaline stabilization is to maintain the pH level at a certain range that is optimum for destroying the pathogens. This is accomplished by adding alkaline material (usually lime) to the sludge. To achieve Class B biosolids, the pH of the mixture of sludge and alkaline material is maintained at or above 12 for a contact time of 2 hours. For achieving Class A biosolids, the pH of the mixture is maintained at 12 or above and the contact time is at least 72 hours. During the contact time, the temperature must be at 52°C for at least 12 hours (Walton & White 2015).

The resulting solids from alkaline stabilization are soil-like products with a significantly reduced amount of pathogens. Alkaline stabilization is one of the most cost-effective methods for

stabilizing sludge. A disadvantage of this method is that adding the alkaline material to the sludge leads to an increase in the product mass that is to be disposed (Metcalf & Eddy 2014).

2.2.3.2 Aerobic digestion

Aerobic digestion is a biological process in which organic matter reacts in the presence of air. This process is usually operated in open top tanks. Compared to the other biological methods for sludge digestion (i.e., anaerobic digestion), aerobic digestion is easier to operate; however, it does not produce biogas (methane) for energy recovery. Typical sludge retention time during aerobic digestion is 40-60 days depending on the digestion temperature. In all operating conditions, the dissolved oxygen (DO) levels should be maintained at 1 mg/L or above. This process requires high energy supplementation due to the need for mixing and aeration and as a result has a high operating cost. Biosolids generated from aerobic digestion have poor dewatering characteristics (Metcalf & Eddy 2014). However compared to anaerobically digested sludge, the dewaterability of aerobically digested sludge is better (Novak et al. 2001).

2.2.3.3 Anaerobic digestion

Anaerobic digestion is a biological process in which organic matter goes through fermentation in a reactor in the absence of oxygen. The fermentation process occurs in a heated sealed reactor. One of the end products of anaerobic digestion is biogas rich in methane, which can be used to generate electricity and heat. The methane gas resulting from anaerobic digestion is used to recycle the energy in the WWTP by generating heat and power through a co-generation unit or a boiler. The biosolids produced from this process has the potential to be utilized in land application. In contrast with aerobic digestion which is simple to operate, anaerobic digestion can experience process upsets easily and doesn’t recover quickly. Therefore, it needs skilled operation (Metcalf & Eddy 2014).

2.2.3.4 Composting

Composting is a biological process in which solid organic matter is kept in an enclosed reactor or pile with biological reactions occurring. A bulking agent is added in order to prepare an environment appropriate for biological activity. The disadvantage of this method is that the final product of composting has a higher volume than the influent sludge that goes through the composting process. Composting can be combined with aerobic or anaerobic digestion to increase the pathogen destruction and to provide Class A biosolids. However, using composting independently can also produce Class A biosolids through in-vessel or aerated static pile composting processes. The composting process is extremely odorous; therefore, it needs serious odor control (Msunar & Stentiford 2009; Metcalf & Eddy 2014).

Among the four methods for sludge stabilization which were described above, anaerobic digestion is the most commonly used one. The following section of this chapter introduces anaerobic digestion in more detail.

2.3 Fundamentals of anaerobic digestion

Anaerobic digestion can be divided into three principal categories based on operational temperature: mesophilic digestion (the most commonly used one), thermophilic digestion, and phased digestion. Many of the new developments in anaerobic digestion are involved in phased digestion.

Anaerobic digestion consists of three main steps of chemical and biochemical reactions including 1. hydrolysis, 2. acidogenesis, and 3. methanogenesis. Figure 2.3 indicates the principal steps in anaerobic digestion. The major chemical reactions occurring at different stages of anaerobic digestion are as follows:

Acidogenesis: $3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O$

Acetogenesis: $CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$

Methanogenesis: $CH_3COOH \rightarrow CH_4 + CO_2$, $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

Important environmental parameters affecting the process of anaerobic digestion are: Temperature, hydraulic retention time, solid retention time, pH, alkalinity, inhibitory substances (toxic material), and availability of trace metals and nutrients (Chen 2010; Singh et al. 2018).

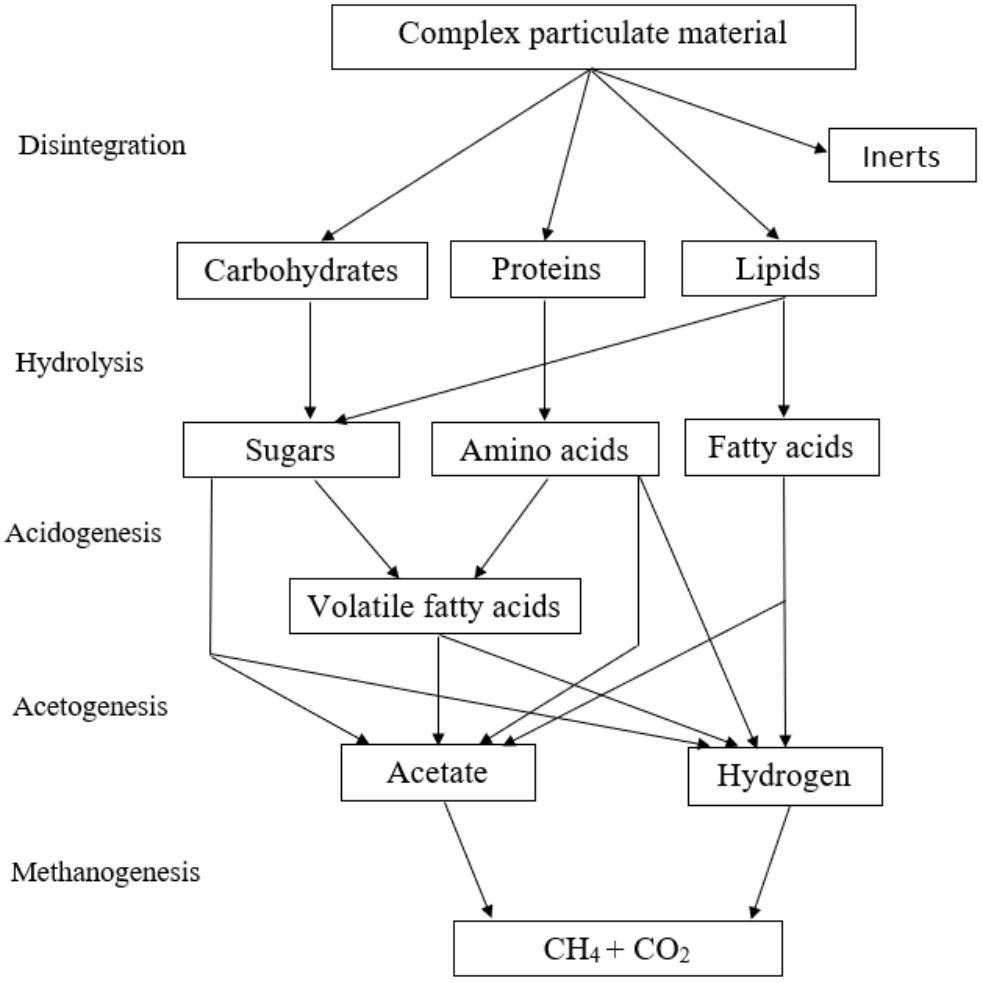


Figure 2.3. Schematic diagram of principal steps of anaerobic digestion (adapted from (Batstone et al. 2002)*)*

The first three factors mentioned above, affect the process selection. Alkalinity is dependent on the feed and affects the control of digestion process. The presence of nutrients and trace metals is necessary for biological growth (Metcalf & Eddy 2014). Anaerobic digestion starts with the conversion of complex organic materials to soluble biopolymers (e.g. lipids, carbohydrates and proteins). In Figure 3, disintegration consists of extracellular reactions and is done through enzymatic and non-enzymatic decay as well as mechanical, chemical, and thermal decomposition (Batstone et al. 2002). Breaking down the particulate organic polymers prepares them for the following hydrolysis step. At this stage, fermentative microorganisms convert biopolymers to building blocks for fermentation (e.g. amino acids, monosaccharides and long-chain fatty acids). These building blocks are soluble in water and have lower molecular weight. Exo-enzymes released from the microorganisms are responsible for these conversions (Haandel & Lubbe 2007).

The following step is acidogenesis. Acidogenesis or acidification is an anaerobic microbial process that produces acids and doesn't need external electron exchange (Gujer & Zehnder 1983). At this stage, organic compounds like VFAs, H_2, CO_2, alcohols, aldehydes, and ammonia gas are produced from the hydrolysis products (Haandel & Lubbe 2007). At high concentrations of hydrogen or formate (also at high biomass production) acidogenic reactions take place since there is no need for additional electron acceptors and more free energy is produced (Batstone et al. 2002).

Bacteria participating in acidification are mostly obligate anaerobes and oxygen, that may be present in the environment, is poisonous for them. Acidification products are then converted to acetic acid, CO_2 and H_2, in the acetogenesis step. The first three stages of anaerobic digestion are called acid fermentation. During the whole process of acid fermentation, organic matter is converted to a substrate which is used in the final methanogenesis phase (Haandel & Lubbe 2007).

In the final step which is methanogenesis, methanogenic archaea consume fermentation products and convert them to carbon dioxide and methane. These methanogens are sensitive anaerobic microorganisms, since among all microbial communities present during anaerobic digestion, they are the most affected by environmental changes. Methanogens are divided into two principal groups: hydroclastic methanogens and acetoclastic methanogens (Bitton & John Wiley & Sons. 2005).

Hydroclastic, also called hydrogenotrophic, methanogens utilize CO_2 and H_2 to produce methane. *Methanobacteriales* and *Methanococcales* are categorized under these hydrogenotropic methanogens (Ritchie et al. 1997). Acetoclastic, also called acetotrophic, methanogens, produce methane and carbon dioxide from acetate. *Methanosarcina* and *Methanosaeta* are categorized under this group of methanogens (Smith & Mah 1978; Ritchie et al. 1997).

Methanogens have a minimum generation time of three days, so they are among the slow-growing microorganisms. Therefore, a longer retention time in an anaerobic digester is required for them to reach to a considerable population count (Gerardi 2003). It is indicated, in the literature, that operating the methanogenic stage at retention times below 8-10 days may result in instability of the process (Bouallagui et al. 2003; Heo et al. 2004). Most anaerobic sludge digesters operate in a sludge retention time range of 15-20 days. A typical organic loading rate (OLR) range for anaerobic digestion of municipal sludge is 1.9-2.5 kg-VS/m^3/day (WEF 2012).

Anaerobic digestion is divided into two groups based on the optimum temperature ranges for it. Mesophilic range with an optimum temperature of 35°C and thermophilic range with an optimum temperature of 55°C. If the temperature is not within these ranges, then solids removal efficiency or rate of anaerobic digestion decreases significantly (Henze et al. 2002); although, there are psychrophilic digesters operating in a range of 0 to 20°C in remote areas (Kashyap et al. 2003).

2.4 Advanced anaerobic digestion

Advanced processes for anaerobic digestion were developed with the purpose of increasing VS removal, increasing biomethane recovery at a faster biodegradation rate, and producing Class A biosolids (high quality biosolids which can be used beneficially). Conventional anaerobic sludge digestion is a single-stage mesophilic digestion. Some of the advanced anaerobic digestion processes are: thermophilic digestion, staged mesophilic digestion, staged thermophilic digestion, temperature phased digestion, and mesophilic or thermophilic digestion coupled with sludge pretreatments.

2.4.1 Thermophilic anaerobic digestion

The optimum temperature range for thermophilic digestion is between 50°C to 57°C. This range of temperature provides the appropriate conditions for thermophilic bacteria. Thermophilic digestion is faster than mesophilic digestion, because increasing the digestion temperature will increase the biochemical reaction rates (every 10°C increase in temperature doubles the rate until reaching the limiting temperature) (Metcalf & Eddy 2014). Increase in reactions rate will lead to a reduction in the digester volume requirement; therefore, saving in capital cost.

Thermophilic digestion improves pathogen destruction and produces biosolids of higher quality compared to mesophilic digestion. Producing Class A biosolids is possible using this method. Since thermophilic digesters are operated at higher temperatures than mesophilic digesters, more thermal energy is required to heat them. This makes the system more complex, because heat recovery is required to make the system energy efficient.

Biosolids produced from thermophilic digestion may not dewater as well as they do in mesophilic digestion. Also, ammonia concentration increases in the dewatering side stream. Dewatered cake from thermophilic digestion has a higher odor potential (Metcalf & Eddy 2014).

2.4.2 Staged mesophilic digestion

In a staged mesophilic digestion process, one large reactor is followed by one or more smaller ones operating at mesophilic temperatures. The advantage of staged mesophilic digestion over single-stage digestion is that this process has a higher VS reduction as well as more biogas production (Torpey & Melbinger 1967; Garber 1997).

Previous research comparing a two-stage mesophilic digester with single-stage mesophilic anaerobic digestion indicate that the two-stage mesophilic digestion has the potential to produce more stable biosolids that are easier to dewater and have less odor (Schafer & Farrell 2000a).

2.4.3 Staged thermophilic digestion

The staged thermophilic digestion process includes a large reactor followed by one or two smaller ones operating at thermophilic temperatures. Its aim is to increase pathogen removal and produce Class A biosolids (Metcalf & Eddy 2014).

2.4.4 Temperature phased digestion

In temperature phased anaerobic digestion (TPAD), a thermophilic reactor is followed by a mesophilic reactor. This process is able to achieve the advantages of the two categories of anaerobic digestion at the same time, while avoiding the drawbacks of both systems. It utilizes the advantage of a higher digestion rate through the thermophilic stage, as well as enhancing the stabilization through the mesophilic stage.

The sludge retention time (SRT) in the first reactor (thermophilic digester) is in the range of 2 to 5 days and for the second stage (mesophilic digester) is 10 days or more. The TPAD process can tolerate shock loadings better than single-stage digestions (Metcalf & Eddy 2014). Previous studies on TPAD has shown that the VS removal in a TPAD process is 15 to 25 percent higher than single-stage digestion under mesophilic conditions (Schafer & Farrell 2000a).

2.4.5 Sludge pretreatment for anaerobic digestion

Pretreatment of sludge involves increasing the extent of the hydrolysis step of anaerobic digestion using some form of energy applied to the sludge. Increasing the hydrolysis leads to more biogas production, VS removal, and in some cases the production of Class A biosolids (Metcalf & Eddy 2014). There are five common forms of sludge pretreatment: physical, chemical, thermal, biological (enzymatic), and electrical.

2.4.5.1 Thermal hydrolysis pretreatment

Thermal hydrolysis (TH) is a thermal conditioning method which is implemented prior to anaerobic digestion. This process functions at a relatively high temperature range (150-200°C).

The advantages of this pretreatment method include: (1) breaking down long-chain organics into shorter chain organic matter to improve the hydrolysis step in anaerobic digestion (improving digestion and gas production), (2) producing Class A products, (3) enhancing digestate quality in terms of odor, texture, and dewatering properties, and finally (4) reducing the volume required for the digester.

There are various types of thermal pretreatment that can be implemented on sludges, of which microwave (MW) and conventional heating (CH) are the most commonly used. Pretreatment temperatures in the range of 60-270°C have been implemented in bench-scale and pilot-scale

anaerobic digestion. However, temperatures ranging from 60°C to 180°C are the prevalent temperatures applied to sludge (Ferrer et al. 2008). Previous studies reported that implementing temperatures above 180°C for thermal pretreatment can result in inhibition in anaerobic digestion. This occurs because of the formation of toxic intermediates such as recalcitrant melanoidins which are produced in Maillard reactions occurring at or above 170°C. Furthermore, thermal pretreatment above 200°C can result in the formation of other inhibitors for anaerobic digestion such as nitrogen (ammonia) and phosphorous in the liquid fraction of sludge (Wilson & Novak 2009).

CH pretreatment is done through thermal conductivity and usually implements an electric oven. In this method, the heat flow starts from the surface of the sludge. The rate of the heating is controlled by two factors: thermal properties of the sludge and the temperature differential (Valo et al. 2004). CH usually applies high pressure and temperature. Both the temperature and the reaction time in a CH process, contribute to the extent of chemical and physical changes in the sample (Hosseini Koupaie & Eskicioglu 2016). This traditional method of thermal pretreatment has some drawbacks such as producing unwanted temperature gradients in the sample as a result of non-uniform heating. Another drawback is the long reaction time required in some cases.

MW pretreatment technology is a relatively new method of thermal pretreatment. This method is applied before the anaerobic digestion process and uses various frequencies. MW is a more practical thermal method than CH due to the following reasons. MW pretreatment is more rapid than CH method. It is also able to heat selectively. The MW method has an instantaneous on/off control which results in enhanced energy efficiency compared to the CH method and also makes it possible to control the reaction rate (Tyagi & Lo 2013). In a study comparing MW and CH methods, CH achieved higher solubilization of COD and sugars. The reason for this result is that CH needs a longer exposure time to maintain the temperature at a certain point. The study

concluded that an accurate comparison between CH and MW thermal methods is not possible with present technology (Eskicioglu et al. 2006). Another recent study reported that when applying the same heating rates for CH and MW methods, both systems result in identical organic matter solubilization and methane production. This study concluded that deciding to opt a thermal pretreatment method to have a more effective system, should be based on the energy input requirement (Koupaie & Eskicioglu 2015).

2.4.5.2 Physical, chemical and electrical pretreatment

Pretreatment methods are generally applied to sludge produced from the secondary treatment process since this type of sludge typically does not digest well anaerobically. Pretreatment of this sludge is accomplished through the application of ultrasonic waves, mechanical shear, electrical pulses, pressure drops, or electrical fields. The application of these different treatment methods has resulted in various degrees of success in enhancing sludge digestion. For the application of the pretreatment process to be practically effective, the amount of sludge entering the digester from secondary treatment must be more or less the same as the sludge produced from primary treatment (Yin et al. 2004; Carrère et al. 2010; Ariunbaatar et al. 2014).

2.5 The studies comparing TPAD with single-stage anaerobic digestion

Among different sludge treatment options, the TPAD system (the topic of this research project) has shown to be more energy efficient as well as more effective in terms of VS and pathogen removal. Also, TPAD has a relatively low capital cost as less volume is required for the digesters in this system (Riau et al. 2010; Coelho et al. 2011). The two important parameters affecting the performance of a TPAD system are the temperature of the thermophilic stage and the sludge retention time in the system (Riau et al. 2012; Ge et al. 2011b; Lv et al. 2016). There are only a

few studies which investigated the effect of different temperatures and sludge retention times on the same system.

Table 2.3 summarizes the previous studies comparing TPAD and conventional single-stage anaerobic digestion for sludge treatment. A study conducted by Ge et al. (2011) compared TPAD (thermophilic (50-70°C)-mesophilic (35°C)) with the dual stage mesophilic-mesophilic anaerobic digestion (both 35°C). Both systems had identical hydraulic retention time (HRT) configurations. The methane production in TPAD systems increased when the thermophilic temperature was increased from 50 to 60°C but decreased when the temperature was further increased to 65 and 70°C. VS destruction was 11-30% higher in the TPAD process compared to the mesophilic-mesophilic anaerobic digestion (Ge et al. 2011b). Another study conducted by Ge et al. (2011) investigated two-stage digestion with a batch thermophilic pre-treatment (Stage 1), conducted at different temperatures (50, 60, 65, 70°C), pH (4, 5, 6, 7), and retention times (1, 2, 4 days). The first stage was followed by mesophilic digestion (Stage 2), conducted uniformly at 37°C. It was observed that the overall process was more effective with short pre-treatment retention times (1–2 days) and neutral pH compared to longer retention time (4 days) and low pH (4–5). Furthermore, increasing the temperature of the thermophilic stage in TPAD improved the degradability of waste activated sludge. The results also showed that 1st-stage thermophilic digestion of TPAD with shorter retention times (e.g., 1 and 2 days) could achieve similar or better degradability as longer retention times (e.g., 4 days). Also, it was observed that the 1st-stage thermophilic temperature has a direct impact on the overall degradability. This means that increasing the thermophilic temperature within a range of 50°C to 70°C leads to an increase in overall degradability (Ge et al. 2011a).

A study suggests that temperature dictates the bacterial community in TPAD systems and there must be an optimum range of temperature for the presence of key bacterial populations (Pervin et al. 2013). According to this study, changing the thermophilic temperature from 50°C to 65°C changed the microbial composition of the thermophilic stage of TPAD considerably. Members of *Thermotogae*, *Lutispora*, and *Coprothermobacter* are the key populations which dominate thermophilic reactors, especially at temperatures of 60°C and 65°C. Almost 10% of the total population in the thermophilic reactor at 60°C consisted of archaeal populations related to the *Methanosarcinaceae*. The majority of variations in microbial composition in the thermophilic reactors of TPAD systems were related to the thermophilic temperature. In the temperature range of 50°C to 60°C, *Thermotogae* was dominant. In the temperature range of 60°C to 65°C, *Lutispora thermophila* was dominant and at 65°C, *Coprothermobacter* was more abundant. Within each temperature level, the bacterial community composition did not change considerably (Pervin et al. 2013).

Previous studies have shown that microbial community composition directly affected the performance of the reactors. At a thermophilic temperature of 50°C, hydrolysis in the thermophilic reactor had no improvement over the mesophilic AD. Hydrolysis increased by increasing the temperature to 60°C and 65°C. The dominating microbial community shifted from mixed communities and *Thermotogae* to *Lutispora thermophila* and *Coprothermobacter* when the temperature increased from 60°C to 65°C. It is concluded that increasing the thermophilic temperature leads to an increase in digestion performance of TPAD (Zahedi et al. 2016; Pervin et al. 2013).

Another study investigated the effect of staged digestion (thermophilic-thermophilic), TPAD, and microwave pretreatment on the AD. The investigated systems included: single-stage anaerobic

digestion (mesophilic and thermophilic), temperature-phased (TPAD) and thermophilic-thermophilic staged digestion. The systems were operated at various SRTs including 20, 15, 10 and 5 d. The thermophilic and mesophilic digestion temperatures in all systems were 55°C and 35°C, respectively. The results of this study showed that two-stage thermo-thermo reactors treating microwaved sludge produced more biogas than all other reactors and removed more VS. Also, all the two-stage systems that were treating pretreated sludge, produced sludge free of pathogen indicator bacteria (even at a total system SRT of only 5 d) (Coelho et al. 2011).

In another study, a TPAD system (65°C + 55°C) was compared to a single stage mesophilic (35°C) and a single stage thermophilic (55°C) AD. The results showed that by increasing the digestion temperature, the reactor performance improved (e.g., COD removal in mesophilic conditions: 35%, in thermophilic conditions: 45%, and in the TPAD system: 55%). The specific biogas (methane) production also increased significantly in the TPAD system compared to the thermophilic anaerobic digestion and mesophilic anaerobic digestion (Bolzonella et al. 2012).

Many researchers also combined physical or mechanical pretreatments with TPAD systems to further improve the TPAD's performance. Another study by Wahidunnabi (2017) compared the single-stage mesophilic anaerobic digestion with TPAD and HPH-TPAD (a TPAD which is fed with high pressure homogenized sludge) at three overall SRTs (20, 14 and 8 days). Mesophilic and thermophilic reactors were operated at 35°C and 55°C, respectively. This study revealed that HPH+TPAD had the best performance among the studied systems as it had maximum specific methane production and solids removal. The optimum scenario which could be selected for full scale was the HPH+TPAD system with 14 d SRT (Wahidunnabi 2015). Research by Riau et al. (2015) investigated the effect of ultrasound pretreatment on TPAD. They discovered that the total methane production and VS removal improved significantly in comparison to the regular TPAD

system (Riau et al. 2015). Another research paper investigated a NT-TPAD (neutral pH in thermophilic digester) at different volume ratios between thermophilic and mesophilic digesters (1:2 and 1:1) and compared it to the conventional TPAD system. In the NT-TPAD system, acidogenesis and methanogenesis in the thermophilic digester are balanced. The thermophilic and mesophilic temperatures analyzed were 55°C and 35°C, respectively. At the same overall retention time, 1:2 and 1:1 volume ratios between thermophilic and mesophilic digesters produced similar biogas results and achieved the same VS removal. Based on these results, smaller SRTs for the thermophilic stage of the TPAD are preferred, because the smaller the SRT, the smaller the volume requirement for the digester; therefore, less input energy is required for heating (Lv et al. 2016). Finally, researchers investigated the effect of MW and sonication pretreatments on TPAD. They concluded that although using pretreatments before TPAD systems further increases the organic removal efficiency, it is not a feasible option for industrial applications, because the biogas production does not compensate the energy input (Pilli et al. 2015; Akgul et al. 2017). In terms of pathogen destruction, TPADs achieved significantly higher removal percentage and met Class A biosolids criteria compared to single stage mesophilic anaerobic digestion (Akgul et al. 2017).

Table 2.3. The studies comparing TPAD with single-stage anaerobic digestion

Source	Scale	Influent	Pre-treatment	Digestion stage	Effluents	Conclusion
(Akgul et al. 2017)	Lab scale	Mixed sludge (TWAS[a] and FPS[b] with a volume % ratio of 67:33, respectively)	Microwave (MW) irradiation, Ultrasonication (US)	TPAD[c] Total SRTs: 14 & 20 d Acid phase SRT[d]: 2 d, the rest of the required SRT time was performed in methane phase thermophilic temperature: 55^{o}C mesophilic temperature: 35^{o}C	At SRTs of 20 and 14 days TPAD achieved (39-45%) higher methane production, pathogen destruction (4-log), digester volume reductions compared to the single-stage AD[e] (control). Acid phase stages of the TPAD systems contained the highest amount of odor-causing VSCs[f] (2000-4000 ppm) and VFAs[g] (4400 ppm) to accumulate	The increased methane production did not compensate for the energy input for pretreatments. Pretreatment is infeasible for industrial applications for single-stage and TPAD. TPADs provided the highest pathogenic removal and met the Class A biosolids fecal coliform requirements.
(Gianico et al. 2015)	Lab scale	Waste activated sludge	Low-energy sonication	Sequential mesophilic/ thermophilic anaerobic digestion Mesophilic step: HRT[h]=3-5 days & T=37°C Thermophilic step: HRT=10 days & T=55°C test 1: 1st stage OLR[i]=3.9 2nd stage OLR=1.2 (OLR_{tot}=1.7 kg VS/m^3/day) test 2: 1st stage OLR=10 2nd stage OLR=2.5 (OLR_{tot}=3.1 kgVS/m^3/day)	Either untreated or ultrasonic pretreated showed better performances in terms of VS[j] removal (ranging from 44 to 55%, compared to the 35–40% of the conventional single-stage digestion). High volatile solid removals, up to 55%. High methane production (+11 %) due to sonication (observed at high loading rate) Positive energy balances (possible exploitation of this innovative two-stage digestion)	Two-stage digestion compared to conventional single-stage processes: reducing the volume of sludge requiring disposal improving the final sludge quality enhancing methane production

Source	Scale	Influent	Pre-treatment	Digestion stage	Effluents	Conclusion
(Lv et al. 2016)	Bench-scale	Dairy cattle manure	No pretreatment	TPAD NT-TPAD[k]: balanced acidogenesis and methanogenesis in thermophilic digester. Overall HRT: 15 days Two tests: 5-day and 7.5-day HRT for the thermophilic digester (the rest of the SRT was supplied by the second stage) Temperatures: 50°C thermophilic, 35°C mesophilic	Similar system performance for both tests: (36–38% VS removal, 0.21–0.22 L methane/g VS fed) Thermophilic digester had a greater volumetric biogas yield at 5-day HRT than at a 7.5-day HRT (6.3 vs. 4.7 L/L/d) Mesophilic digester had a stable volumetric biogas yield for both tests (about 1.0 L/L/d).	NT-TPAD system had a greater volumetric biogas production rate than AT-TPAD[l]. At the same overall HRT/SRT, 1:2 and 1:1 volume ratios between thermophilic and mesophilic digesters, similar biogas production and VS removal was achieved Volume ratio of 1:2 are preferred, because a smaller thermophilic digester and less energy input is needed to maintain the thermophilic temperature.
(Leite et al. 2016)	Pilot-scale	Waste activated sludge	No pretreatment	Two-phase AD Comparing single and two-phase digesters at the same conditions of organic loading and retention time. Methanogenic reactor (thermophilic-2nd step digester): HRT of 18 days Fermenter reactor (1st step, pretreatment): HRT of 2 days Temperature: both fermenter & digester at 55°C	Two-phase AD showed higher organic matter removal and biogas production compared to the single-stage AD. VS removal rose from 34% in the single-stage to 38% in the two-phase system. Biogas production increased 32% (from 0.21 to 0.31 m^3/kgVS.d). Two-phase AD produced 15% more energy.	The heat produced in a CHP unit satisfied all heat requirements insuring more than the complete energetic sustainability of the process. The digestate after the single-stage AD presented the poorest dewatering trend while the filterability of the two-phase digestate was also reachable without chemical conditioning. This improvement was due to the lower solids content on this digestate (4.2% TS).

Source	Scale	Influent	Pre-treatment	Digestion stage	Effluents	Conclusion
(Fernández-Rodríguez et al. 2016)	Lab-scale	Organic Fraction of Municipal Solid Waste (OFMSW)	No pretreatment	TPAD Tthermophilic reactor (55–57°C) Mesophilic reactor (35–37°C) Two TPAD tests: 4:10 and 3:6 (the first digit means the SRT used in the first thermophilic phase, the second digit is the SRT used in the second mesophilic phase).	TPAD 4:10 was better than TPAD 3:6, with higher productivity of methane (35–45%) and removal of organic matter (6–19%).	The best results were obtained for the TPAD 4:10. However, TPAD 3:6 reaches a high productivity of methane, 2.45 LCH_4/(L reactor/day), which together with the significant decreasing in the overall SRT, also makes it an interesting industrial option.
(Riau et al. 2015)	Lab scale	Waste activated sludge (WAS)	Ultrasonic	TPAD Thermophilic digester: 55°C Mesophilic digester: 35°C No differences were found when ultrasound was applied before or after the thermophilic stage of the TPAD system	By applying ultrasound, total methane production was increased by more than 42% and volatile solid removal more than 13% in comparison to control system	In spite of the increase in the initial VFA concentration due to sonication, the second mesophilic stage reduced the VFA concentration in the final effluent by 95% in both sonicated and control systems.
(Li 2015)	Lab scale	Raw sludge effluent after the thickening process	No pretreatment	TPAD at 45°C Temperatures: 1st stage 45°C and 2nd stage 35°C Two tests: 1. SRT (2.5 d + 10 d) 2. SRT (7.5 d + 10 d)	Comparing two experiments: TPAD 55-35°C and TPAD 45-35°C. Best operating condition is for TPAD Run 2 (45°C-7.5days+35°C-10days) which showed relatively high methane yield, sufficient VS reduction, sufficient pathogen deactivation, less acetate and VFA accumulation, well-buffered system, and stable operating process.	Except for COD reduction, all environmental parameters associated with 45°C-35°C TPAD were observed with better performance than 55°C-35°C TPAD. Also there is a lower energy requirement than conventional TPAD.

Source	Scale	Influent	Pre-treatment	Digestion stage	Effluents	Conclusion
(Wahidunnabi 2015)	Lab scale	Mixed sludge	High pressure homogenization (HPH)	TPAD Three overall SRTs: 20: (2(pretreatment)+9+9) d, 14: (2(pretreatment)+6+6) d, 8: (2(pretreatment)+3+3) d Mesophilic and thermophilic temperatures: 35 ± 2°C and 55 ± 2°C Digesters were fed with a mixed sludge of FPS and high pressure homogenized TWAS at a volume ratio of 33:67%	Digested sludge from all digestion systems were qualified as Class B biosolids Mesophilic TPAD at 20 d SRT nearly meet Class A biosolids Daily methane production from the digester increased with shorter SRTs as OLRs were higher	Comparing control, TPAD and HPH-TPAD shows that HPH+TPAD has the maximum specific methane production (L CH_4/g COD added) and maximum organic removal efficiencies. HPH pretreatment was not effective for coliform reduction and has the potential to reduce emissions of VSCs. Without pretreatment, TPAD system at 14 d SRT, itself will provide 24% higher biodegradation and 1.75 log and 36% reduction in pathogen and digester volume requirement, respectively, compared to control digester.
(Pervin et al. 2013)	Lab scale	Waste activated sludge	In one system, the pre-treatment was performed under mesophilic conditions at 35°C (MP), while the other was operated under thermophilic conditions at 50°C for 186 days, 60°C for 100 days and 65°C for 60 days (TP).	TPAD 1st stage HRT: 2 days 2nd stage HRT: 14 days Two tests: 1) mesophilic pretreatment (35°C) 2) thermophilic pretreatment: (50, 60, 65°C)	The mesophilic pre-treatment reactor bacterial communities were heavily influenced by the feed, while the thermophilic reactor was less diverse, and had dominant populations of Thermotogae sp., Lutispora thermophila, and Coprothermobacter, shifting progressively from the first to the last as the temperature was increased from 50°C to 65°C.	Functionality was higher at 60°C and 65°C, showing that while temperature can direct community, there will be optimums related to the emergence of key populations.

Source	Scale	Influent	Pre-treatment	Digestion stage	Effluents	Conclusion
(Bolzonella et al. 2012)	Pilot scale	Waste activated sludge	No pretreatment	TPAD TPAD temperature: (65 + 55°C) Total HRT: 20 days -1st reactor: HRT 2 d -2nd reactor: HRT 18	The extreme thermophilic reactor (working at 65°C) showed a high hydrolytic capability and a specific yield of 0.33 gCOD (soluble) per gVSfed.	The COD removal increased from 35% in mesophilic conditions, to 45% in thermophilic conditions, and 55% in the two-stage TPAD system. The specific biogas production increased from 0.33 to 0.45 and to 0.49m3/kg VS fed at 35, 55, and 65 + 55°C, respectively.
(Ge et al. 2011a)	Lab scale	Waste activated sludge	Batch thermophilic pre-treatment	TPAD Two-stage digestion: a batch thermophilic pre-treatment (Stage 1), conducted at different conditions (50, 60, 65, 70°C), pH (4, 5, 6, 7) and retention time (1, 2,4days); and a subsequent mesophilic digestion (Stage 2), conducted uniformly at 37°C.	The overall process was more effective with short pre-treatment retention times (1–2 days) and neutral pH compared to longer retention time (4 days) and low pH (4–5). Increased temperature in the thermophilic stage in TPAD improves degradability of waste activated sludge.	Pre-treatment temperature (thermophilic temperature) had a strong impact on the whole process, increasing overall degradability from 0.3 to 0.5 as temperature increased from 50 to 65°C, with apparent hydrolysis coefficient increasing from 0.1 to 0.4d−1. Pre-treatment for shorter retention times (1 and 2 days) could achieve similar or better degradability as a longer retention time (4 days). The combined TPAD process was more effective at pre-treatment of pH 6–7 with 33–48% degradability, compared to low pH (4–5) with 21–42% degradability. The thermophilic temperature had a stronger impact on degradability, which was increased from 21% to 49% with temperature increased from 50 to 65°C.

Source	Scale	Influent	Pre-treatment	Digestion stage	Effluents	Conclusion
(Coelho et al. 2011)	Lab scale	TWAS	Microwave	Staged digestion SRTs: 20, 15, 10 and 5 d Temperatures: Mesophilic=35°C, Thermophilic=55°C Three tests: 1- single-stage (mesophilic and thermophilic), 2- temperature-phased (TPAD) 3- thermophilic-thermophilic	Two-stage thermo-thermo reactors treating pretreated sludge produced more biogas than all other reactors and removed more VS. All the two-stage systems treating microwaved sludge produced sludge free of pathogen indicator bacteria (even at a total system SRT of only 5 d).	MW pretreatment and staging reactors allowed the application of very short SRT (5 d) with no significant decrease in performance in terms of VS removal. Association of MW pretreatment and thermophilic operation: improves dewaterability of digested sludge. MW pretreatment caused the solubilization of organic material in sludge but also allowed more extensive hydrolysis of organic material in downstream reactors.
(Ge et al. 2011b)	Lab scale	WAS	No pretreatment	TPAD An experimental thermophilic (50-70°C)-mesophilic (35°C) system was compared against a control mesophilic-mesophilic (35°C both) system. Thermophilic stages (0.6 L, 2 days HRT), mesophilic stages (4.2 L, 14 days HRT).	Hydrolysis coefficient significantly enhanced at 60, 65 and 70°C, but was not improved under thermophilic reactor of 50°C. Higher NH_4^+-N was released during thermophilic reactors and further increased by increasing the thermophilic temperature. In TPAD with thermophilic temperature of 50 to 60°C, a large amount of methane was produced, but decreased with further increase of temperature to 65 & 70°C.	VS destruction was 11-30% higher in TPAD process (except thermophilic pre-treatment of 50°C) compared to mesophilic-mesophilic system. Solubilisation was improved during thermophilic pre-treatment relative to mesophilic pre-treatment (maximum of 27% at thermophilic pre-treatment of 60°C). Methane production from the pre-treatment stage was heavily inhibited at acidic conditions (pH 5). Increasing thermophilic pre-treatment temperature had no impact on the overall degradability.

[a]Thickened waste activated sludge, [b]Fermented primary sludge, [c]Temperature-phased anaerobic digestion, [d]Sludge retention time, [e]Anaerobic digestion, [f]Volatile sulfur compounds, [g]Volatile fatty acids, [h]Hydraulic retention time, [i]Organic loading rate, [j]Volatile solids, [k]Neutral thermophilic TPAD, [l]Acidified thermophilic TPAD

2.6 Summary of literature review

Among different sludge treatment options, the TPAD system, which is the topic of this research project, has shown to be more energy efficient. It is also more effective in terms of VS and pathogen removal. Moreover, TPAD has a relatively low capital cost due to the lower volume required for the digesters in this system (Riau et al. 2010; Coelho et al. 2011). The two important parameters affecting the performance of a TPAD system are the temperature of the thermophilic stage and the sludge retention time in the system (Riau et al. 2012; Ge et al. 2011b; Lv et al. 2016).

The benefits of using TPAD aligns with the needs of sludge digestion at Lulu Island WWTP. It can be an improvement for current single-stage mesophilic anaerobic digestion at this plant if the proper thermophilic temperature and retention time is applied. There are only a few studies which investigated the effect of different temperatures and sludge retention times on the same system. This study intended to evaluate the effect of two TPAD systems on treatment of municipal sludge and to compare them with conventional single-stage mesophilic anaerobic digestion. One TPAD system is operated at the minimum temperature of the thermophilic range (55°C) and the other is operated at the maximum temperature of the thermophilic range (70°C) for the acid phase. The effect of three overall SRTs (30, 20 and 15 days) on the process performance of the TPAD is also investigated.

Chapter 3: Materials and methods

This chapter provides a list of materials and equipment that were used in this research. Furthermore, it provides experimental design and a thorough explanation of the methods used for running the experiments.

3.1 Materials

The list of chemicals used in this research is provided in Table 3.1. In addition to the chemical materials listed in this table, biological materials are also used in this research including mixed sludge as digester feed and anaerobic culture as inoculum.

3.1.1 Feed sludge

The mixed sludge used for feeding the digesters was collected from Lulu Island WWTP located in Richmond (B.C., Canada). This facility provides primary and secondary treatment to wastewater and then uses conventional single-stage anaerobic digestion (AD) to stabilize the sludge produced during the process of wastewater treatment. Thickened waste secondary sludge (TWSS) and thickened primary sludge (TPS) are mixed together using 35:65% volumetric ratio of TWSS to TPS in the treatment facility to form the mixed sludge. This mixed sludge was used as digester feed for this study.

After collection, the mixed sludge was shipped to UBC's Bioreactor Technology Group's Laboratory in coolers packed with dry ice via overnight courier bi-weekly and was stored in a fridge at 4^{o}C in order to maintain the physical and chemical characteristics. The mixed sludge was characterized bi-weekly and the characterization results are presented in Table 3.2.

Table 3.1. List of chemicals

Materials	Manufacturer	Purity
Acetic acid	Sigma-Aldrich	99%
Acetonitrile	Fisher Scientific	Optima
Ammonium molybdate	MP Biomedicals	ASC grade
Antimony potassium tartate	Fisher Scientific	ASC grade
Ascorbic acid	Fisher Scientific	ASC grade
Bovine Serum albumin	Fisher Scientific	ASC grade
Butyric acid	Sigma-Aldrich	99%
Distilled water	-	0.0 ppm salt
Folin-Ciocalteu phenol reagent	Fisher Scientific	ASC grade
Glutamic acid	Acros Organics	99%
Humic acid	Sigma-Aldrich	99%
Isobutyric acid	Sigma-Aldrich	99.5%
Mercuric sulphate	Fisher Scientific	ASC grade
Methanol	Fisher Scientific	Optima
Phenol	Fisher Scientific	ASC grade
Potassium acid phalate	Fisher Scientific	ASC grade
Potassium dichromate	Fisher Scientific	ASC grade
Potassium dihydrogen phosphate	Fisher Scientific	ASC grade
Potassium nitrate	Sigma-Aldrich	99.99%
Potassium persulphate	Fisher Scientific	ASC grade
Potassium sodium tartrate	Sigma-Aldrich	99%
Propionic acid	Sigma-Aldrich	99.95%
Sodium citrate, dihydrate	Fisher Scientific	99%
Sodium hydroxide	Fisher Scientific	ASC grade
Sodium nitroferricyanide	Fisher Scientific	99.9%
Sodium tripoly phosphate	Fisher Scientific	ASC grade
Sulphuric acid	BDH	ASC grade
Triclosan	Sigma-Aldrich	99.1%

Table 3.2. Mixed sludge characterization

Parameter	Average value	(St. dev., number of replicates)
Total solids (TS) (% w/w)	4.06	(0.31, 35)
Volatile solids (VS) (% w/w)	3.56	(0.26, 35)
Total chemical oxygen demand (TCOD) (mg/L)	63,486	(5,566, 27)
Ammonia (mg/L)	610	(161, 27)
Alkalinity (mg/L)	1,603	(295, 27)
Volatile fatty acids (VFA) (mg/L)	3,179	(352, 22)
pH	5.70	(0.21, 41)

3.1.2 Anaerobic inoculum

The mesophilic (38ºC) anaerobic inoculum used in the digestion set-up was sampled from the full-scale anaerobic digesters (SRT of 30 days) at the Lulu Island WWTP. The thermophilic (55ºC) anaerobic inoculum was sampled from the effluent stream of a bench-scale anaerobic digester which had been operating for 18 months (SRT of 18 days) using Annacis Island WWTP's (B.C., Canada) mixed sludge as feed. Originally, the thermophilic inoculum was sampled from the full-scale thermophilic anaerobic digesters at the Annacis Island WWTP.

3.2 Equipment

Table 3.3 presents the list of the equipment used for the experiments in this research.

Table 3.3. List of equipment

Equipment	Type	Manufacturer
Balance	XS204DR	Mettler Toledo
Capillary suction apparatus	440	Fann
Centrifuge	Sorvall Lengend XT	Thermo Scientific
Dual channel pH/ion meter	Accumet excell XL25	Fisher Scientific
Gas chromatograph – A	7890A	Agilent
Gas chromatograph – B	7820A	Agilent
Gas monometer	Custom Built	-
Incubator/shaker	Innova 44R	New Brunswick Scientific
Microplate	96 well	Fisher Scientific™
Microplate reader	BioTek Synergy HT	BioTek Instruments
Muffle furnace	W-13	Paragon Industries
pH Probe	13-636-XL25	Fisher Scientific
Pipettes	Various	Fisher Scientific
Spectrophotometer	Genesys 10	Thermo Electron Corporation
Thermotron	S-1.5-3200	Thermotron

3.3 Lab-scale anaerobic digesters

In this study, two TPAD scenarios along with one conventional mesophilic single-stage anaerobic digestion were investigated and compared to assess the advantages of TPAD over the single-stage AD. The single-stage mesophilic anaerobic digestion was considered as the control reactor to simulate the full-scale anaerobic digestion at the Lulu Island WWTP (baseline conditions) side by side (Figure 3.1).

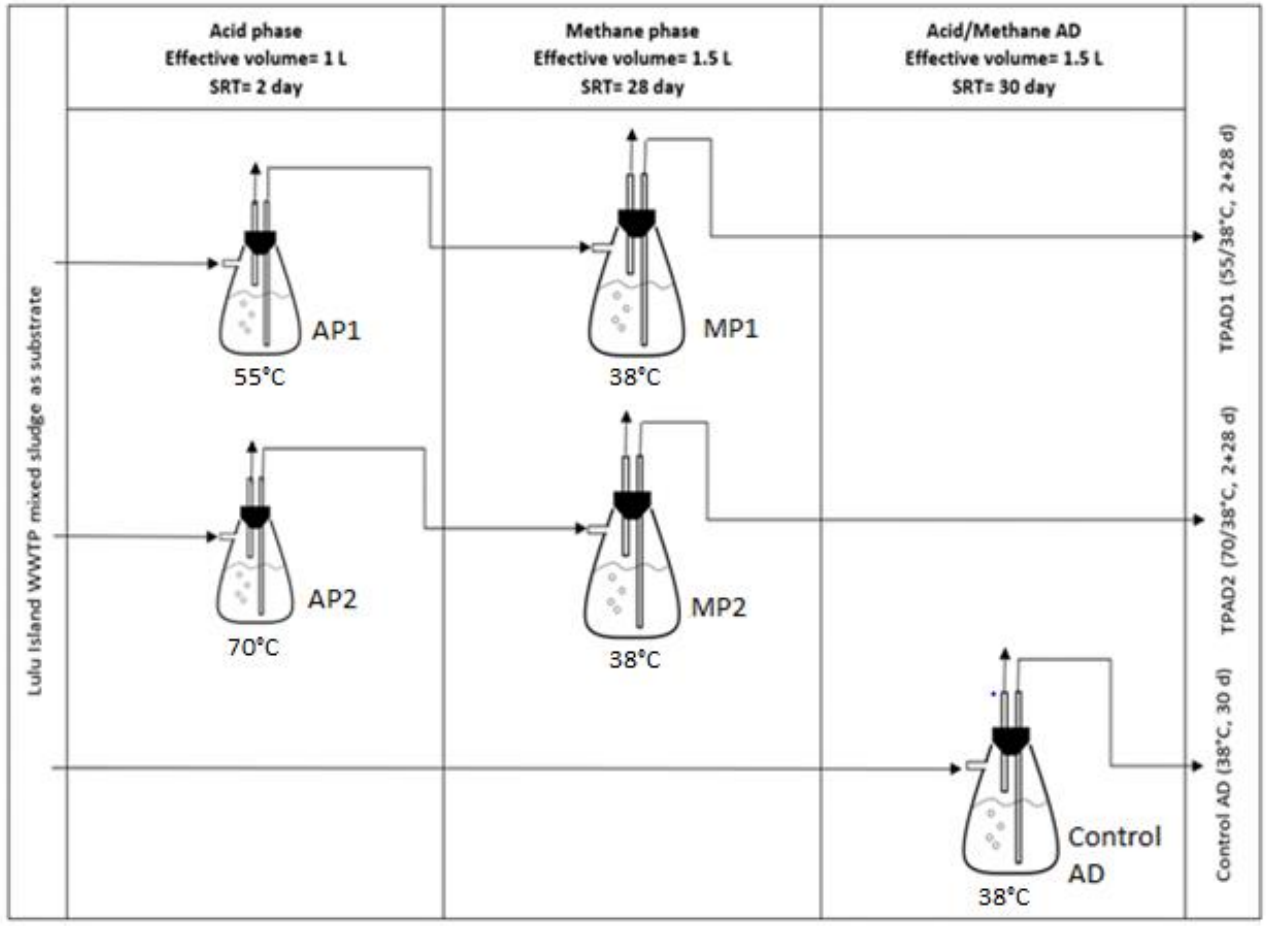


Figure 3.1. Experimental set-up of anaerobic digesters
(AP=acid phase digester, MP=methane phase digester)

The control digester was operated at a mesophilic temperature. The acid phase and methane phase (first stage and second stage) of the TPAD system were operated under thermophilic and mesophilic temperatures, respectively. The digester volume of the methane phase and the total SRT between the single-stage anaerobic digestion and TPAD systems were kept identical for comparison.

Five semi-continuous flow (fed once/day, 7 days/week) anaerobic digesters were made of 2 L side-armed Erlenmeyer flasks (Figure 3.2 and Figure 3.3). The effective volume of the single-stage (control) digester was 1.5 L and for the TPAD systems the effective volume for acid and methane

phases were 1 L and 1.5 L respectively in different SRTs. The mouth of the flasks was covered with rubber stoppers to provide anaerobic condition inside the flasks. Two glass rods were inserted into the stopper for withdrawing the sludge as well as collecting biogas. A 2 L Tedlar® bag was attached to one of the rods using a rubber hose to collect biogas. Rubber tubing was attached to the other rod for extracting the digested sludge every day. The side arm of the digester was connected to additional tubing and was used as the feed inlet of the digester. For sealing purposes, all connective tubing was shut by using clamps. Every 24 h, the volume of biogas collected in the Tedlar® bags was measured at room temperature by using a U-tube manometer. The results were then standardized considering standard temperature and pressure as 0°C and 1 atm.

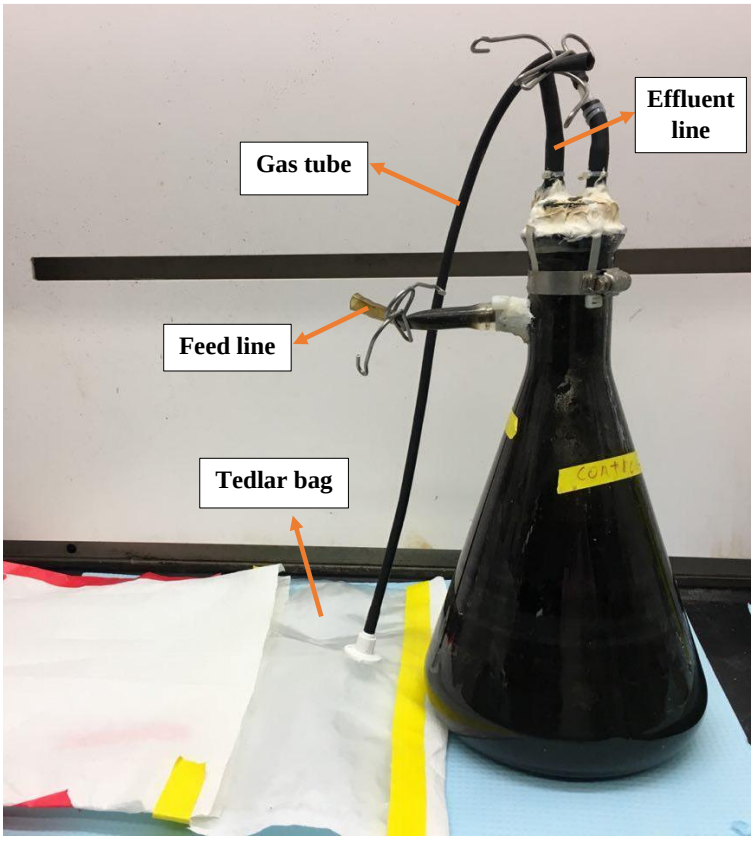


Figure 3.2. Configuration of a semi-continuous bench-scale anaerobic digester

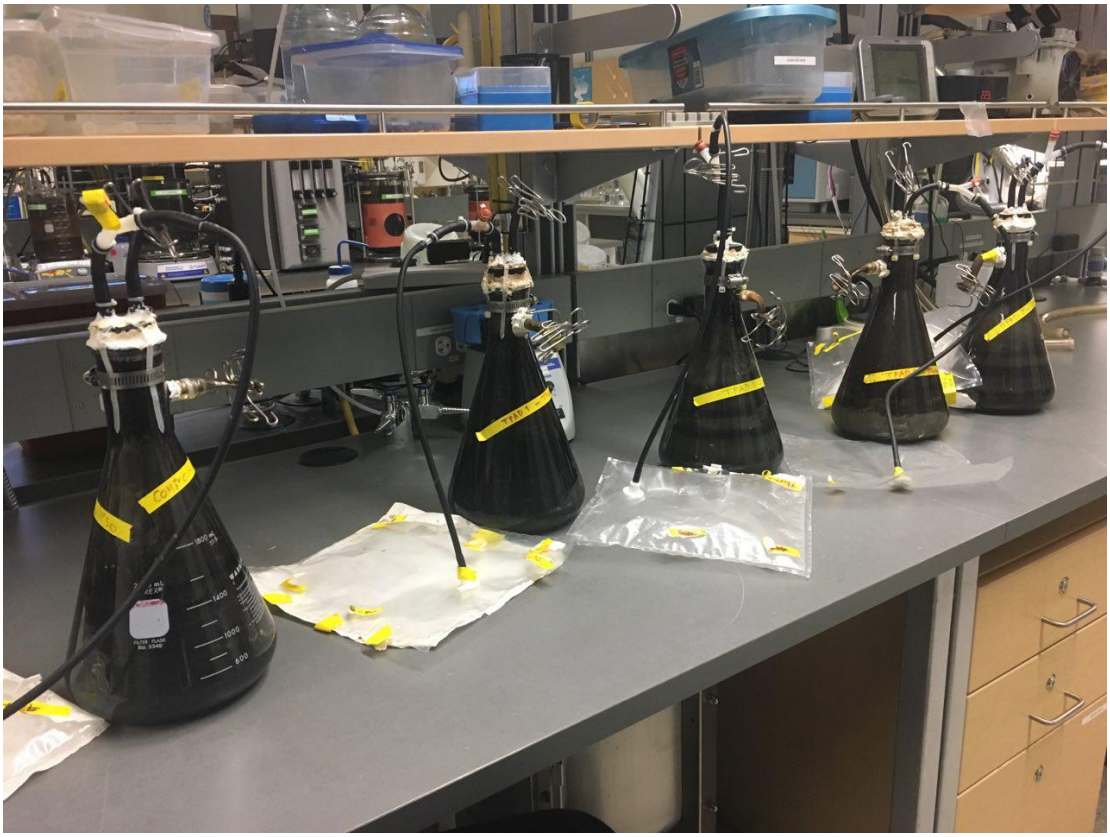


Figure 3.3. Image of the bench-scale anaerobic digesters used in this experiment

Three temperature-controlled shakers were used for incubating the digesters at the desired temperatures as well as providing uniform mixing (Figure 3.4). The shakers were rotating at 90 rpm and were adjusted to 35 ± 1ºC for mesophilic ADs, and to 55 ± 1ºC and 70 ± 1ºC for thermophilic ADs.

Figure 3.4. Image of the temperature-controlled shakers

The initial SRT of each digestion system was adjusted to 30 d, in order to simulate the full-scale digesters at the plant. The digesters were operated at this SRT for 211 days. After that, the SRT of all systems was decreased to 20 d and then to 15 d and these SRTs were maintained for 80 days each. The SRT of TPAD configurations was assigned as follows: the SRT of acid phase (first stage) was kept at 2 d for the entire experiment and the rest of the required SRT was performed in the methane phase (second stage) digesters.

Table 3.4 presents the conventional parameters that were analyzed for performance assessment of anaerobic digestion experiment and the frequency of each test. Furthermore, Figure 3.5 shows the sampling points for the experiments.

Table 3.4. Conventional parameters analyzed in the anaerobic digestion experiments

Parameter	**Frequency**	**Sample location**
Biogas volume	Daily	AD headspace
Biogas percentage composition (CH_4, CO_2, N_2, O_2)	Once a week	AD headspace
pH	Daily	Influent/Effluent of each digester
Total solids	Once a week	Influent/Effluent of each digester
Volatile solids	Once a week	Influent/Effluent of each digester
Total chemical oxygen demand	Once a week	Influent/Effluent of each digester
Soluble chemical oxygen demand	7 sampling during the SRT of 30 d	Influent/Effluent of each digester
Ammonia	Once a week	Influent/Effluent of each digester
Alkalinity	Once a week	Influent/Effluent of each digester
Total and soluble proteins	7 sampling during the SRT of 30 d	Influent/Effluent of each digester
Total and soluble humic acid	7 sampling during the SRT of 30 d	Influent/Effluent of each digester
Total and soluble sugars	7 sampling during the SRT of 30 d	Influent/Effluent of each digester
Volatile fatty acids	Every two weeks	Influent/Effluent of each digester
Fecal coliforms	Minimum 3 sampling during each SRT	Effluent of final stage digesters
Dewaterability	Minimum 3 sampling during each SRT	Effluent of final stage digesters
Heavy metals	Minimum 3 sampling during each SRT	Effluent of final stage digesters

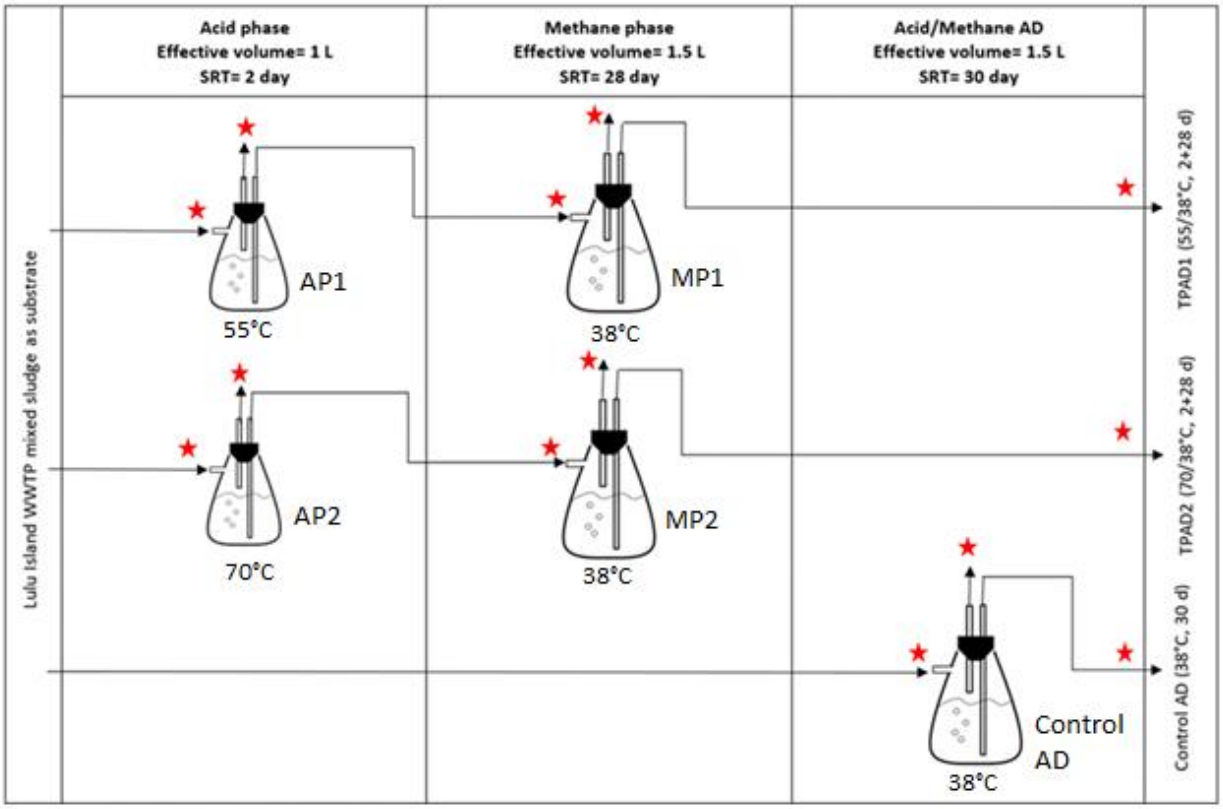


Figure 3.5. Sampling points in the anaerobic digestion experiments

(★ indicates sampling point)

3.4 Analytical methods for sample characterization

In order to compare the performance of the TPAD systems with the single-stage anaerobic digestion (control digester), several experiments were conducted on the digester influent and effluent sludge streams as well as on the headspace biogas. In this section, the analytical methods applied for sample characterization are explained in detail.

3.4.1 Total solids and volatile solids

Total solids (TS) and VS content of the sludge samples were measured according to Standard Methods 2540 B and 2540 E, respectively (APHA 2005). The equipment used for this analysis included ceramic crucibles (CoorsTek™ porcelain), an analytical balance (Thermo Fisher

Scientific, Mettler-Toledo Excellence XA-105), a convection oven, and a muffle furnace. Before the analysis, the crucibles were prepared by soaking them in a 20% sulfuric acid solution for 2 hours for cleaning. Then the crucibles were scrubbed and rinsed with water. After the crucibles were dried in air, they were heated at 550°C for half an hour and then transferred to desiccator for cooling down to room temperature. After the crucibles were ready to use, about 15 g of sludge sample was added to each of them. The samples were then heated at 98°C ± 2°C in the convection oven overnight and then the temperature was increased to 105°C ± 2°C the following morning. Samples were dried at this temperature for 2 hours. Then the crucibles were transferred to the desiccator to cool down to room temperature. Then sample masses were recorded, and TS content was calculated based on equation (1).

$$Total\ Solids\ \left(\%, \frac{g}{g}\right) = \left(\frac{Wet\ mass\ (g) - Dry\ mass\ (g)}{Wet\ mass\ (g)}\right) \times 100 \quad (1)$$

The final step was burning the dried samples at 550°C for at least half an hour. After this step, the crucibles were transferred to the desiccator to come to room temperature. Finally, the sample masses were recorded and VS content was calculated based on equation (2).

$$Volatile\ Solids\ \left(\%, \frac{g}{g}\right) = \left(\frac{Dry\ mass\ (g) - Burned\ mass\ (g)}{Wet\ mass\ (g)}\right) \times 100 \quad (2)$$

TS and VS content were reported in units of percentage by weight (% wt.).

3.4.2 Chemical oxygen demand (COD)

In order to measure total and soluble chemical oxygen demand (tCOD and sCOD), the Closed Reflux Colorimetric Method (Standard Methods 5220 D) was applied (APHA 2005). The equipment and materials used for this analysis included calibration standard solutions (prepared using potassium hydrogen phthalate (>99.95%, Sigma BioXtra)), digestion solution (containing

mercuric sulfate, potassium dichromate, and concentrated H_2SO_4 (>98%)), catalyst solution (containing silver sulphate and concentrated H_2SO_4 (>98%)), 12 mL glass vials, an analytical balance (Thermo Fisher Scientific, Mettler-Toledo Excellence XA-105), a spectrophotometer (Thermo Fisher Scientific, GENESYS™ 10S UV-Vis Spectrophotometer), an oven (Thermotron Industries, Thermotron S-1.5C), and miscellaneous glassware for volumetric measurements. The reagents used in this analysis were ASC grade or of better quality.

The COD levels in the sludge streams of the digestion systems (feed and effluent) were much higher than the maximum range of the calibration curve (100 - 700 mg COD/L); therefore, sludge samples were diluted with RO water. Afterward the diluted samples were thoroughly mixed using a benchtop homogenizer (Kinematica™ Polytron™, PT 10-35 GT) for 5 minutes at 7000 rpm. Then 2.5 mL of the homogenized sample was transferred to 12 mL glass vials and mixed with 3 mL of digestion solution and 1.5 mL of catalyst solution. Then the mixtures were digested at 150°C ± 0.1°C for 3 hours. When the digestion was completed, samples were transferred to a dark place to cool down to room temperature. Afterwards, the absorbance of the samples was measured at the wavelength of 600 nm using the spectrophotometer. The COD content of each sample was then calculated based on the calibration standard curve (Appendix A, Figure A.1).

For the sCOD analysis, the influent and effluent sludge samples were first centrifuged at 10000 rpm for 30 min. Then a 0.45 mm pore size filter was used to filter the supernatant for sCOD measurements. The filtrated supernatant samples were diluted using RO water. The digestion and incubation steps then followed the same protocol as in the total COD measurement method.

3.4.3 Dewaterability

A commonly used method for measuring the dewatering rate of sludge is the capillary suction test (CST). In order to measure the CST, the method from APHA 2710 G was applied (APHA 2005).

There was one exception from the standards methods used in this experiment in which the sample volume was reduced to 5 mL using a 5 mL syringe, since this measurement is more accurate than measuring 6.4 mL by a 10 mL syringe.

The equipment and materials used for this analysis include chromatography paper (Whatman®, Type 17) and a CST apparatus (Fann Instrument Company, Model 440). In this test, 5 mL of sludge sample was placed into a reservoir which rested on a filter paper. The liquid portion of the sample drained into the filter paper by capillary action while the solid portion was maintained on top of the filter. The filter paper was connected to a digital timer that measured the time that the liquid took to travel between two sensors. This time indicated the CST. CST is dependent on the TS content of the sample. In order to make the results comparable, CST was normalized by TS content. The CST test was performed at room temperature (23°C) and on a leveled bench surface. Each digester was tested for the CST at least three sampling times at the steady state.

3.4.4 pH

pH is among the most important operational parameters for anaerobic digestion. To keep the pH of the digester in a safe range (neutral range), pH of the effluent stream of the digesters were monitored daily using the Standard Methods 4500-Hþ B (APHA 2005). The equipment used for pH measurement is a pH electrode attached to an Accumet™ Excel XL25 pH/mV/Temperature/ISE meter (Thermo Fisher Scientific, Inc., Ottawa).

3.4.5 Alkalinity

To measure alkalinity, the influent and effluent sludge samples were first centrifuged at 10000 rpm for 30 min. Then the supernatant was used to measure the alkalinity according to Standard Methods 2320B (APHA 2005). In this method, the supernatant of the sludge sample was titrated with a

diluted solution of sulfuric acid (normality of 0.1) to reach a pH of 4.6. The acid volume consumed in the titration was then used to calculate the amount of alkalinity according to equation (3).

$$Alkalinity\ \left(\frac{mg}{L}\ as\ \mathrm{CaCO3}\right) = \frac{A \times N \times 50{,}000}{mL\ of\ sample} \quad (3)$$

A = mL of standard acid added

N = normality of standard acid

Approximately 10 mL of the supernatant of each sludge sample (the exact volume was recorded) was placed in a 40 mL beaker. The beaker was placed on a magnetic stir plate and a stir bar was placed into it. A 50 mL burette, filled with dilute sulfuric acid was used for the titration process.

3.4.6 Ammonia

For measuring the ammonia, the influent and effluent sludge samples were first centrifuged at 10000 rpm for 30 min. Then the supernatant was used to measure the dissolved ammonia concentration according to Standard Methods 4500D (APHA 2005).

For the ammonia analysis, a set of standard solutions were prepared using 1000 ppm ammonia stock solution to create a standard curve with a range of 50 to 1000 mg/L (Appendix A, Figure A.2). Afterward, the supernatant of the samples were diluted to bring the ammonia levels within the detection range of the calibration curve. Roughly 20 mL of each diluted sample was transferred into an Erlenmeyer flask and then 0.5 mL of NaOH (10 N) was added to raise the pH above 11. Raising the pH transformed the dissolved ammonia (NH_3 (aq) and $NH4^+$) into NH_3 (aq). Then the ammonia concentration was measured using an ammonia selective electrode from a dual channel pH/ion meter (Accumet excell XL25, Fisher Scientific).

3.4.7 Volatile fatty acids

In order to measure the VFAs including acetic, propionic, and butyric acid concentrations in the digesters sludge streams, the samples were first centrifuged at 10000 rpm for 30 min. Then a 0.2 μm nylon filter was used to filter the supernatant. Then 0.5 mL of filtered sample was pipetted into a 1.5 mL glass vial and 0.5 mL of iso-butyric acid as an internal standard solution was added to it (Ackman 1972). The samples were stored at -20°C to be analyzed in batches. VFA measurement was done in batches by injecting the samples to an Agilent 7890A Gas Chromatograph (GC) with a capillary column (Agilent 19091F-112, HP-FFAP polyethylene glycol TPA column length x ID: 25 m x 320 mm), a flame ionization detector, and an autosampler. In order to test the recovery of the GC, a standard solution prepared from acetic acid, propionic acid, and butyric acid with a total concentration of 2000 mg/L was injected to the GC with each batch. The carrier gas for the VFA method was helium and the flowrate was 40 mL/min.

3.4.8 Biogas volume

The volume of biogas produced from each digester was measured daily using a manometer. The biogas was collected in Tedlar® bags attached to the digesters. To measure the biogas, the Tedlar® bag was detached from the digester while the gas line was clamped (to avoid the biogas escape), and then attached to the manometer. Then the collected biogas was pumped into the manometer. The manometer was calibrated with a known volume of gas injected into it and a calibration curve was created. The volume of biogas samples was calculated based on this calibration curve which was then corrected to the standard temperature and pressure (STP). Temperature and pressure of the lab environment were recorded daily by a thermometer and taken from the local airport respectively. The final value of daily biogas volume was then corrected for the daily sampling time difference.

3.4.9 Biogas composition

For determining the biogas composition in the headspace of the digesters, an Agilent 7820A GC was used. The GC was equipped with a packed column (Agilent G3591-8003/80002) and thermal conductivity detector in which the carrier gas was helium with a 25 mL/min flowrate. The method was established by van Huyssteen (van Huyssteen 1967) and it was able to detect methane, carbon dioxide, nitrogen, and oxygen content in the biogas sample in percentage.

For each test used to determine biogas composition, 0.5 mL of the biogas sample was manually injected into the GC with a gas tight syringe. For GC for calibration, a certified standard gas mixture containing carbon dioxide (20%), nitrogen (7%), and methane (73%) was used.

3.4.10 Fecal coliforms and heavy metals

For analyzing the fecal coliform content of the sludge digestate (effluent of the digesters), sludge samples were saved in sterilized containers and the analysis was performed in less than 2 hours after sampling. In order to bring the fecal coliform levels of the sludge sample below the maximum range of the method quantification, samples were diluted using Type 1 water and sterilized glassware.

Type 1 water is exceptionally pure water which is produced through a distillation process followed by an ion exchange resin and then is filtered through 0.2 µm filters. By going through these steps, all impurities and ions in the water are extracted (ASTM 2011).

Then 0.45 mm membrane filters were used to filter the diluted sludge samples and then MFC media plates (mFC Nutrient Pad Sets, Sartorius, Germany) were used as growth media. The filter papers were placed on the media plates and the plates were then incubated at 44.5 ± 0.1°C for 30 hours in a Thermotron S-1.5C benchtop environmental chamber (Thermotron Industries, Holland,

Michigan). This led to the growth of dark blue colonies which showed the presence of fecal coliforms. Then the Standard Methods 9222D (APHA 2005) was used to count the colonies and report them as colony forming units (CFU). In order for the results to be comparable and reportable as per US Environmental Protection Agency (EPA) and Organic Matter Recycling Regulation (OMRR 2008) of BC, the TS content of each sludge sample was also measured to normalize the CFU results based on that.

Heavy metals analysis was carried out in digestate samples by a local laboratory by applying an EPA method 6020B using inductively coupled plasma mass spectrometry (USEPA, 2014). The sludge samples were first digested with HCI and HNO_3 during sample preparation for heavy metal testing by the laboratory.

3.4.11 Protein and humic acid

In order to asses the effect of TPAD on solubilization of organics, the quantification of protein and humic acid (HA) concentrations were performed on both total and soluble fraction of the substrate and digestate of each digestion system.

For the soluble protein and HA analysis, samples from the influent and effluent sludge streams (feed and digestates) were first centrifuged at 10000 rpm for 30 min via a Fisher Scientific Sorvall Legend XT centrifuge. Then a 0.45 µm membrane pore size filter was used to filter the supernatant (liquid fraction) of the centrifuged samples. The filtered samples were then diluted using RO water in order to bring the protein and HA levels within the detection limit of the method.

For the total protein and humic acid analysis, since samples from the influent and effluent sludge streams (feed and digestates) have very high levels of total protein and HA, they were diluted using RO water in order to bring the protein and HA levels within the detection range of the method.

For each test, a set of standard solutions was prepared to make calibration curves (Appendix A, Figures A.3 and A.4). For the protein test, bovine serum albumin was used to prepare the standards and for the humic acid test, humic acid standards were used. Standard solutions were prepared every time that the test was conducted to make sure that the reagents stayed fresh.

The method used for protein and humic acid analysis was the modified Lowry protein assay (Frølund et al. 1996). This method was performed as follows: after diluting the samples for both total and soluble analysis, 0.5 mL of each diluted sample was transferred to borosilicate glass culture tubes. Then 2.5 mL of a reagent solution (protein reagent for protein test and HA reagent for humic acid test) was added to the sample. The tubes were then capped, properly mixed, and left to rest in a dark place for 10 minutes. Afterward, 0.25 mL Folin-Ciocalteu phenol reagent was added to each tube, then tubes were capped again, vortexed and left to rest in a dark place for half an hour to react. After 30 minutes, 250 μL of the sample in each glass vial was transferred into a well on a Fisher Scientific™ 96 well microplate in triplicate. The absorbance of each sample was measured at 750 nm using BioTek Synergy HT microplate reader.

Equations (4) to (7) indicate how the protein and humic acid absorbances was calculated with the method proposed by (Frølund et al. 1996).

$$A_{Total} = A_{Protein} + A_{Humic\ Acid} \quad (4)$$

$$A_{Blank} = 0.2A_{Protein} + A_{Humic\ Acid} \quad (5)$$

$$A_{Protein} = 1.25(A_{Total} - A_{Blank}) \quad (6)$$

$$A_{Humic\ Acid} = A_{Blank} - 0.2A_{Protein} \quad (7)$$

3.4.12 Sugars

In order to asses the effect of TPAD on solubilization of sugars, the quantification of the sugar concentration was performed on both total and soluble fraction of the substrate (mixed sludge) and digestate of each digestion system.

For the soluble sugar analysis, samples from the influent and effluent sludge streams (feed and digestates) were first centrifuged at 10000 rpm for 30 min via a Fisher Scientific Sorvall Legend XT centrifuge. Then a 0.45 µm membrane pore size filter was used to filter the supernatant (liquid fraction) of the centrifuged samples. The filtered samples were then diluted using RO water in order to bring the sugar concentration within the detection limit of the method.

For the total sugar analysis, since samples from the influent and effluent sludge streams have very high levels of sugar, they were diluted using RO water in order to bring the sugar levels within the detection range of the method.

For each test, a set of standard solutions was prepared to make calibration curves Appendix A, Figure A.5). Glucose solution was used for preparing the standards. Standard solutions were prepared every time that the test was conducted to make sure that the reagents stayed fresh.

The method used for measuring total and soluble sugar concentration was proposed by Dubois et al. (DuBois et al. 1956). This method is a colorimetric method which works based on comparing the absorbance of each unknown sample with the absorbance of standard samples that have known concentrations of sugar. The absorbance was measured at 490 nm using an Evolution 60S UV-Vis spectrophotometer.

3.5 Statistical analysis of data

In the anaerobic digestion experiments, three main goals were pursued: increasing the solids removal, biogas production, and pathogen destruction by TPAD systems compared to the single-stage AD. For statistical analysis of data, the responses (outputs) to be analyzed were considered as follows: TS removal, VS removal, specific daily methane production, and pathogen destruction. Each response was analyzed based on two factors: Total sludge retention time (SRT) and type of digestion system. Each factor contained three experimental levels as follows: Total SRT: 15 days, 20 days, and 30 days. Digestion system: control (38°C), TPAD1 (55°C, 38°C), TPAD2 (70°C, 38°C). For investigating the impact of each experimental level on the response factors, the multi-factor analysis of variance (ANOVA) at 95% confidence level ($\alpha = 0.05$) was employed using Minitab 18 software.

Chapter 4: Results and discussion

The results and discussion are presented in two subsections: The acid phase hydrolysis performance of TPAD and overall biodegradation performance of both AD systems. The results of the TPAD's hydrolysis stage are presented in the first section, then the impact of the improved hydrolysis by the acid phase of the TPADs on the performance of AD is provided in the second section.

4.1 TPAD hydrolysis performance

The primary objective of using TPAD instead of the single-stage AD is to increase the hydrolysis rate and as a result to reduce the reactor volume. As mentioned in Chapter 1: hydrolysis step of anaerobic digestion is done in the acid phase of TPAD. In this thesis, the effect of acid phase temperatures of 55°C and 70°C at the SRT of 2 days on the hydrolysis performance was monitored. The extent of hydrolysis can be assessed based on the solubilization of biopolymers and particulate COD. The higher the ratio of soluble to total biopolymers in the effluent sludge from the acid phase, the higher the hydrolysis extent.

The presented results characterize the hydrolysis performance based on the solubilization of COD, proteins (P), humic acids (HA) and sugars (S) in all digestion systems at the overall SRT of 30 days. The following sections focus on the result and discussion of the improvement in hydrolysis through TPAD systems over the control AD. The average concentration of total and soluble biopolymers and COD of the feed sludge and the AD systems are shown in Table 4.1 and Table 4.2.

Table 4.1. Total biopolymers and COD concentration

	Feed (mixed sludge)	Control AD (38°C)	AP[c]1 (55°C)	MP[d]1 (38°C)	AP2 (70°C)	MP2 (38°C)
COD[a] (mg/L)	63708 (5003, 7)*	19382 (1599, 7)	65383 (5936, 7)	22928 (2160, 7)	65070 (6705, 7)	20313 (1697, 7)
Protein (mg/L)	1107 (123, 7)	524 (55, 7)	754 (69, 7)	609 (50, 7)	823 (75, 7)	520 (46, 7)
HA[b] (mg/L)	1951 (187, 7)	1433 (169, 7)	2047 (187, 7)	1502 (137, 7)	2132 (192, 7)	1370 (120, 7)
Sugar (mg/L)	2684 (297, 7)	288 (24, 7)	2221 (201, 7)	238 (26, 7)	2534 (236, 7)	326 (28, 7)

**Data represent arithmetic mean of measurements (standard deviation, number of samples)*
[a]Chemical oxygen demand, [b]Humic acid, [c]Acid phase of TPAD, [d]Methane phase of TPAD

Table 4.2 Soluble biopolymers and COD concentration

	Feed (mixed sludge)	Control AD (38°C)	AP[c]1 (55°C)	MP[d]1 (38°C)	AP2 (70°C)	MP2 (38°C)
COD[a] (mg/L)	7660 (848, 7)*	491 (51, 7)	14105 (1801, 7)	513 (62, 7)	13793 (1608, 7)	541 (65, 7)
Protein (mg/L)	66 (7, 7)	8 (1, 7)	172 (22, 7)	10 (1, 7)	258 (31, 7)	13 (2, 7)
HA[b] (mg/L)	240 (29, 7)	48 (5, 7)	564 (65, 7)	54 (6, 7)	747 (93, 7)	56 (5, 7)
Sugar (mg/L)	28 (3, 7)	4 (0.6, 7)	61 (8, 7)	7 (1, 7)	155 (17, 7)	5 (0.8, 7)

*Data represent arithmetic mean of measurements (standard deviation, number of samples)
[a]Chemical oxygen demand, [b]Humic acid, [c]Acid phase of TPAD, [d]Methane phase of TPAD

4.1.1 Soluble to total COD ratio

As shown in Figure 4.1, soluble to total COD ratio is 12.2% in the feed (mixed sludge). TPAD1 and TPAD2 acid phases increased the solubility of COD in the sludge significantly (i.e., 25.2% and 25.3% at 55°C and 70°C in 2 days, respectively) by breaking large molecules into smaller

molecules (< 0.45 µm). According to an ANOVA test analysis, the temperature level of acid phase (55 vs 70°C) did not make a difference statistically in SCOD/TCOD ratio at constant SRT (P-value > 0.05). In anaerobic digestion, soluble organics are consumed more easily. So, the acid phase of TPAD accelerates the digestion of solubilized organics in the subsequent methane phase. As a result, there is less soluble to total COD ratio in the methane phase effluent of TPAD1 and TPAD2 (i.e., 2.3% and 2.1%, respectively) compared to the effluent sludge from control digester (2.9%).

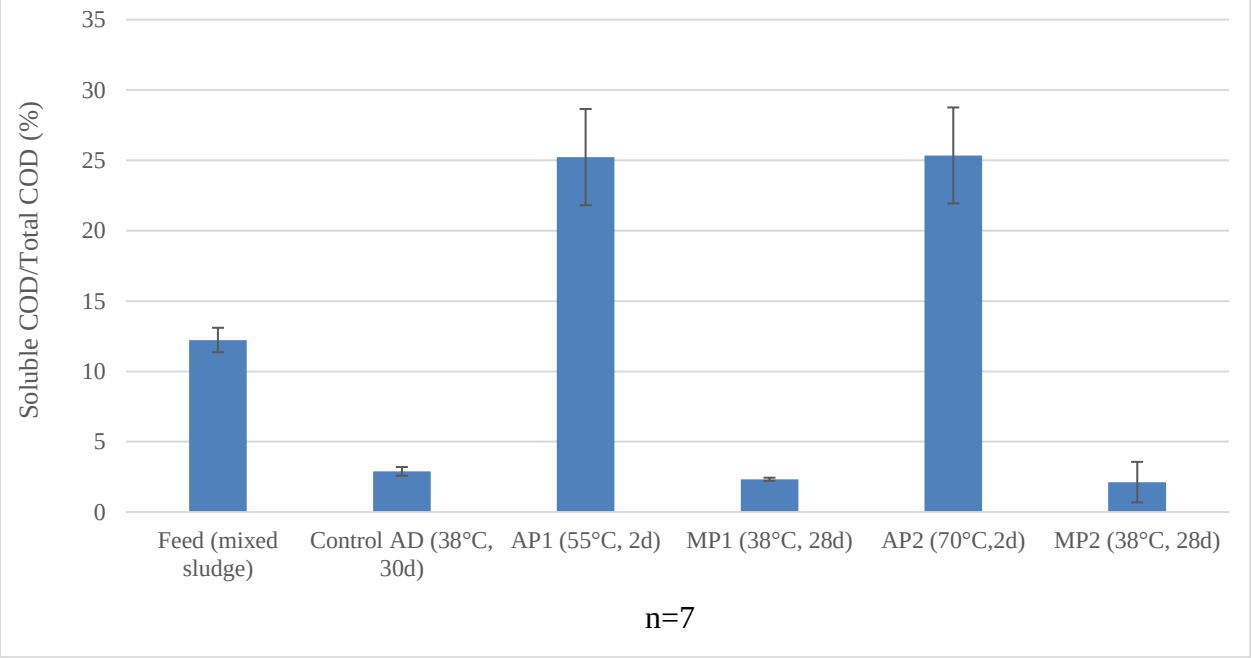


Figure 4.1 Soluble to total COD ratios in the raw and digested sludge (n=number of data points, AP=acid phase digester, MP=methane phase digester, Data represent the arithmetic mean and error bars represent the standard deviation of the measurements)

Recent studies investigating the effect of various pretreatment methods on sludge digestion, have shown that microwave (MW) (with temperatures up to 160°C), sonication (with up to 30 min sonication time for a specific energy input of 11,343 kJ/kg TS), conventional heating (with temperatures up to 160°C) and high pressure homogenization (HPH) (with homogenizing pressure of 12000 psi) pretreatments have the potential to increase the COD solubilization up to 15%, 17%, 22% and 27%, respectively (Hosseini Koupaie & Eskicioglu, 2016; Islam, 2015; Wahidunnabi,

2015). Acid-phase digester of the TPAD systems in this study, achieved up to 25.3% COD solubilization which shows that acid phase digestion is equally or more effective than MW, sonication and conventional heating pretreatments in terms of sludge hydrolysis.

4.1.2 Soluble to total protein ratio

TPAD acid phase increased the solubility of proteins in the sludge very significantly as shown in Figure 4.2. The average ratio of soluble to total protein increased from 5.9% in the feed (mixed sludge) to 21.8% in TPAD1 and 31.4% in TPAD2 acid phases. As seen in Figure 4.2, increasing thermophilic temperature increased the concentration of soluble protein. The soluble proteins were then consumed in the digestion process and as a result, the soluble to total protein ratio in the effluent of the control, TPAD1 and TPAD2 methane phase digesters was very low (1.5%, 1.6%, and 2.5%, respectively).

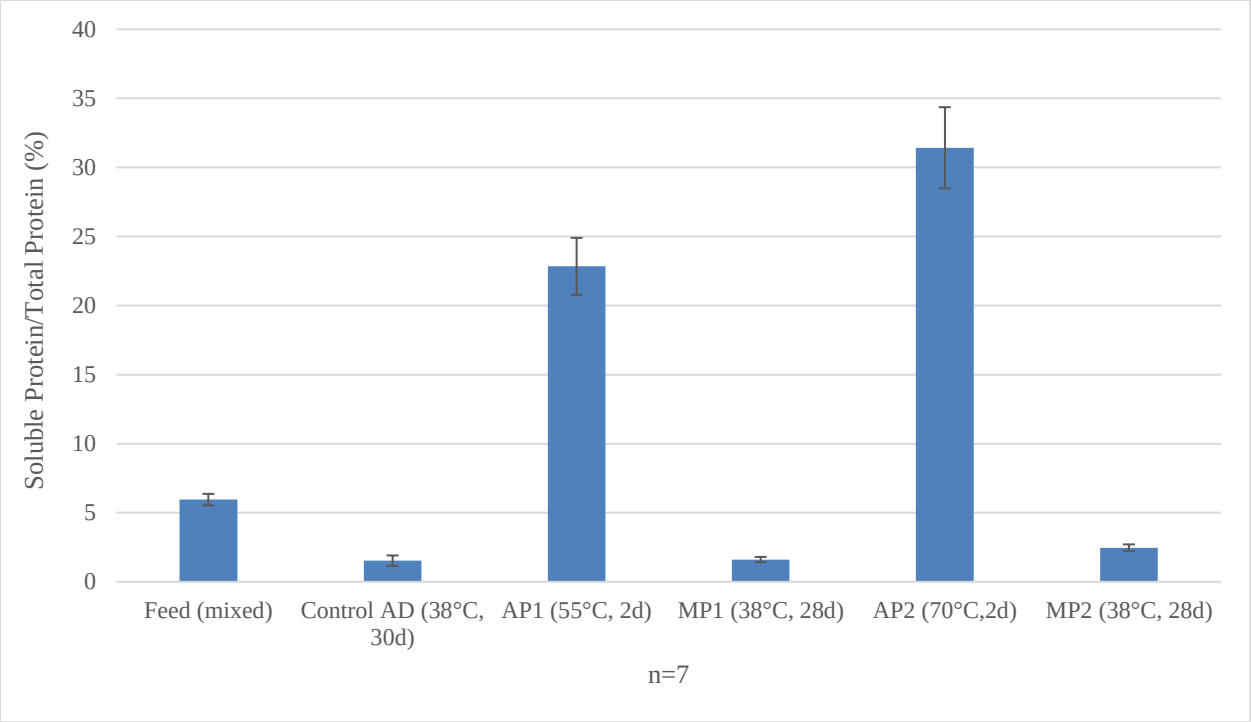


Figure 4.2 Soluble to total protein ratio in the raw and digested sludge (n=number of data points, AP=acid phase digester, MP=methane phase digester, Data represent arithmetic mean and error bars represent standard deviation of the measurements)

Recent studies which investigated the effect of various pretreatment methods on sludge digestion, have shown that HPH (with homogenizing pressure of 12000 psi), MW (with temperatures up to 160°C), conventional heating (with temperatures up to 160°C) and sonication (with up to 30 min sonication time for a specific energy input of 11,343 kJ/kg TS) pretreatments have the potential to increase the protein solubilization up to 18%, 26%, 31% and 47%, respectively (Islam 2015; Hosseini Koupaie & Eskicioglu 2016; Wahidunnabi 2015). Acid-phase digester of the TPAD systems in this study, could achieve up to 31.4% protein solubilization which shows that it is more effective than HPH and MW pretreatments in terms of sludge hydrolysis.

4.1.3 Soluble to total humic acid (HA) ratio

Similar to the solubilization results for proteins shown in section 4.1.2, TPAD acid phase increased the solubility of humic acid content of the sludge significantly as well. As shown in Figure 4.3, the

average ratio of soluble to total humic acid increased from 12.3% in the feed (mixed sludge) to 27.6% in TPAD1 acid phase and 35% in TPAD2 acid phase. The soluble fraction of the humic acids were then consumed in the digestion process which leads to low levels of soluble to total humic acid ratio in the effluent of the control, TPAD1 and TPAD2 methane phase digesters (3.3%, 3.6%, and 4.1%, respectively).

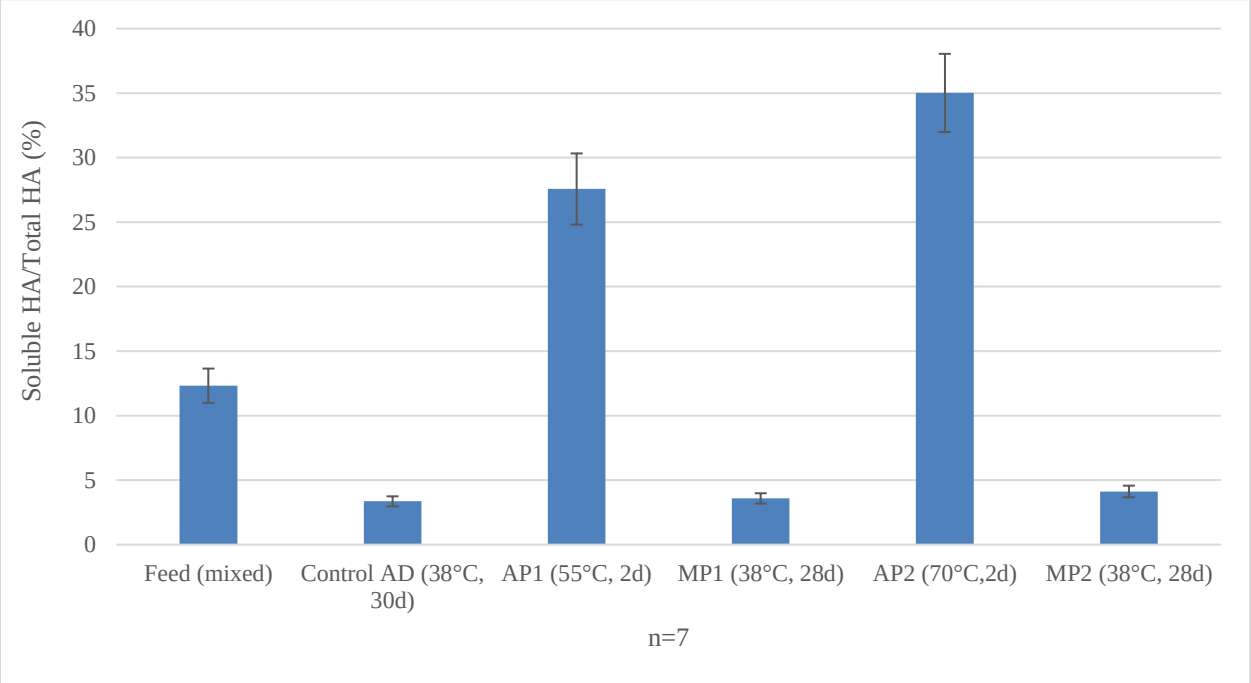


Figure 4.3. Soluble to total HA ratio in the raw and digested sludge (n=number of data points, AP=acid phase digester, MP=methane phase digester, Data represent arithmetic mean and error bars represent standard deviation of the measurements)

Recent studies have shown that MW (with temperatures up to 160°C), HPH (with homogenizing pressure of 12000 psi) and conventional heating (with temperatures up to 160°C) pretreatments have the potential to increase the humic acid solubilization up to 25%, 31% and 34%, respectively (Hosseini Koupaie & Eskicioglu, 2016; Islam, 2015; Wahidunnabi, 2015). Acid-phase digester of the TPAD systems in this study, could achieve up to 35% humic acid solubilization which shows that it is more effective than HPH and MW pretreatments in terms of sludge hydrolysis.

4.1.4 Soluble to total sugar ratio

The effect of TPAD on solubilizing sugar was not as significant as other biopolymers, although there was a minimal increase in the soluble to total sugar ratio in TPAD acid phases compared to the feed (mixed sludge) as shown in Figure 4.4. While this ratio was 1.05% in the feed, it increased to 2.8% and 6.2% in TPAD1 and TPAD2 acid phases, respectively. However, soluble sugars were not consumed considerably in the digestion process in the control and TPAD1 methane phase.

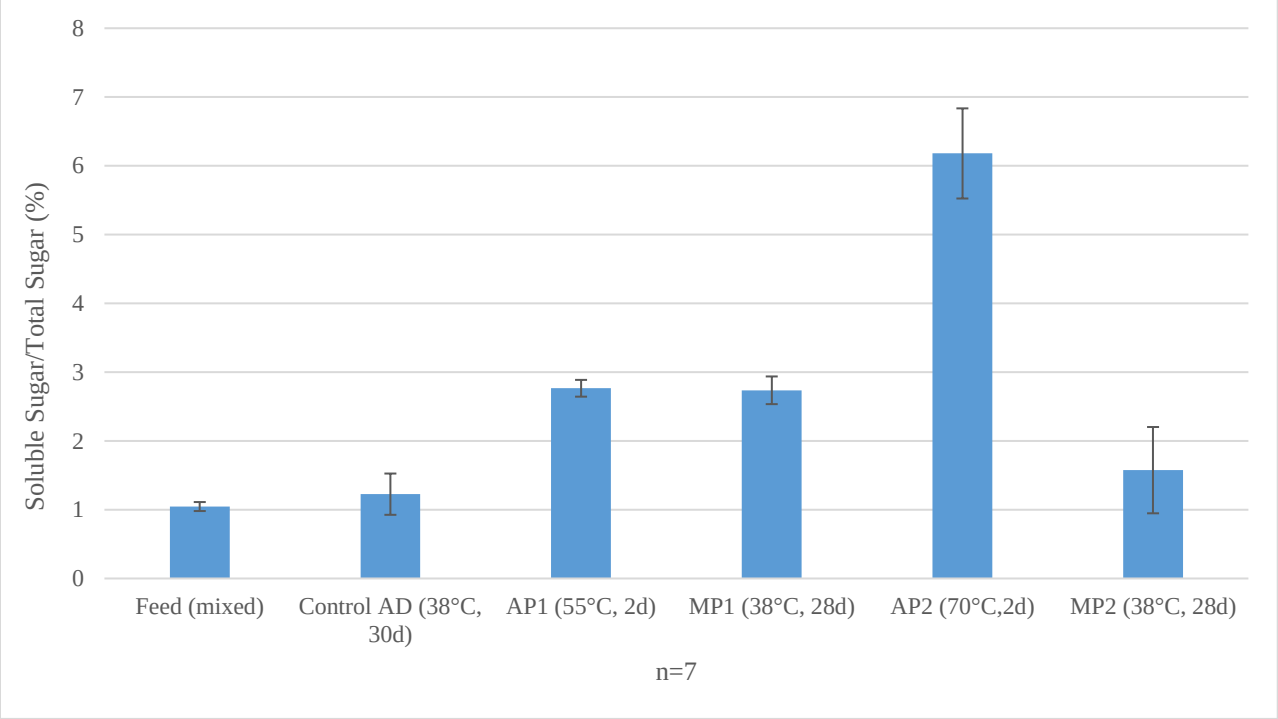


Figure 4.4. Soluble to total sugar ratio in the raw and digested sludge (n=number of data point, AP=acid phase digester, MP=methane phase digester, Data represent arithmetic mean and error bars represent standard deviation of the measurements)

The results of this study on sugar solubilization during the hydrolysis, is similar to the findings of the recent studies reported on implementing pretreatment methods (i.e. MW, HPH, conventional heating, sonication) for improving the sludge hydrolysis. According to these studies, sugar shows the least solubilization ratio among all biopolymers in the hydrolysis stage (Islam 2015; Hosseini Koupaie & Eskicioglu 2016; Wahidunnabi 2015).

4.1.5 Soluble to total organics ratio

Figure 4.5 displays the soluble to total ratio of several types of organics (i.e. COD, protein, sugar, and humic acid) for all digestion systems side-by-side. As the trend shows, the soluble to total ratio for all organics increased in TPAD acid phase with 2-d SRT by increasing the digester temperature from 55 to 70^{o}C. Due to the limitation with running number of digester scenarios side-by-side, this study could not include other acid phase vessels operating at middle temperatures (i.e. 60 and 65^{o}C, Figure 4.6) to verify this linear trend. Future studies in Bioreactor Technology group will assess other acid-phase temperature/SRT configurations to optimize sludge hydrolysis.

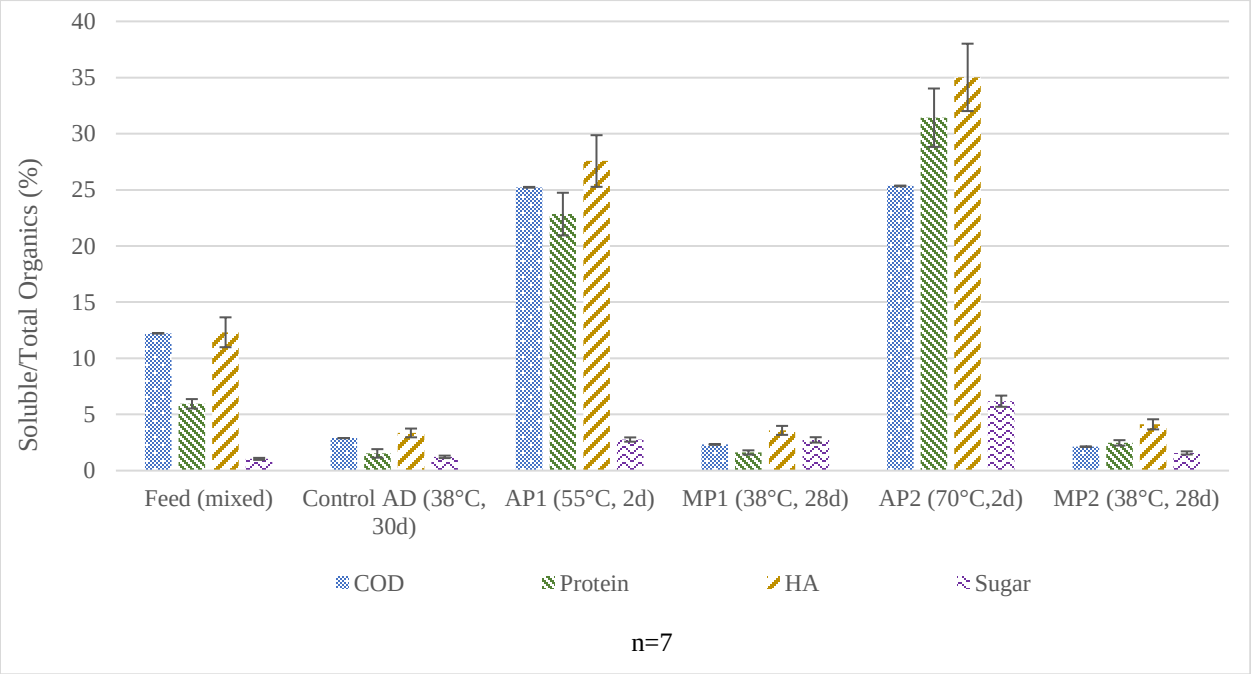


Figure 4.5. Soluble to total organics ratio in the raw and treated sludge (n=number of data points, AP=acid phase digester, Data represent arithmetic mean and error bars represent standard deviation of the measurements)

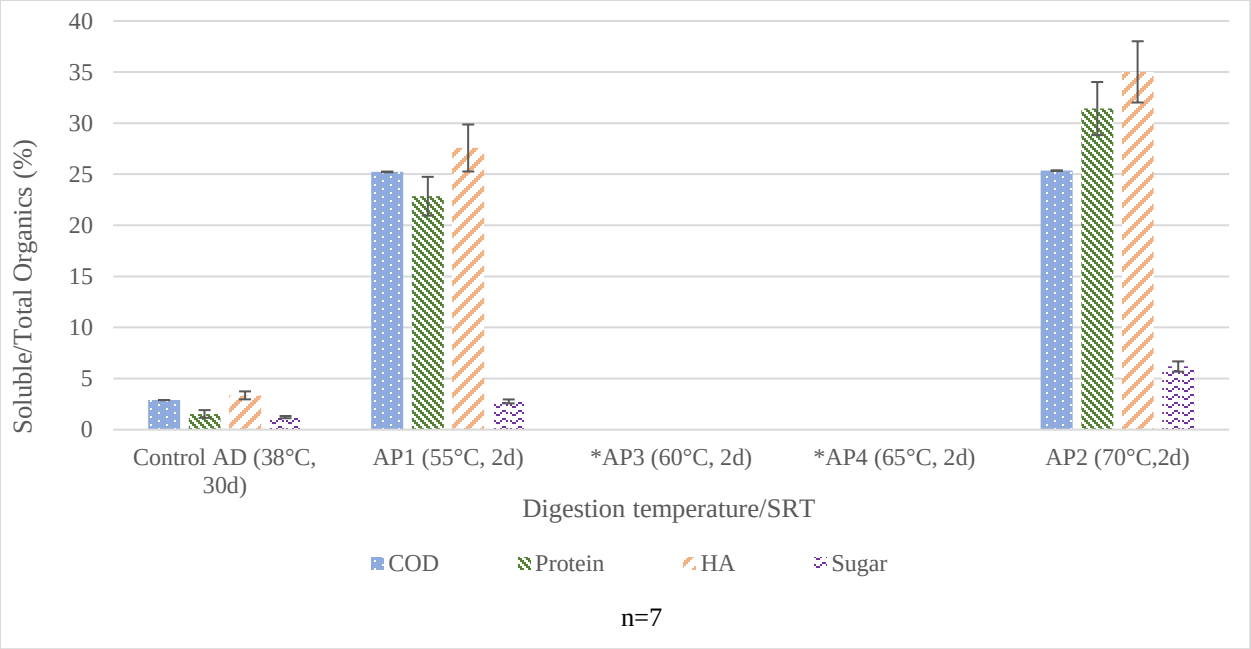


*Figure 4.6. Relation between TPAD thermophilic temperature and solubilization ratio (n=number of data points, AP=acid phase digester, Data represent arithmetic mean and error bars represent standard deviation of the measurements, *Scenarios to be investigated in future work)*

4.2 AD performance

Section 4.2 provides the results from performance evaluation of the control and TPAD systems investigated in this research. Figure 4.7 and Figure 4.8 present daily biogas (mL) and specific daily biogas (mL/g VS fed) yields from the digesters at all three SRTs, respectively.

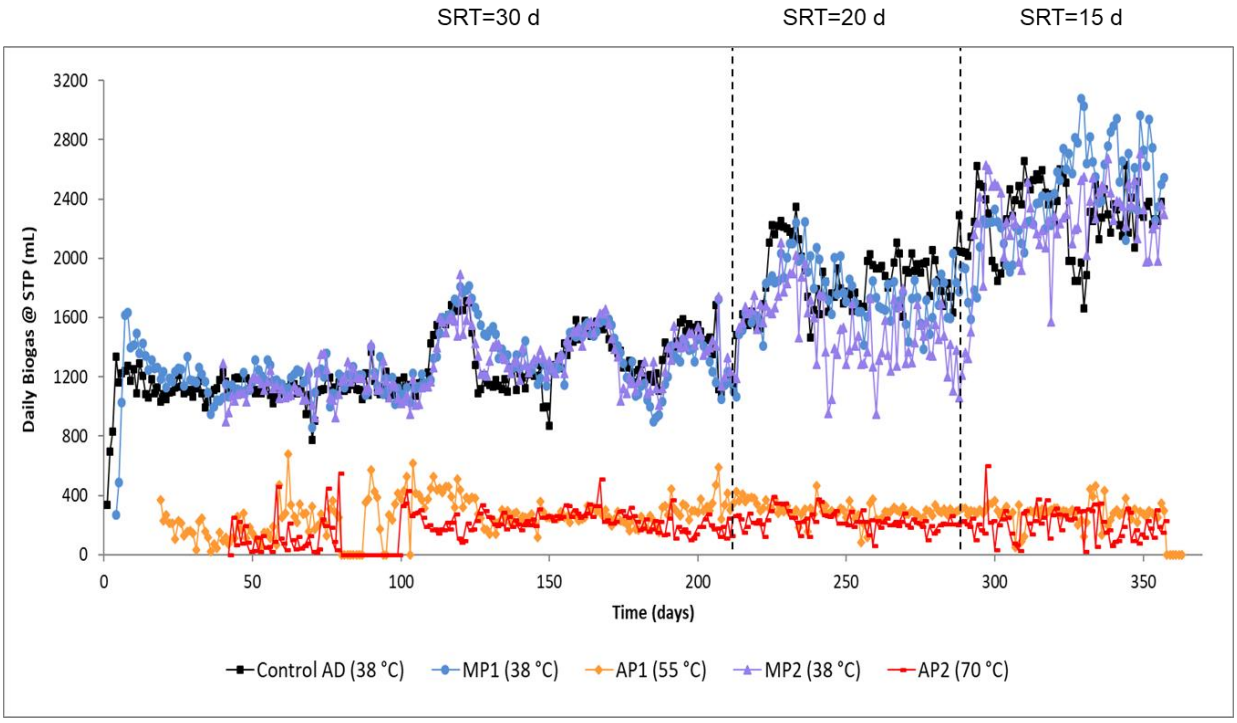


Figure 4.7 Daily biogas yield from all digestion systems

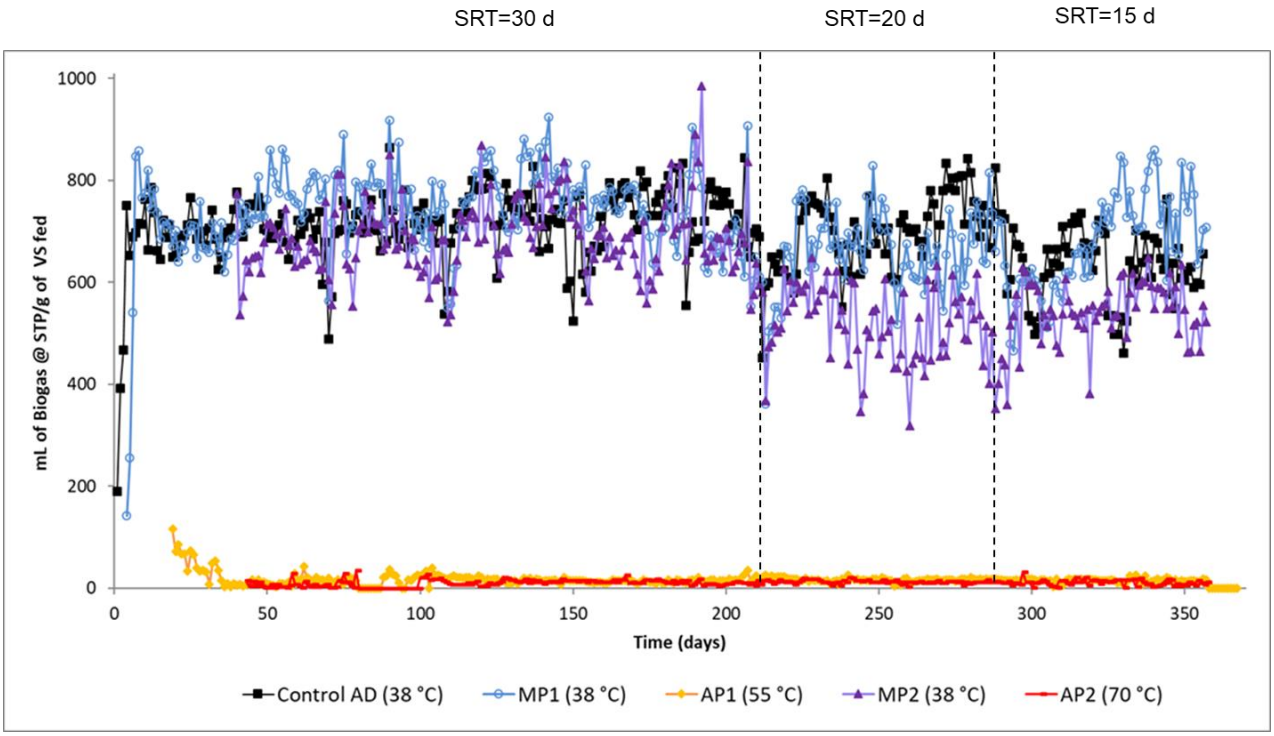


Figure 4.8 Specific daily biogas yield from all digestion systems

As it can be seen from the figures, upon start-up, the digesters reached to steady state approximately within 15 days at the SRT of 30 days and they were stable for a digester operation of 360 days without any process upset. The majority of the biogas was generated in the methane phase of the TPAD systems while the acid-phases (at SRT of 2 d; OLR of 26.1 g-VS/L/d) produced negligible amount of specific biogas volumes per VS fed (Figure 4.8). As expected, at the longest SRT of 30 days (OLR of 1.74 g-VS/L/d) the digesters were the most stable, and higher variations in daily biogas productions were observed in the methane phase of TPAD systems as well as in control as SRT was gradually shortened to 20 and 15 days (OLRs of 2.61 g-VS/L/d, and 3.48 g-VS/L/d, respectively).

By looking at Figure 4.7, the overall trend shows that by decreasing the SRT from 30 to 15 days, the amount of daily biogas production increased, which is due to increase in organic loading rate (from 1.74 g-VS/L/d at SRT of 30 days to 3.48 g-VS/L/d at SRT of 15 days). Higher organic loading rate provides more organic matter to be consumed by microorganism and leads to more biogas production. However, as shown Figure 4.8, by decreasing the SRT from 30 to 15 days, the amount of specific daily biogas decreased, which is due to more organic loading rate at smaller SRTs. According to the biogas graphs, the gap of biogas production between TPAD1 and the other two digestion systems increased in smaller SRTs. Therefore, the maximum improvement in biogas production was observed at the smallest SRT (SRT of 15 days) in TPAD1.

According to the hydrolysis performance provided in section 4.1, the solubilization levels of organics in AP2 (70°C/2-d SRT) is higher than AP1 (55°C/2-d SRT). Therefore, more biodegradability and as a result, more biogas production is expected in TPAD2 system. However, as it is shown in Figure 4.7 and Figure 4.8 the amount of biogas produced from MP1 is higher than MP2. This could be due to the higher temperature in the acid phase of TPAD1, since some

microorganisms are not able to survive at temperature of 70℃ and this leads to less diversity of microorganisms in TPAD2 system. As a result, the activity of microorganisms is less in the methane phase of TPAD2 and less biogas is produced. Table 4.3 to Table 4.5 below summarizes the influent and effluent characterization data for digesters at each SRT during the steady state. These tables are used as references during the discussion.

Table 4.3. Influent and effluent characterization of AD systems during the SRT of 30 days

			TPAD 1		**TPAD 2**	
Parameters	Feed	Control (38°C/30-d)	AP[e]1 (55°C/2-d)	MP[f]1 (38°C/28-d)	AP2 (70°C/2-d)	MP2 (38°C/28-d)
TS[a]%	3.93 (0.58, 14)*	1.68 (0.08, 14)	3.71 (0.68, 14)	1.89 (0.47, 14)	4.08 (0.69, 14)	1.70 (0.35, 14)
VS[b]%	3.48 (0.49, 14)	1.24 (0.06, 14)	3.24 (0.59, 14)	1.34 (0.30, 14)	3.56 (0.57 14)	1.24 (0.25, 14)
TCOD[c] (mg/L)	62505 (9950, 11)	20160 (1331, 11)	60291 (11572, 11)	22634 (5784, 11)	63158 (8972, 11)	20677 (5648, 11)
Ammonia (mg/L)	509 (170, 10)	1489 (201, 10)	1038 (415, 10)	1481 (265, 10)	548 (189, 10)	1393 (282, 10)
Alkalinity (mg/L)	1651 (444, 10)	5815 (450, 10)	2794 (837, 10)	5874 (459, 10)	1579 (369, 10)	5679 (516, 10)
VFA[d] (mg/L)	3132 (130, 7)	15 (2, 7)	4855 (206, 7)	11 (1, 7)	2890 (139, 7)	14 (1, 7)
pH	5.7 (0.1, 28)	7.7 (0.2, 101)	5.9 (0.5, 97)	7.6 (0.2, 82)	5.7 (0.2, 62)	7.6 (0.2, 58)

*****Data represent arithmetic mean of measurements (standard deviation, number of samples)
[a]Total solids, [b]Volatile solids, [c]Total chemical oxygen demand, [d]Volatile fatty acids, [e]Acid phase, [f]Methane phase

Table 4.4. Influent and effluent characterization of AD systems during the SRT of 20 days

			TPAD 1		TPAD 2	
Parameters	Feed	Control (38°C/20-d)	AP[e]1 (55°C/2-d)	MP[f]1 (38°C/18-d)	AP2 (70°C/2-d)	MP2 (38°C/18-d)
TS[a]%	4.08 (0.25, 12)*	1.75 (0.08, 12)	3.72 (0.30, 12)	1.74 (0.09, 12)	4.06 (0.26, 12)	1.74 (0.09, 12)
VS[b]%	3.59 (0.19, 12)	1.23 (0.04, 12)	3.21 (0.23, 12)	1.22 (0.05, 12)	3.54 (0.18, 12)	1.22 (0.04, 12)
TCOD[c] (mg/L)	63880 (3090, 7)	23034 (1287, 7)	60443 (3649, 7)	21975 (1115, 7)	61450 (3227, 7)	22076 (1410, 7)
Ammonia (mg/L)	486 (180, 9)	1390 (137, 9)	1065 (167, 9)	1420 (171, 9)	559 (152, 9)	1349 (152, 9)
Alkalinity (mg/L)	1368 (271, 9)	4881 (230, 9)	2633 (241, 9)	4877 (351, 9)	1450 (229, 9)	4670 (348, 9)
VFA[d] (ppm)	2743 (501, 8)	20 (3, 8)	4696 (340, 8)	13 (2, 8)	2694 (470, 8)	17 (4, 8)
pH	5.6 (0.1, 15)	7.6 (0.2, 22)	5.8 (0.5, 22)	7.5 (0.1, 22)	5.6 (0.2, 22)	7.5 (0.1,22)

*****Data represent arithmetic mean of measurements (standard deviation, number of samples)
[a]Total solids, [b]Volatile solids, [c]Total chemical oxygen demand, [d]Volatile fatty acids, [e]Acid phase, [f]Methane phase

Table 4.5. Influent and effluent characterization of AD systems during the SRT of 15 days

			TPAD 1		TPAD 2	
Parameters	Feed	Control (38°C/15-d)	AP[e]1 (55°C/2-d)	MP[f]1 (38°C/13-d)	AP2 (70°C/2-d)	MP2 (38°C/13-d)
TS[a]%	4.16 (0.11, 9)*	1.97 (0.11, 9)	3.62 (0.08, 9)	1.92 (0.14, 9)	4.16 (0.12, 9)	1.88 (0.15, 9)
VS[b]%	3.61 (0.1, 9)	1.43 (0.1, 9)	3.09 (0.08, 9)	1.40 (0.11, 9)	3.60 (0.1, 9)	1.36 (0.11, 9)
TCOD[c] (mg/L)	64072 (3658, 9)	23174 (498, 9)	62913 (3100, 9)	22891 (1754, 9)	65778 (2238, 9)	22490 (2269, 9)
Ammonia (mg/L)	834 (133, 8)	1516 (246, 8)	1629 (300, 8)	1517 (297, 8)	875 (86, 8)	1478 (273, 8)
Alkalinity (mg/L)	1791 (169, 8)	5439 (611, 8)	3012 (449, 8)	5455 (726, 8)	1853 (156, 8)	5197 (785, 8)
VFA[d] (ppm)	3662 (424, 7)	8 (1, 7)	5718 (397, 7)	8 (2, 7)	4031 (456, 7)	19 (2, 7)
pH	5.6 (0.1, 22)	7.7 (0.2, 24)	5.8 (0.5, 24)	7.5 (0.1, 24)	5.7 (0.2, 24)	7.6 (0.1,24)

*****Data represent arithmetic mean of measurements (standard deviation, number of samples)
[a]Total solids, [b]Volatile solids, [c]Total chemical oxygen demand, [d]Volatile fatty acids, [e]Acid phase, [f]Methane phase

Considering other studies on TPAD systems digesting municipal sludge, the data collected for pH, alkalinity, ammonia and VFAs in this research are within the expected ranges for these parameters (Riau et al. 2012; Coelho et al. 2011; Bolzonella et al. 2012; Wahidunnabi 2015).

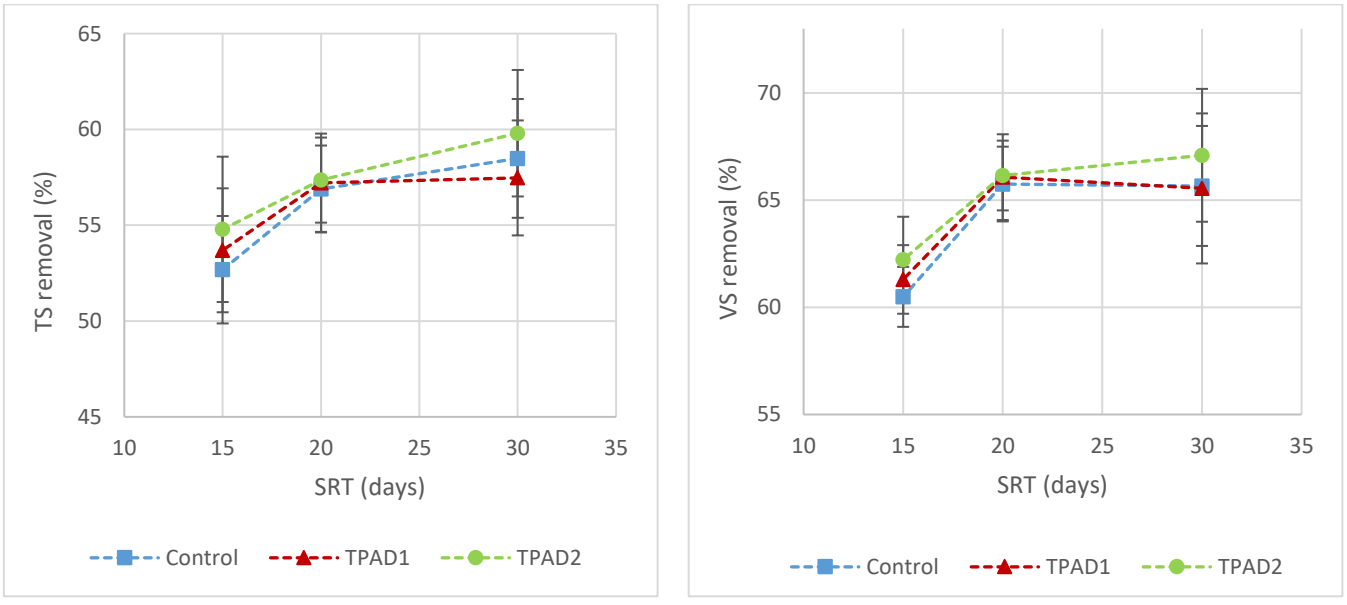
According to the hydrolysis performance provided in section 4.1, the solubilization levels of organics in AP2 (70℃/2-d SRT) is higher than AP1 (55℃/2-d SRT). Since hydrolysis of biopolymers and COD leads to production of ammonia and VFA, higher levels of these parameters are expected in AP2. However, as it is reported in Table 4.3 to Table 4.5, the amount of VFA and ammonia in AP2 are lower than AP1. This could be due to losing some ammonia and VFA from

liquid phase to gas phase because of evaporation at high temperature. Also, temperature of 70℃ may cause inhibition for ammonification and lead to less ammonia production in AP2.

4.2.1 Solids removal

Figure 4.9 (a) and (b) shows the average solids removal efficiencies of the digestion systems at three different SRTs. By reducing the SRT from 30 to 15 days, all digestion systems showed lower removal efficiencies in both total and volatile solids. This is due to less available time in shorter SRTs for the microorganisms to consume organic matter (volatile solids) in the sludge which leads to accumulation of organic matter in the digesters.

At all three SRTs, TPAD2 showed the highest removal efficiency and achieved the highest improvement over control at the SRT of 15 days (4% improvement in TS removal and 2.9% improvement in VS removal).



(a) (b)

Figure 4.9. Average removal efficiencies, (a) Total Solids (TS), (b) Volatile Solids (VS) (error bars indicate standard deviation of data)

According to an ANOVA test analysis (Table 4.6 and Table 4.7), the effect of different SRTs on total and volatile solids removal of anaerobic digestion was statistically significant (P-value < 0.05), however configuration of digestion systems (i.e. single-stage mesophilic or TPAD), and the interaction between SRT and digester's configuration did not have a significant effect on solids removal (P-value > 0.05). As a result, using TPAD incorporating an acid phase (2-d SRT) at temperature of 55°C or 70°C did not improve the overall solids removal in anaerobic digestion significantly.

The results of this study on solids removal from the TPAD systems, support the findings of the recent studies reported on using TPAD for digesting municipal sludge. According to these studies, the typical range of TS and VS removal in TPAD systems are 40-60% and 50-70%, respectively (Wahidunnabi 2015; Fernández-Rodríguez et al. 2016; Lv et al. 2016; Akgul et al. 2017).

Table 4.6 Analysis of variance for total solids removal

Factor	Type	Levels	Values
Digester	Fixed	3	Control, TPAD1, TPAD2
SRT (d)	Fixed	3	15, 20, 30

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SRT	2	464.963	232.481	10.41	0.001
Digester	2	12.519	6.259	0.28	0.759
Digester*SRT	4	9.037	2.259	0.1	0.981
Error	18	402	22.333		
Total	26				

DF: degrees of freedom; SS: sum of square; MS: adjusted mean of square; F: observed F value, P: probability value

Table 4.7. Analysis of variance for volatile solids removal

Factor	Type	Levels	Values
Digester	Fixed	3	Control, TPAD1, TPAD2
SRT	Fixed	3	15, 20, 30

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SRT	2	591.63	295.815	19.39	0
Digester	2	5.63	2.815	0.18	0.833
Digester*SRT	4	6.37	1.593	0.1	0.98
Error	18	274.667	15.259		
Total	26				

DF: degrees of freedom; SS: sum of square; MS: adjusted mean of square;
F: observed F value, P: probability value

4.2.2 Methane production

During the SRT of 30 d, the TPAD1 (55^{o}C, 38^{o}C) showed higher specific methane production (ml methane per gram of VS fed) compared to control (5.6% improvement), however, the TPAD2 (70^{o}C, 38^{o}C) showed no improvement over the control (Figure 4.10). Similarly, during the SRT of 20 days, TPAD1 showed higher improvement over the control in terms of methane production (9.5%), however, TPAD2 not only did not show any improvement, but also had a negative impact on methane production and compared to the control by producing 11% less specific methane volume. During the SRT of 15 d, TPAD1 showed its maximum improvement (14.7% improvement), while TPAD2 did not have any improvement over the control.

Overall, among all three digestion systems (control, TPAD1, TPAD2), TPAD1 showed the best performance in terms of specific methane production in all three SRT periods. There is also a

clear relationship between the SRT of the system and the methane production improvement, so that by reducing the SRT from 30 d to 15 d, the methane production improvement in TPAD1 increased from 5.6% to 14.7% (Figure 4.10).

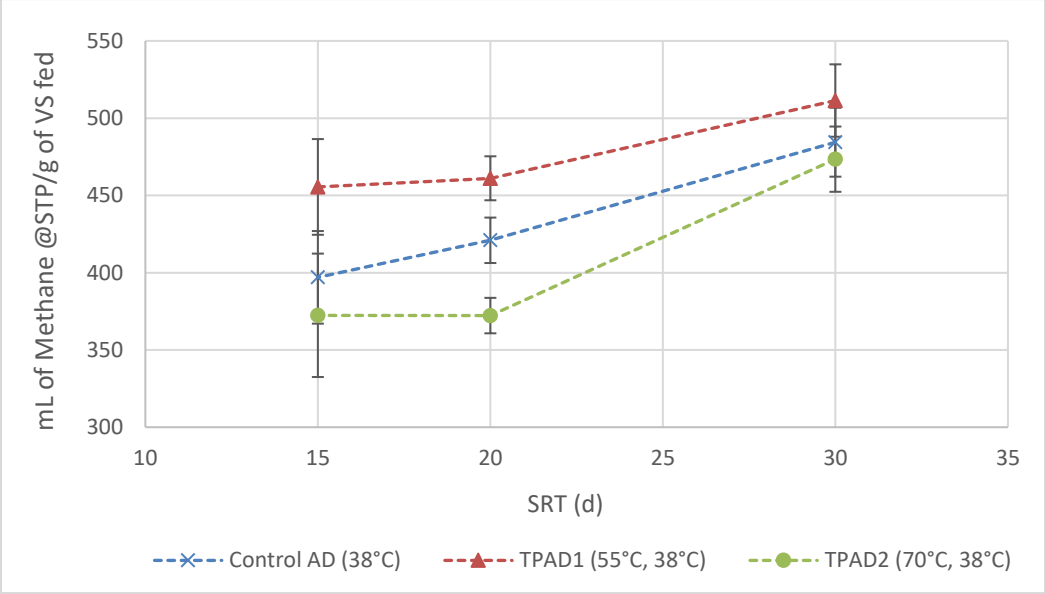


Figure 4.10. Average specific methane production (error bars indicate standard deviation of data)

According to an ANOVA test analysis (Table 4.8), the effect of SRT, digester configuration, as well as their interaction on methane production of anaerobic digestion was statistically significant (P-value < 0.05).

Table 4.8. Analysis of variance for methane production

Factor	Type	Levels	Values
Digester	Fixed	3	Control, TPAD1, TPAD2
SRT	Fixed	3	15, 20, 30

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Digester	2	39285	19642.7	76.58	0.000
SRT	2	44151	22075.4	86.07	0.000
Digester*SRT	4	3328	832.0	3.24	0.036
Error	18	4617	256.5		
Total	26	91381			

DF: degrees of freedom; SS: sum of square; MS: adjusted mean of square; F: observed F value, P: probability value

By decreasing the SRT (e.g. from 30 d to 15 d and corresponding OLR increased from 1.74 to 3.48 g-VS/L/d), there is less available time for the microorganisms to consume the organic matter in the substrate (mixed sludge) to produce methane and this may lead to an incomplete anaerobic digestion in short SRTs (e.g. 15 d) in single-stage digesters where hydrolysis/acid formation/methane formation are occuring simultaneously. From the literature, it is known that the first step of the anaerobic digestion process is hydrolysis, and this is the rate-limiting step of this process. Using TPAD instead of a single-stage AD increases the hydrolysis rate through the acid phase at elevated temperatures and leads to higher methane production. For this reason, at shorter SRTs, the effect of TPAD on improving the methane production is more discernable (i.e., by decreasing the SRT from 30 d to 15 d, the improvement in methane production of TPAD1 over control has increased from 5.6% to 14.7%).

The possible reason for not having any improvement in TPAD2 could be because of the very high temperature (70°C) of the acid phase. Based on the literature there is an optimum range of temperature for bacterial activity in the AD. If the temperature goes over the optimum limit, some of the methanogens may be killed in the first stage and this leads to less methanogenesis in the second stage of the TPAD2. Also high temperature in the acid phase may cause the generation of potentially inhibitory substances in the AD process (Sahlström 2003; Ge et al. 2011a; Ge et al. 2011b; Pervin et al. 2013).

4.2.3 Effluent dewaterability

Dewaterability is one of the important parameters for sludge disposal, because it is directly related to the cost of biosolids storage and transport. In the sludge, there is free water trapped in its floc structure or bounded water with extra cellular polymeric substances (EPS) through hydrogen bonds (Zhou et al. 2002). The rate at which sludge releases its water is known as Capillary suction time (CST). The lower the CST, the faster the dewaterability of the digestate (Yu et al. 2008). Figure 4.11 presents the results from the CST test on the sludge digestates from three digestion systems in this experiment. The results are normalized by TS content (% wt.) of the digester effluents. The CST test was performed three times for each digestion system and in triplicate, at steady state. As shown in Figure 4.11, TPAD1 (55°C, 38°C) showed the fastest dewaterability rate among all digestion systems. TPAD acid phase releases the bound water and leads to enhancing the dewaterability.

According to an ANOVA test analysis, the effect of SRT, digester configuration, as well as their interaction on dewaterability of the digestate was statistically significant (P-value < 0.05).

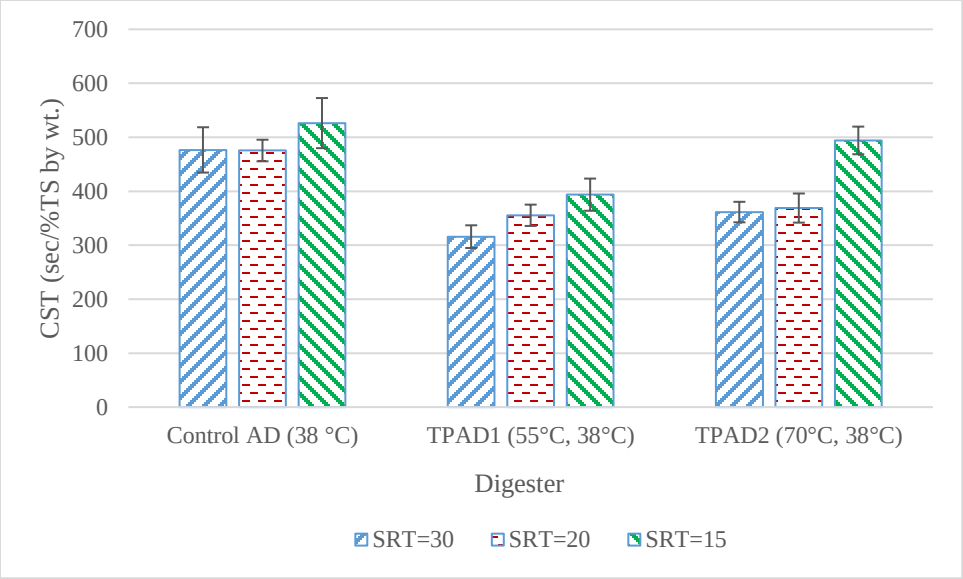


Figure 4.11. Specific capillary suction time for the final effluent of the reactor systems (Data represent arithmetic mean and error bars represent standard deviation of the measurements)

4.3 Digestate quality for land application

4.3.1 Fecal coliform content

One of the main applications for biosolids produced from sludge treatment is land application as soil amendment, however, these biosolids must meet specific criteria before being land applied. Fecal coliform levels in biosolids indicate the pathogen contamination. The land application of biosolids is regulated by BC Ministry of Environment by a regulation called Organic Matter Recycling Regulation (OMRR). The OMRR divides biosolids in categories of Class A and Class B. These categories are defined based on the concentrations of trace heavy metals and fecal coliform (OMRR 2008).

According to OMMR of British Columbia, biosolids must be classified as Class A in order to be used in land application without any restrictions. The fecal coliform level in Class A biosolids is less than 1,000 MPN/g dry solids (OMRR 2008). The concentration limit for Class B biosolids is

2,000,000 MPN/g dry TS which is much higher compared to Class A limit, therefore there are restrictions for land application of Class B biosolids.

In this study, pathogen removal in different sludge treatment systems were analyzed through fecal coliform counting in the feed and effluent sludge streams of the digesters. Figure 4.12 indicates average fecal coliform counts at various SRTs. TPAD 1 constantly meets Class A biosolids criteria because it contains less than 1000 MPN per gram of dry solids at all three different SRTs. TPAD2 and control AD did not meet Class A limit and are both classified as Class B biosolids.

The major reason of having the highest pathogen removals in TPAD1 is that the acid phase of TPAD1 had the highest amount of VFAs among all digesters, and high concentration of VFAs lead to low pH levels so as a result helps deactivating pathogens (Berg & Berman 1980; Abdul & Lloyd 1985; Sahlström 2003). Also, elevated temperature in acid phase could be another reason for more pathogen removal in TPADs over control AD (Sahlström 2003; Riau et al. 2010; Ge et al. 2011a). Also, there is an obvious reverse relation between number of fecal coliforms and the SRT, so that by reducing the SRT from 30 d to 15 d, there is a continuous increase in the number of fecal coliforms in all three digestion systems.

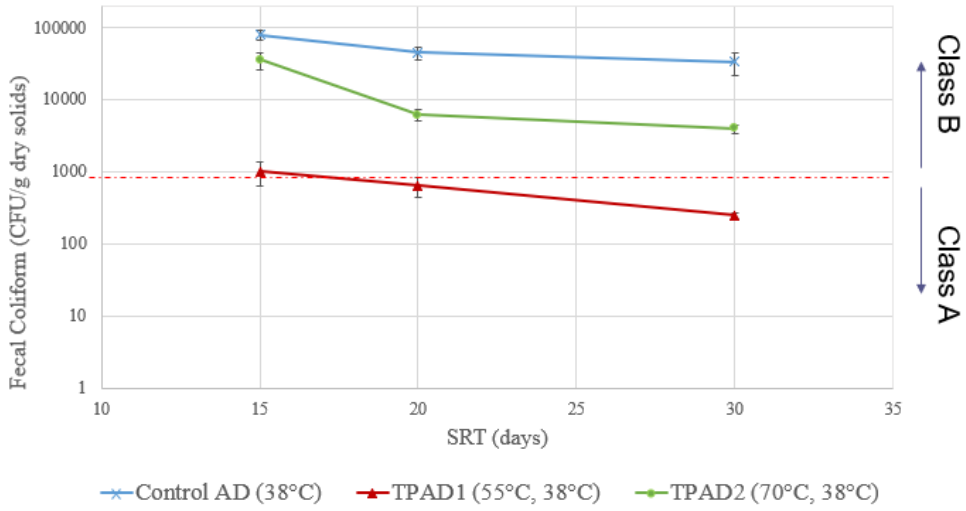


Figure 4.12. Average number of fecal coliforms at various SRTs
(CFU: colony forming unit, error bars indicate standard deviation of data)

According to an ANOVA test analysis (Table 4.9), the effect of SRT, digester configuration, as well as their interaction on fecal coliform removal in anaerobic digestion was statistically significant (P-value < 0.05).

Table 4.9. Analysis of variance for fecal coliform counts in digester effluents

Factor	Type	Levels	Values
Digester	Fixed	3	Control, TPAD1, TPAD2
SRT	Fixed	3	15, 20, 30

Source	DF	Adj SS	Adj MS	F-Value	P-Value
SRT	2	2.08E+09	1.04E+09	22.11	0
Digester	2	1.65E+10	8.26E+09	175.41	0
Digester*SRT	4	1.13E+09	2.83E+08	6.01	0.003
Error	18	8.47E+08	47066577		
Total	26				

DF: degrees of freedom; SS: sum of square; MS: adjusted mean of square;
F: observed F value, P: probability value

4.3.2 Heavy metals content

Heavy metal content in the biosolids is an important parameter for land application. The major source of heavy metals in the wastewater sludge is industrial wastewater. OMRR regulates a group of heavy metals including arsenic, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, selenium and zinc (OMRR 2008).

Table 4.10 to Table 4.12 presents the results of heavy metals analysis for the three SRTs which were investigated in this study. The final digestate from all three digestion systems could meet Class A biosolids in terms of heavy metals concentration, during all operating SRTs.

Table 4.10. Heavy metal concentration in AD digestates (SRT=30 d)

Parameter	OMRR[a] Criteria				
	Class A biosolids	**Class B biosolids**	**Control (38°C, 30 d)**	**TPAD1 (55°C-2 d, 38°C-28 d) 2nd stage**	**TPAD2 (70°C-2 d, 38°C-28 d) 2nd stage**
Arsenic (mg/kg)[b]	75	75	4.0	4.2	4.5
Cadmium (mg/kg)	20	20	3	3.1	3.5
Chromium[c] (mg/kg)	–	1060	23	27	24
Cobalt (mg/kg)	150	150	3	4	4
Copper[c] (mg/kg)	–	2200	444	469	471
Lead (mg/kg)	500	500	23	26	25
Mercury (mg/kg)	5	15	1.0	1.4	1.1
Molybdenum (mg/kg)	20	20	6.0	6.1	6.0
Nickel (mg/kg)	180	180	23	28	24
Selenium (mg/kg)	14	14	4.0	4.1	4.1
Zinc (mg/kg)	1850	1850	892	947	957

[s]Organic Matter Recycling Regulations (OMRR 2016), [b]mg per kg of dry solids, [c]Chromium and copper should not exceed Class B levels to be categorized as Class A or Class B.

Table 4.11. Heavy metal concentration in AD digestates (SRT=20 d)

Parameter	OMRR[a] Criteria				
	Class A Biosolids	**Class B Biosolids**	**Control (38 °C, 20 d)**	**TPAD1 (55 °C-2 d, 38 °C-18 d) 2nd stage**	**TPAD2 (70 °C-2 d, 38 °C-18 d) 2nd stage**
Arsenic (mg/kg)[b]	75	75	3.0	3.1	3.2
Cadmium (mg/kg)	20	20	3.1	3.1	3.2
Chromium[c] (mg/kg)	–	1060	23	24	23
Cobalt (mg/kg)	150	150	3	3	3
Copper[c] (mg/kg)	–	2200	321	327	332
Lead (mg/kg)	500	500	17	19	19
Mercury (mg/kg)	5	15	0.8	1	1
Molybdenum (mg/kg)	20	20	4.7	4.9	5.0
Nickel (mg/kg)	180	180	19.9	20.1	20.2
Selenium (mg/kg)	14	14	4	6	3
Zinc (mg/kg)	1850	1850	763	775	777

[s]Organic Matter Recycling Regulations (OMRR 2016), [b]mg per kg of dry solids, [c]Chromium and copper should not exceed Class B levels to be categorized as Class A or Class B.

Table 4.12. Heavy metal concentration in AD digestates (SRT=15 d)

Parameter	OMRR[a] Criteria				
	Class A Biosolids	**Class B Biosolids**	**Control (38 °C, 15 d)**	**TPAD1 (55 °C-2 d, 38 °C-13 d) 2nd stage**	**TPAD2 (70 °C-2 d, 38 °C-13 d) 2nd stage**
Arsenic (mg/kg)[b]	75	75	3.4	3.3	3.2
Cadmium (mg/kg)	20	20	2.9	2.8	2.6
Chromium[c] (mg/kg)	–	1060	24	23	22
Cobalt (mg/kg)	150	150	2.9	2.7	2.5
Copper[c] (mg/kg)	–	2200	338	328	316
Lead (mg/kg)	500	500	17	16	16
Mercury (mg/kg)	5	15	0.8	0.7	0.8
Molybdenum (mg/kg)	20	20	5.3	5.2	4.8
Nickel (mg/kg)	180	180	17	17	14
Selenium (mg/kg)	14	14	3.4	3.3	3.0
Zinc (mg/kg)	1850	1850	789	775	740

[s]Organic Matter Recycling Regulations (OMRR 2016), [b]mg per kg of dry solids, [c]Chromium and copper should not exceed Class B levels to be categorized as Class A or Class B.

Chapter 5: Conclusions, limitations and future work

In this research, the performance assessment of a TPAD system for the sludge digestion unit of Lulu Island WWTP was investigated. The effect of two TPAD systems with different thermophilic temperatures in the acid phase, on anaerobic digestion of mixed sludge from Lulu Island WWTP was experimented. One mesophilic single-stage AD as a control reactor was also operated to simulate the existing full-scale digesters at the plant. The digesters were monitored for about 13 months for solids removal, pathogen destruction, methane production and dewaterability. Following the collection and analysis of data, the following conclusions were obtained:

Among the three digestion systems of the single-stage AD, TPAD1 (55°C, 38°C) and TPAD2 (70°C, 38°C), TPAD1 had the best performance in terms of specific methane production, pathogen destruction and dewaterability rate.

Using TPAD instead of conventional single-stage AD did not have a statistically significant effect on solids removal, however, in general, the improvement on organic solids removal from TPADs over the control increased as the SRT was decreased as a result of control being challenged at higher OLRs.

The digestate from TPAD1 (55°C, 38°C) met the Class A biosolids criteria at all three SRTs (30 d, 20 d and 15 d), however after shortening the SRT, fecal coliform counts became closer to the limit line of the Class A biosolids.

Although there were previous studies on TPAD, there were few studies who investigated various thermophilic temperatures and SRTs on the same system. This study successfully assessed thermophilic temperatures of 55 and 70°C in TPAD for the specific mixed sludge produced from Lulu Island WWTP. Overall, by considering the improvements in methane production, pathogen

destruction, solids removal and volume reductions, TPAD1 (55°C, 38°C) at an overall SRT of 15 days could be selected for full-scale implementation to replace current single-stage mesophilic (38°C) digesters operating at 30-d SRT.

Due to the limitation with running number of digester scenarios side-by-side, this study could not include acid phase vessels operating at middle temperatures (i.e., 60 and 65^{o}C, Figure 4.6) to verify if there is a linear trend between organics solubilization and thermophilic temperature. Future studies in Bioreactor Technology group will assess other acid-phase temperature/SRT configurations to optimize sludge hydrolysis. Having the result from this, the full range of thermophilic temperature (50-70^{o}C) for TPAD would be investigated, and the relation between the solubilization ratio and the temperature, as well as hydrolysis rate constant in TPAD systems could be determined.

Also, since the microbial fingerprinting analysis was not feasible in the Bioreactor Technology group, a comprehensive assessment of the microbial community was not provided. However, samples were prepared and stored in a freezer to be analyzed in the future by a research group with genomics expertise to provide insights about the types of metabolic pass ways occurring.

For decision making regarding the full-scale application of TPAD scenarios, a detailed cost analysis is necessary. The capital and maintenance cost for the equipment used in TPAD systems, as well as the cost associated with the process energy requirement must be included.

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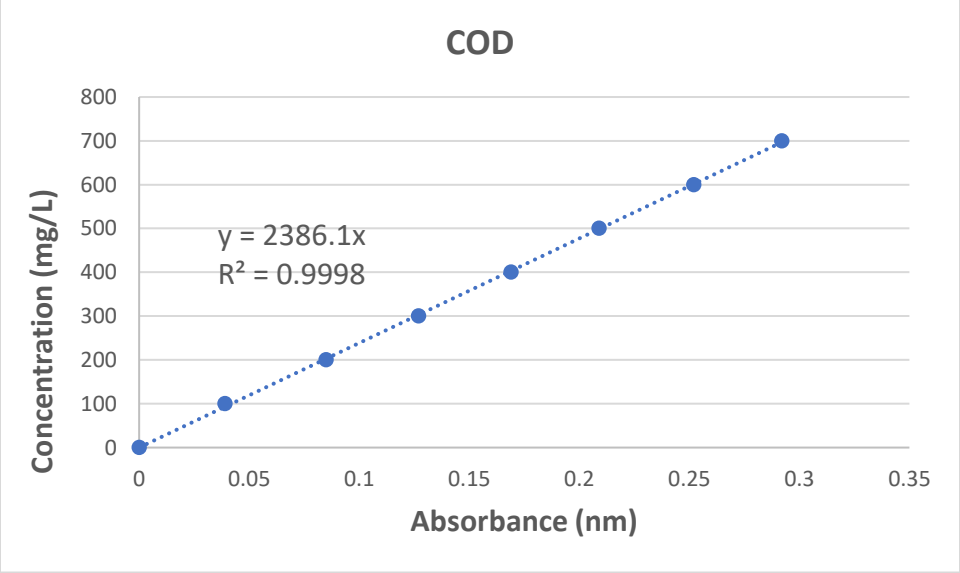
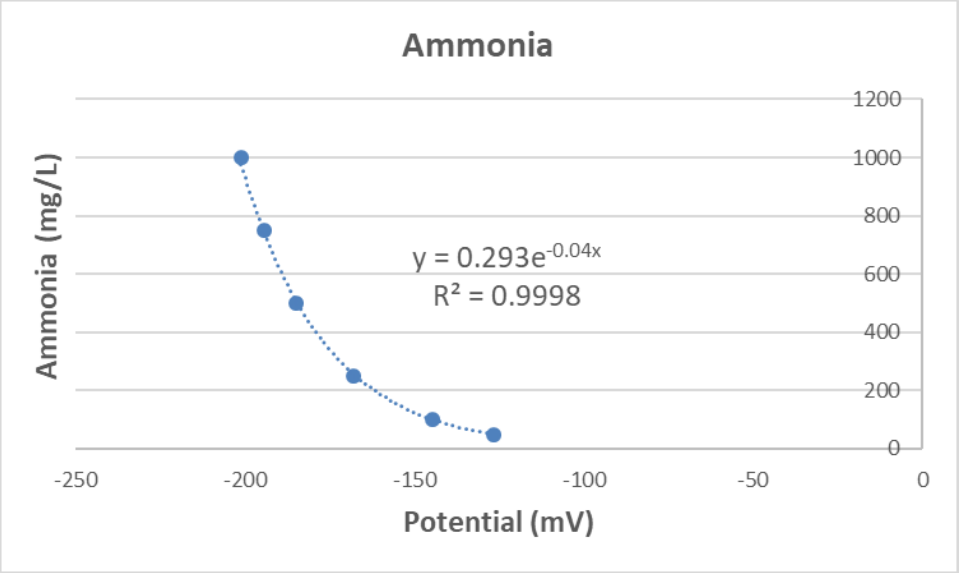
Appendices

Appendix A. Sample calibration curves

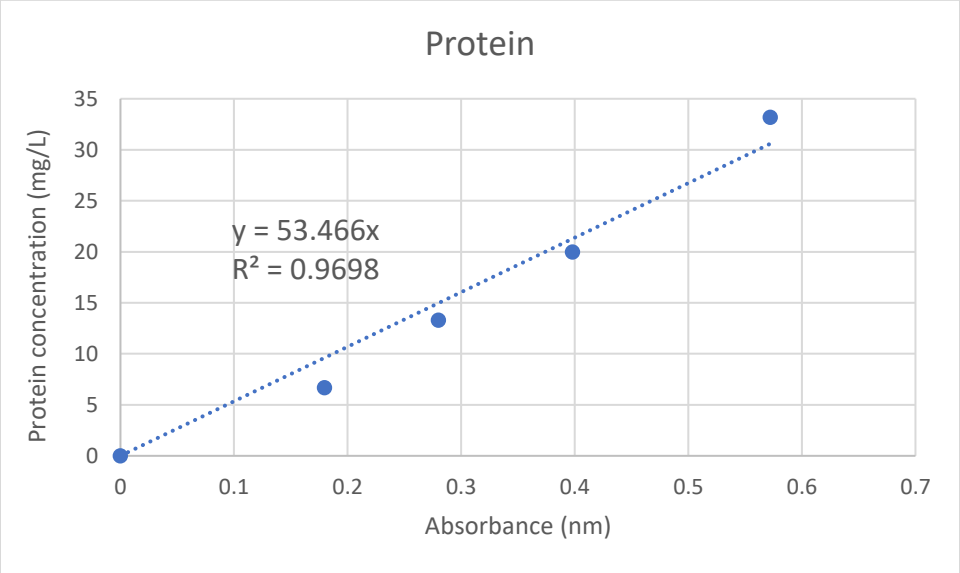
A.1. Chemical oxygen demand calibration curve



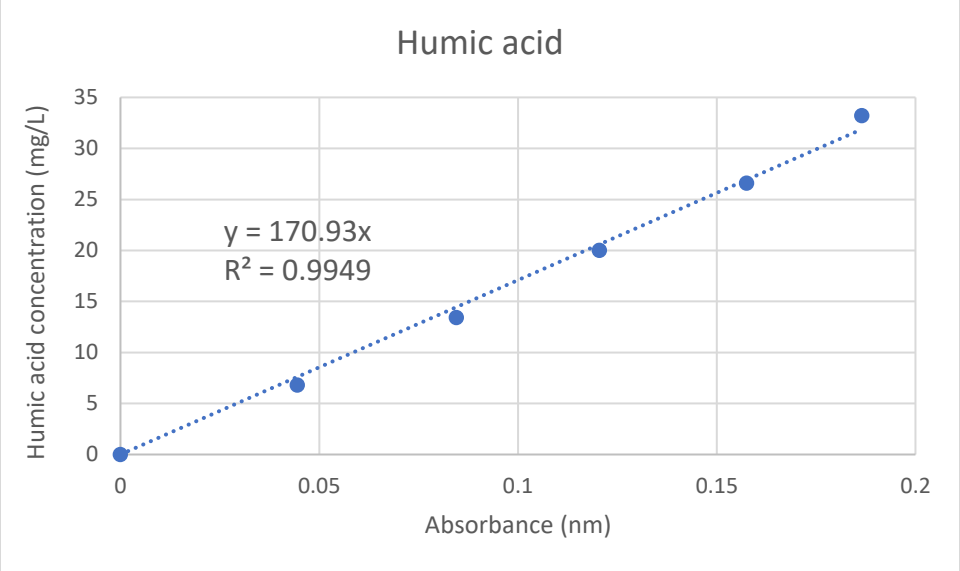
A.2. Ammonia calibration curve



A.3. Protein calibration curve



A.4. Humic acid calibration curve



A.5. Sugar calibration curves