Anaerobic Digestion System Incorporating Intermediate Thermal Treatment: A Laboratory Scale Investigation into Enhancing Methane Productivity

by

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ABSTRACT

ANAEROBIC DIGESTION SYSTEM INCORPORATING INTERMEDIATE THERMAL TREATMENT: A LABORATORY SCALE INVESTIGATION INTO ENHANCING METHANE PRODUCTIVITY

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Anaerobic digestion with intermediate thermal hydrolysis (AD+ITH) is a new configuration currently under investigation due to the evolution of the pre-treatment TH technology. This project used batch and semi-continuous laboratory scale anaerobic digestion tests to investigate the possibility for enhanced methane productivity and volatile solids destruction. A semi continuous AD+ITH reactor was operated for 210 days, thermal treating sludge at 165°C for 30 minutes. The AD+ITH produced more methane and volatile solids reduction than conventional anaerobic digestion by 1.5 times. However, an energy balance estimated that the inclusion of a TH unit without thickening would not result in a self-sustaining process and have a negative energy balance. Further investigations are required at pilot-scale, and to determine whether the cost of extra methane is decreased through the added complexity of this AD+ITH.
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ABBREVIATIONS

AD- Anaerobic Digestion
BMP- Biochemical Methane Potential
CH$_4$- Methane
CHP- Combined Heat and Power
CLRS- Continuous Measurements with Liquid Replacement
CO$_2$- Carbon Dioxide
COD- Chemical Oxygen Demand
DS- Dry Solids
GC- Gas Chromatography
HRT- Hydraulic Retention Time
ITH- Intermediate Thermal Hydrolysis
LRS- Liquid Replacement System
OLR- Organic Loading Rate
PS- Primary Sludge
PTR- Post Thermal Treatment Recycling
SCOD- Soluble Chemical Oxygen Demand
SIR- Substrate to Inoculum Ratio
SRT- Solid Retention Time
STP- Conditions of Standard Temperature and Pressure
TH- Thermal Hydrolysis
THP- Thermal Hydrolysis Process
TS- Totals Solids
TSS- Totals Suspended Solids
VFA- Volatile Fatty Acid Concentration
VS- Volatile Solids
VSS- Suspended Volatile Solids
WAS- Waste Activated Sludge
WWTP- Wastewater Treatment Plant
CHAPTER 1- INTRODUCTION

Anaerobic digestion (AD) is one of the oldest process for sludge stabilization, and currently plays a key role in the recovery of renewable energy from waste materials (Carlsson, 2015; Metcalf and Eddy, 2014). Pretreatment of AD substrates results in higher biogas production, reduced retention times and a reduction in the quantity of bio solids for disposal (Carlsson, 2015). In the past decades, AD pretreatment in the form of chemical, biological, electrical and thermal have been gaining attention in the scientific community (Carlsson et al., 2012). Out of these options, thermal hydrolysis (TH) has been the most frequently applied pretreatment for wastewater treatment plant (WWTP) secondary and combined sludge (Barber, 2016).

Applying thermal treatment to anaerobic digestate (AD$_1$-TH-AD$_2$) is a new configuration currently under investigation due to the evolution of the TH technology (Barber, 2016; Mills, 2015; Shana, 2015). The goal of this configuration is to be more energy efficient than pre-treatment. Instead of applying energy to both digestible and non-digestible material ahead of digestion, energy should be focused on the material resilient to digestion (Takashima, 2008). But so far literature on intermediate thermal treatment remains limited. There is a need for studies investigating the system’s behavior to enhance energy recovery.

Aims and Objectives

The aim of this research was to investigate the possibility of enhancing the methane potential of an anaerobic digester system using intermediate thermal hydrolysis. The detailed objectives of the research were:

1. Develop a biochemical methane potential test methodology for the investigation of this thesis.
   a. What is the current status of BMP testing?
   b. What areas of BMP testing are currently under investigation to eliminate systematic errors?
   c. How is a BMP test designed to provide accurate and reliable results?
   d. What are the future aspects of BMP testing?
2. Use the BMP tests to evaluate the methane potential of thermal treated anaerobic digestion.
   a. How does thermal treatment of anaerobic digestate compare to the thermal treatment of primary sludge or thickened waste activated sludge?
   b. How does the first digester retention time impact the thermal treated digestate methane yield?
   c. What are the optimal thermal treatment temperature and time conditions?
   d. What is the impact of thermal treatment on the methane yield of liquid and solid fractions of anaerobic digestate?

3. Use a lab-scale semi-continuous anaerobic digester with intermediate thermal hydrolysis to evaluate the performance and feasibility of an up-scaled process.
   a. How does the first digester retention time impact the performance of an anaerobic digestion system with ITH?
   b. How does an anaerobic digester with ITH compare with conventional anaerobic digestion and anaerobic digestion with pre-treatment based on methane productivity and volatile solid destruction?
   c. Would an anaerobic digestion system with intermediate thermal hydrolysis without thickening before the thermal unit have a positive energy balance?

**Scope of Papers**
The scope of this investigation focused on the AD substrate level of thermal treatment. The framing of this investigation therefore excludes major engineering factors for integration into wastewater treatment plants (WWTP) such as volume of final biosolids or impacts on recycling steams, cost, energy, and the environment. The framing also excludes impacts on the solids train such as thickening, dewatering and disposal.

**Thesis Organization**
This thesis consists of the following Chapters:

- Chapter Two provides a literature review of (1) the wastewater treatment process, (2) anaerobic digestion, (3) thermal hydrolysis as a pre-treatment, (4) intermediate thermal hydrolysis experiments, and (5) post-digestion thermal hydrolysis with re-
digestion experiments. This chapter ends with recommendations into areas needed for further development.

- **Chapter Three** presents the materials and methodologies used to conduct this research. This includes site information, feed and seed characteristics, batch and semi-continuous anaerobic digester setups, thermal hydrolysis reactor, and analytical methodologies used for characterisation of sludge physical properties.

- **Chapter Four** presents the Biochemical Methane Potential Assay Method for Anaerobic Digestion Research that was developed for the use for this project. This paper consists of two sections: (1) background information about the BMP method, and (2) the BMP serum bottle syringe method used for this project.

- **Chapter Five** provides experimental results for anaerobic batch tests investigating intermediate thermal hydrolysis process influence on digestate methane yield. Four separate BMP tests were carried out in this section, each investigating a different aspect of how thermal treatment may influence the anaerobic digestion process.

- **Chapter Six** presents an assessment of a lab-scale anaerobic digester treating TWAS with intermediate thermal hydrolysis. The reactor was operated for 210 days monitoring methane productivity, VS reduction and final digestate quality.

- **Chapter Seven** summarizes the completed research for the project.
CHAPTER 2- LITERATURE REVIEW

2.1. Introduction

Wastewater is defined as used water discharged from homes, business, industries or agriculture that potentially contains numerous pathogens, nutrients, or toxic compounds, rendering the water unsuitable for most uses without treatment (Metcalf and Eddy, 2014). To protect public health and the environment, wastewater should be immediately removed from sources of generation and sent for treatment. Only after the wastewater has been cleaned to a stringent standard removal level of physical, chemical and biological properties, can the wastewater effluent be returned to the environment or safely reused. For example, the liquid effluent may be used for land irrigation, while the removed biosolids may be applied to agriculture.

Throughout history the required degree of wastewater treatment objectives and goals have changed. From 1900 to 1970s, the wastewater treatment objectives were concerned with (1) the removal of suspended and floatable material, (2) the treatment of biodegradable organics, and (3) the elimination of pathogenic organics (Metcalf and Eddy, 2014). But in the 21st century, the view of wastewater as a waste requiring disposal shifted to accommodate the pressures of increasing populations, reduced available land and climate change. Wastewater became a viewed as a “renewable recoverable source of energy, resources and potable water.”

To accommodate new economic, social and political concerns, the wastewater treatment process changed to address issues with the collection, treatment and reuse of water. Wastewater treatment now focused on providing higher levels of treatment when discharged to the environment, while maximizing energy efficiency and minimizing odour, noise and visual impacts to neighboring communities (Metcalf and Eddy, 2014).

To provide perspective and background knowledge to the reader, the following section will provide a brief overview of a typical municipal wastewater treatment process. The following section is split into a discussion on (1) liquid phase treatment and (2) solid phase treatment. After introducing the liquid phase, the residual sludge processing will be discussed with relationship to waste activated sludge and anaerobic digestion.
2.2. Overview of Wastewater Treatment Processes

As wastewater is removed from residential homes, commercial or industrial sites, it travels through a sewer system and pumping station to a central treatment center (Metcalf and Eddy, 2014). The constituents found in wastewater are then removed by physical, chemical and biological unit processes. Often to prevent pollution of surface water from organic matter and concentrations of suspended solids, wastewater is separated into liquid and solid phases. The liquid phase treatment goal is to clear the water of suspended solids and disinfect for discharge. To accomplish this, liquid phase treatment involves four stages: (1) preliminary treatment, (2) primary treatment, (3) secondary treatment and (4) tertiary treatment.

Preliminary treatment prepares wastewater influent for further treatment. Common treatment steps include comminution, screening, grit removal and pre-aeration. Example constituents removed from wastewater that may give maintenance or operational problems with the treatment operations, processes and ancillary system can include rages, sticks, grit and grease (Mills, 2015). These waste materials collected from the screens are put into the garbage and the liquid constituents are sent to primary treatment.

Primary sedimentation is customarily the first step in the wastewater treatment process in which significant amounts of suspended solids and organic material from the wastewater are removed by gravity sedimentation. In the settlement tank, solids sink to the bottom to form a separate sludge stream called primary sludge, while fats and oils rise to the top creating a scum layer that gets removed. The wastewater then flows over a weir at the top of the tanks and continues to secondary treatment (Metcalf and Eddy, 2014).

In secondary treatment, the biodegradable organic matter and suspended solids not removed in primary treatment are removed in aeration tanks. Here microorganism eat up the rest of the organics that are left in the wastewater and the microorganisms that settle to the bottom are called return activated sludge and returned back to the aeration tank. After the biological treatment, the wastewater enters the final settlement tanks. The biological sludge mass is allowed to sink to the bottom forming sludge which can be pumped. In this stage more than 70% of the sludge is returned to the aeration basin to ensure sufficient sludge retention time.
Tertiary removes residual suspended solids after secondary treatment, usually by granular medium filters, cloth filters or microscreens. In this last stage before the final effluent is discharged, nearly all the tiny remaining particles are removed. The wastewater gets filtered through sand in a polishing process called a sand filter. Then any disease-causing bacteria are killed with chlorine. Bisulphite gets added to remove the leftover chlorine before discharge (Metcalf and Eddy, 2014).

2.2.1. Residual Solid Processing
Sludge is any material produced during primary, secondary or advanced wastewater treatment that has not undergone any process to reduce pathogens or vector attraction. As stated by Tchobanoglous et al., (1991), its processing, reuse and disposition presents perhaps the most complex problem in the field of wastewater treatment. Common types of sludge include primary sludge, enhanced primary sludge, waste activated sludge and secondary sludge. The sludge and biosolids resulting from wastewater treatment processes are usually in the form of a liquid or semisolid liquid containing 0.25 to 12% solids by weight. The problems of dealing with sludge are complex. The organic matter contained in the wastewater represents only a small part of the sludge solid matter, and as it decomposes it become offensive. The management of solids and concentrated contaminants removed by treatment has been and continues to be one of the most expensive problems in the field of wastewater engineering (Tchobanoglous et al., 1991).

2.2.2. Waste activated sludge
Waste activated sludge (WAS), is the residual semi-solid material containing principally water, organic matter, undegradable particles and living organisms left over from biological treatment at secondary stage in WWTP. As a source for human health problems, WAS remains a challenging issue for processing, reuse, and disposal (Raheem et al., 2018). Globally, WAS production continues to increase as a result of a growing population and industrialization. Raheem et al., (2018) reported the WAS quantity produced in the European Union-15 countries was around 10 million of dry tons in 2010, China producing 6.25 million dry ton per year with an annual growth rate of 13%, and the United states generating over 8 million dry tones of WAS per year. Meanwhile environmental quality requirements for WAS are becoming increasingly stringent. As disposal outlets decrease, and economic and social
pressures build, there is a need for innovative and cost effective solutions (Raheem et al., 2018).

Sewage sludge stabilization is required prior to discharge to reduce biological activity, odour and prevent the release of harmful chemicals into the environment. The success in achieving these objectives is related to the effects of the processing of the volatile or organic fraction of the sludge (Metcalf and Eddy, 2014). Currently, there is a diverse treatment range and valorization technologies available for safe disposal, resource recovery, and power generation. Common treatment options include anaerobic digestion, incineration, pyrolysis and gasification. Recently, thermochemical pathways (incineration, pyrolysis and gasification) have been found to improve efficiencies with fast processing and versatile end applications. But they are all energy intensive for high moisture wet sludge, and flue gases require expensive clean-up prior to emission (Raheem et al., 2018).

Anaerobic digestion has been marketed as the best process for the stabilization of sludge generated from aerobic wastewater treatment. As stated by Nges and Liu, (2010), AD potential advantages of other stabilization processes include: (1) the production of energy as methane, (2) a reduction of 30-50% of sludge volume required for ultimate disposal, (3) the production of sludge residue generally free from offensive odours when full digested, and (4) a high rate of pathogen destruction. But, despite these advantages AD has limitations that continue to limit AD usage (Appels et al., 2008): (1) only a partial decomposition of the organic fraction, (2) slow reaction rates and associated large volumes and high costs of the digesters, (3) the vulnerability to the process to various inhibitors, (4) poor supernatant quality produced, (5) the presence of other biogas constituents such as carbon dioxide, hydrogen sulphide and excess moisture, (6) the possible presence of volatile siloxanes in the biogas, and (7) the increased concentration of heavy metals and various industrial organics in the residual sludge due to significant reduction of the organic fraction during digestion. Thus, there is a need for enhanced AD processes.

Today, the theory and technology of biogas production and volatile solids reduction are mature and well developed. The key to further research is optimization (Mao et al., 2015). To understand the potential opportunities to enhance the AD process, it is important to first review the fundamentals. *Fundamentals of Anaerobic Digestion of Wastewater Sludge*
(Parkin and Owen, 1986) was a paper central for providing this understanding and the format to best communicate these factors. The following sections will cover (1) the fundamentals of anaerobic digestion and (2) the stabilization of WAS using anaerobic digestion.

2.2.3. Fundamentals of Anaerobic Digestion
Anaerobic digestion is one of the oldest processes used for stabilization of sludge (Metcalf and Eddy, 2014). The purpose of anaerobic digestion is to convert organic material into methane in the absence of molecular oxygen, thereby reducing the organic solid content of the sludge, its putrescibility and pathogen content. In this section, key factors that are required to understand this process are briefly reviewed. These include (1) process description, (2) important environmental conditions required for efficient and stable digestion, and (3) performance indicators.

2.2.3.1. Process Description
Anaerobic oxidation of waste is carried out through a series of complex microbiological processes. The conversion of wastewater organics to methane involves several groups of bacteria carrying out rather specific reactions (Parkin and Owen, 1986). Often a three stage scheme of hydrolysis, fermentation (acidogenesis and acetogenesis), and methanogenesis are used to explain the breakdown biodegradable material of waste solids (Gonzalez-Fernandez et al., 2015). The starting point depending on the nature of the waste processed.

Hydrolysis is the first step in the digestion process, where extracellular enzymes excreted from different bacteria breakdown complex organic matter (carbohydrates, proteins, and lipids) are converted into monomeric and dimeric compounds to be readily accessible for the acidogenic bacteria (Gonzalez-Fernandez et al., 2015). Hydrolysis is generally considered to be the rate-limiting step for anaerobic digestion of complex substrates. As stated by Lier et al, this is not due to a lack of enzyme activity but due to the availability of free accessible surface area of the particles and structure of solid substrates.

Acidogenesis, the second step and the beginning of fermentation, is the most rapid conversion step in the anaerobic food chain (Lier et al., 2008). Here, dissolved compounds in cells of fermentative bacteria are converted into simple compounds which are then excreted. These compounds produced include VFA, alcohols, lactic acid, CO$_2$, H$_2$, NH$_3$, and H$_2$S and new cell material (Lier et al., 2008).
Acetogenesis, the intermediate step, occurs for some of the VFAs produced from aciogenesis (Metcalf and Eddy, 2014). In this step, propionate and butyrate are converted by fermentation by bacteria to produce acetate, CO$_2$, hydrogen and new cell material (precursors for methane formation). For the reaction associated with the conversion of propionate and butyrate to acetate to occur, hydrogen must be at low concentrations in the system or the reaction will not proceed (H$_2$$<$10$^{-4}$ atm). In addition, short chain fatty acids (SCFA), other than acetate, are further converted to acetate, hydrogen gas and carbon dioxide by the acetogenic bacteria.

Methanogenesis, is the third basic step, and the final stage in the overall anaerobic conversion of organic matter. Methanogenic bacteria convert acetate, hydrogen plus carbonate, formate or methanol to methane, carbon dioxide and new cell material (Lier et al., 2008). Approximately 72% of the methane formed comes from aceticlastic bacteria, while the remaining 28% results from reduction of carbon dioxide using hydrogen as the energy source by carbon dioxide reducing methanogens (Parkin and Owen, 1986). The composition of gas produced from stable fermentation and methanogenesis operations is typically 65% methane and 35% carbon dioxide. In situations where the waste contains a higher lipid fraction, there will be a higher methane fraction in the biogas gas (Metcalf and Eddy, 2014).

Figure 2-1: Modified from Flow-diagram for the anaerobic degradation of a composite particulate material indicating COD fractions (Remigi and Buckley, 2006)
2.2.3.2. Key Factors affecting process efficiency and stability

Bacteria are sensitive to environmental conditions. In order to ensure efficient digestion operation, a balance between the acid-forming and hydrogen-forming bacteria and the methane producers must be maintained (Parkin and Owen, 1986). In general, the methane bacteria and hydrogen producing, and consuming bacteria have slower growth rates and are more sensitive to environmental stress than the fermentative organism. Thus optimal design should provide a large, stable population of these types of bacteria (Parkin and Owen, 1986). Three key factors to efficient digestion include: (1) provide adequate contact between the bacteria and their food sources by efficient mixing; (2) provide a suitable uniform environment, (3) provide sufficient bacterial retention time (SRT).

2.2.3.2.1 Solid Retention Time

Solid retention time (SRT) is regarded as the most important parameter for anaerobic digester design and operation (Parkin and Owen, 1986). SRT accurately defines the relationship between the bacterial system and digester operation conditions. Hydrolysis, fermentation and methanogenesis are directly related to the SRT, where an increase or decrease in SRT results in an increase or decrease in the extent of each reaction. The challenge for the engineer or operator is selecting the optimal SRT for a substrate, to ensure efficient conversion of complex organic matter to methane and carbon dioxide, the population of bacteria in the digester must be of sufficient quantity and concentration. A value chosen closer to the minimum SRT required for each reaction, may have a smaller digester size and increased rate of methane production, but has a trade-off with a lower degree of sludge stabilization and an increased risk of digester failure and an inability to buffer against fluctuations in temperature, and fed or generated toxicity. On the other hand, a longer SRT may have a high degree of stabilization, but to a point lower increments of increase in digester performance, and massive increases in digester sizing and operational costs. The most common choice is for SRT for full-scale plants is within the range of 15-20 days (Metcalf and Eddy, 2014).

2.2.3.2.2. Temperature

Temperature influences the growth rate and metabolism of micro-organism and the population dynamics in the anaerobic reactor, but also effects factors such as gas transfer rates and settling characteristics of biological sludge (Parkin and Owen, 1986). Most anaerobic digesters are operated in either mesophilic (30-38°C) or thermophilic (50-58°C)
temperature ranges. Thermophilic digestion is faster than mesophilic digestion since the biochemical reaction rates increase with increasing temperature. Additional advantages are increased solids reduction, improved dewatering, and increased destruction of pathogenic organism. But the use of thermophilic temperatures has a higher energy requirement, a lower quality supernatant with large quantities of dissolved solids, a higher odour potential and much poorer process stability (Appels et al., 2008). In general, for either temperature range, maintaining a stable temperature is important as bacteria are sensitive to slight changes in temperature, with reported temperature changes greater than 1°C/day affecting process performances.

2.2.3.2.3. Mixing
Proper mixing is one of the most important considerations in achieving optimum process performance (Metcalf and Eddy, 2014). Efficient mixing provides the utilization of the entire digester volume by dispersing metabolic end products and any toxic materials contained in the influent sludge, maintaining intimate contact between the bacteria, bacterial enzymes, and their substrates, preventing stratification and temperature gradients (Parkin and Owen, 1986). Ineffective mixing impacts process kinetics by decreasing the effective system volume and SRT. Ineffective mixing can also cause foaming, scum formation, and excessive solids deposition (Parkin and Owen, 1986).

2.2.3.2.4. Performance Indicators
AD performance is assessed by relating the output of a digestion system to either the input or to the volumetric digester capacity (Carlsson et al. 2012). Common AD performance is evaluated using the TS or VS percent reduction of incoming sludge, and the methane productivity in comparison to the theoretical methane yield of the substrate (Parkin and Owen, 1986). Imbalanced digestion can be triggered by changes in organic or hydraulic loading, changes in organic feed characteristics, changes in temperature, or introduction of toxic substances. During imbalanced digestion, typically volatile acid concentrations increase while bicarbonate alkalinity, pH, gas production, percent methane, and the destruction of organic matter all decrease. Careful monitoring of these variables should allow operations personnel to observe the onset of stress and take appropriate remedial measures to prevent system failure.
2.2.3.2.4. Volatile Acids-Alkalinity-pH
The relationship between VFA-alkalinity-pH must be discussed together (Parkin and Owen, 1986). Bicarbonate ion, \( \text{HCO}_3^- \), is a major source of buffer capacity in anaerobic digestion for maintaining the optimum pH range of 6.5-7.6. It is produced during digestion by the breakdown of nitrogenous organic matter, soaps or organic acid salts. The concentration of \( \text{HCO}_3^- \) in solution is related to the percent of \( \text{CO}_2 \) in the digester gas phase. To maintain pH in the optimum range, Balk values of 1000 to 5000 mg/L as CaCO\(_3\) are required, with \( \text{CO}_2 \) concentration between 25-45%. With higher Balk, the more volatile acids can be neutralized without a pH-drop. Under stable conditions, Balk is approximately equal to TALK. But in imbalanced conditions, VA concentrations increase, reducing BALK and replaces it with acetate alkalinity, leading to a drop in pH. In addition, during imbalanced conditions, the percent \( \text{CO}_2 \) would increase, depressing the pH even more. Therefore, maintaining a high BALK is recommended (Parkin and Owen, 1986).

2.2.3.2.5. Gas Production
Methane production, as a process performance indicator is probably the most sensitive since it is directly related to organic matter destruction (Parkin and Owen, 1986). Typical values of percent methane for digesters operating on municipal wastewater sludge are 60-75%. During system imbalance, methane production and total gas production will decrease, while the percent \( \text{CO}_2 \) will increase. But because methane production is sensitive to normal daily changes in organic and hydraulic loading, it is not the best variable to measure for detection of the start of imbalanced digestion. Daily monitoring of a combination of methane production, percent \( \text{CO}_2 \), VA, pH and TALK will allow the operator to detect early system stresses (Parkin and Owen, 1986).

2.2.3.2.6. Organic Matter Destruction
The destruction of organic matter is the primary objective of anaerobic digestion (Parkin and Owen, 1986). Therefore, COD and VS must be measured to determine the overall process efficiency. But as an indicator of imbalanced digestion, organic matter destruction is not sensitive a measure of process imbalance. It will only confirm what trends VA, pH, TALK and methane production have already shown. Frequent monitoring of influent COD and VS levels may help determine if system imbalance was caused by increased organic loading (reduction in effective SRT), and may help to predict and minimize detrimental effects if the
monitoring is frequent enough (Parkin and Owen, 1986). The destruction of the organic matter was calculated using Equation 1.

\[
Efficiency \% = \frac{S_o - S}{S_o} \times 100
\]

Equation 1-1: Organic Matter Destruction

Where \(S_0\) is the influent COD or total solids (g/L), and \(S\) is the effluent COD or TS (g/L).

2.2.4. WAS and Anaerobic Digestion
The biodegradability of WAS for anaerobic digestion is estimated to range between 30-50%, depending on the activated sludge operating conditions (Parkin and Owen, 1986). The degradable portion of WAS is comprised primarily of active bacterial cells. Parkin and Owen, (1986) reported that the biodegradable fraction of active bacteria is 68%, but this does not mean that WAS is 68% degradable. WAS contains nonbiodegradable debris from dead bacteria and refractory organics. Most of the organic components in WAS are located within the microbial cell (Müller, 2001). The cell wall of microorganism is a stable semi-rigid structure protecting the cell from lysis, causing hydrolysis to be the rate limiting step (Müller, 2001). As a result, WAS is inherently less biodegradable than primary sludge, where long retention times will not reduce COD or cause the VS reduction to exceed 50% or raise WAS biodegradability to the level of primary sludge. **Only through thermal treatment of WAS can it be made as degradable as primary sludge** (Parkin and Owen, 1986).

2.3. Anaerobic Digestion Pre-treatment
In the past decade AD pre-treatment (in the form of chemical, physical, electrical and thermal) has been gaining attention in the scientific community. Zhen et al., (2017) reported that ScienceDirect shows the number of published papers has increased from 36 in 2000, to over 100 papers in 2006, and up to 609 papers in 2015. In addition, the corresponding percentage of “sludge preAD” based papers in all papers related to AD rose from 24.5% in 2000 to 43.6% in 2015.

Although out of the many proposed processes few have been implemented in full-scale wastewater treatment plants, due to the difficult practicality of substrate manipulation (variety of pre-treatment effects intertwined with substrate characteristics and pre-treatment mechanisms) and economic issues (Barber, 2016; Carlsson et al., 2012; Parkin and Owen,
1986). Hendriks and Zeeman, (2009) after analyzing and comparing pre-treatments conclude that (1) chemical pre-treatments are effective, but expensive compared to the effect on lignocellulosic compound digestibility; (2) the limitation to mechanical pre-treatments is that they require the input of a higher-value energy source (electricity), and (3) thermal pre-treatments, such as steam explosion and pressure-cooking, have a high potential to achieve an economically positive balance (Hendriks and Zeeman, 2009). So far, the major pre-treatment methods reported to be employed in full-scale installations have been thermal hydrolysis (TH), enzyme hydrolysis and ultrasound (Takashima and Tanaka, 2014). TH has emerged as the pre-treatment of choice due to its techno-economic benefits (Carlsson et al., 2012). As of 2016, there were 75 facilities on several continents with 39 facilities fully operational (Takashima, 2008).

2.3.1. Thermal Hydrolysis Pre-treatment
2.3.1.1. Thermal Hydrolysis
Thermal hydrolysis is the process of disintegrating both living and dead cells, to permit the release of intracellular matter to be more accessible for anaerobic microorganisms (Barber, 2016). Typical TH tested treatment temperatures are usually within 60 to 270°C (Pilli et al., 2015). As the temperature increases from 100°C to 180°C, the substrate organic matter will progress through different chemical reactions (Barber, 2016). First, the organic matter is solubilized in bulk, second, polysaccharides are released from loosely bound EPS, third, tightly bound ECP is destroyed further releasing polysaccharides, fourth, cell walls are destroyed releasing intracellular proteins and cell wall debris, and finally at temperatures above 180°C, nonbiodegradable products are produced as the polysaccharides react with newly released proteins (Barber, 2016).

2.1.2. Thermal Hydrolysis Pre-treatment Effect on AD
TH has the potential to influence the AD process performance by affecting the rate and extent of degradation (often monitored through methane yield and VS reduction) (Haug et al., 1978; Hii et al., 2013). As the degradation rate is increased by solubilisation or particle size reduction of organic matter that would have been slowly hydrolyses, the extent of degradation is increased by the release or exposure of organic material that was originally
inaccessible to microorganism or the transformation of material that was originally not biodegradable (Barber, 2016; R Cano et al., 2014; Mills, 2015).

Many review papers have been published summarizing the effect of TH on the performance of AD systems using methane yield and VS reduction parameters. The most common optimal temperature range for WAS treatment is within 160-180°C, for 20-40 minutes (Abelleira-Pereira et al., 2015; Barber, 2016; Camacho et al., 2002; Hii et al., 2014; Müller, 2001; Neyens and Baeyens, 2003; Suárez-Iglesias et al., 2017; Tyagi and Lo, 2011). (Pilli et al., 2015) reported results from various researchers using the TPT of WAS in temperature range of 120-170°C for 30-60 minutes. Results generally showed an increased methane production of 22-43%, a methane content increased from 40 to 70%, a total solids reduction up to 59%, an increase in VS degradation up to 23%, COD reductions up to 75%, and improved dewaterability (Bougrier et al., 2008; Carrère et al., 2010; Donoso-Bravo et al., 2011; Sapkaite et al., 2017).

The disadvantages of the process include the large increase in soluble inert fraction, final effluent colour, ammonia inhibition in the main digester due to increased performance, and the risk of poor press solids captured due to an increase in fines (Takashima and Tanaka, 2010). THP also requires external energy sources such as biogas and additional fuel to meet its specific heat demand (Chen et al., 2012). In addition, the overall performance of THP configuration is said to be limited under low digester HRT and high organic loading conditions, where %VS and biogas yield are below the expected performance (Nielsen et al., 2011).

2.3.1.2. Full-scale Application
Full scale TH pre-treatment processes, by CAMBI and BIOHELYS are often cited as two of the most common/established commercial high-temperature options that have been implemented into WWTPs. The main results from these models are increase in biogas production and reduction of volatile solids from 30-45%, to 50-60%, a reduction of sludge volume with digested sludge cake totals solids (TS) content higher than 30%, and increased of digester capacity with organic loading of 5-56 kg VS/m³ d (Pilli et al., 2015; Shana et al., 2013).
CambiTHP™ is a well-established process outside North America with more than two dozen installations (Abu-Orf and Goss, 2012; Pilli et al., 2015). The Cambi™ process consists of three basic units: the Pulper, the Reactor and the Flash Tank (see Figure 2). The residuals must be pre-dewatered to slightly higher than 16% TS before transferred to a storage tank large enough to provide equalization of inflow variations and THP to operate at uniform flow rate. In the pulper residuals are mixed and preheated using recycled steam from the Flash Tank. The preheated residuals are pumped to the Reactor vessel and heated to 165°C and exposed at a pressure of 120-130 psig and held for 30 minutes. The steps for Cambi™ cycle operation takes 90 minutes from fill to empty. The treated residuals (now 3-4% less than the raw dewatered cake) are transferred to the Flash Tank and sent to cooling and dilution (8-12% solid concentration) (Abu-Orf and Goss, 2012).

![Diagram of CambiTH system](image)

Figure 2-2: Main components of CambiTH system (modified image retrieved from (Abu-Orf and Goss, 2012).

Veolia Water Solutions and Technology is also another company providing full-scale TH technology (Hii et al., 2013). Biothelys is like CAMBI, in its batch hydrolysis reactors. In the first stage, dewatered sludge (15-16% DS) are added to the hydrolysis reactor, where the sludge is heated through steam injection for 30-60 min reaching a temperature around 150-180°C. The sludge is then cooled down and added to the anaerobic digester. The advantages of this process allow for up to 80% higher reduction in the quantity of sludge in comparison to conventional mesophilic digestion (Pilli et al., 2015).
ExelysTM by Veolia Water, is another thermal hydrolysis technology, but instead works in a continuous mode, using a progressive cavity pump. This process can hydrolyze higher solids content sludge than traditional batch hydrolysis (22% w/w compared to 16%DS), which allows for less water to heat, and a reduction in 30% less steam. In the first section, the high solid sludge is injected with steam and moved through a steam condenser, to a self cleaning static mixer, and into the reactor where the sludge flows at a very low velocity for plug-flow conditions. After the sludge has been held for the required time, the sludge then passes through a heat exchanger and into the pressure holding pump to pump the hydrolysed sludge to the digestion process (Gurieff et al., 2011).

Figure 2-3: LD Exelys Process Configuration (modified image retrieved from (Abu-Orf and Goss, 2012)).

2.3.1.3. Next Generation Thermal Hydrolysis Process- High Solids THP
Developments are ongoing to improve full-scale TH technology (see Figure 4). As the TH technology has evolved from batch to continuous, new configuration possibilities have become available (Viswanathan et al., n.d.). To improve energy balances, further reduce sludge quantity and increase biogas production, a smaller TH reactor has been suggested to be placed between two anaerobic digesters. In 2009, Veolia Water patented Digester-Lysis-Digestion (ITHP) and Digester-Lysis (PTHR) configurations. Both processes are currently operating at various wastewater treatment plants in Europe. For example, a DLD plant is in operation in Hillerod WWTP in Denmark (2010), Lille Marquette, France (2014), and Billund in Denmark (2013), and a Digester-Lysis with a recycle loop at Versailles WWTP (320 000 PE) in France (2014) (Chauzy et al., 2014a, 2014b, 2014c).
Full-scale operations show the application of each configuration depends on the needs of the municipalities current and future goals, along with answering questions about integration into existing facilities, regulations and laws, and operational costs (Chauzy et al., 2014b). The following sections are focused on the investigation of TH treatment of digestate for the possibility of increasing methane productivity and VS destruction.

Figure 2-4: Evolution of THP (modified image retrieved from (Abu-Orf and Goss, 2012)).

2.3.2. Digestate as a Substrate
Digestate, the residual product of AD, is a liquid to thick slurry containing a significant quality of solids. In this paper, the focus was placed on digestate originating from WWTPs, which typically operate with total solids contents of less than 10% TS for substrates that have a high moisture content such as domestic and industrial wastewater (Sawatdeenarunat et al., 2015). As an example, Elbeshbishy et al., (2012) characterized whole digestate from three operating full-scale anaerobic digesters with solids ranging from 2.0% (w/w) to 5% solids (see Table 1): (1) the primary mesophilic digester (14-18 day HRT, 45% VSS reduction) from Guelph, Ontario WWTP; (2) mesophilic anaerobic digester (17 day HRT, 27 day SRT, 62% VSSr) from the food waste Dufferin Organics processing Facility, Toronto, Ontario (treating 25,000 metric tons/year of source separated organics), and an anaerobic digester (17-20 day HRT/SRT, 49-52% VSSr) from Joint Water Pollution Control Plant, Carson, California (treating approximately 300 million gallons of wastewater per day) (Elbeshbishy et al., 2012).
Table 2-1: Anaerobic digester/ inocula characteristics from three full-scale anaerobic digesters (modified table from (Elbeshbishy et al., 2012)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Guelph’s Inoculum</th>
<th>Toronto’s Inoculum</th>
<th>JWPCP’s Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD</td>
<td>mg/L</td>
<td>18100±2600</td>
<td>70500±1800</td>
<td>19400±110</td>
</tr>
<tr>
<td>SCOD</td>
<td>mg/L</td>
<td>6560±280</td>
<td>31200±700</td>
<td>660±70</td>
</tr>
<tr>
<td>TSS</td>
<td>mg/L</td>
<td>18000±3400</td>
<td>47400±2300</td>
<td>18300±450</td>
</tr>
<tr>
<td>VSS</td>
<td>mg/L</td>
<td>10000±720</td>
<td>37100±1800</td>
<td>11600±280</td>
</tr>
<tr>
<td>TVFA</td>
<td>mg/L</td>
<td>230±60</td>
<td>210±30</td>
<td>26±6</td>
</tr>
<tr>
<td>NH₄</td>
<td>mg/L</td>
<td>540±80</td>
<td>820±20</td>
<td>470±60</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.6±0.2</td>
<td>6.9±0.2</td>
<td>7.2±0.1</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>mg CaCO₃/L</td>
<td>3200±180</td>
<td>5000±220</td>
<td>5400±260</td>
</tr>
</tbody>
</table>

In theory, the goal of AD is to achieve a completely stable digestate product (without the ability to develop health or odour problems), but in practice this is not possible, as the digestate organic matter content and quality depend on the operating conditions of the AD plant (Menardo et al., 2011b; Parkin and Owen, 1986). For example, if the AD plant has a high organic loading rate and a short HRT, the digestate will still contain a considerable amount of undigested organic matter, but if the AD plant has a low OLR and a long HRT then the digestion methane yield could be negligible (Hansen et al., 2006; Menardo et al., 2011b). As stated by Nges & Liu, (2010), the operation of digesters at low residence times are more popular for full-scale plants focusing on biogas production, which then increases the volume of digestate that is not completely depleted of biodegradable organic compounds.

Product stability is typically reported as volatile acid concentrations, or the percent VS destroyed and some measure of digestate sludge odour. As defined by Parkin & Owen, (1986), indications of incomplete and inefficient and unstable products, have volatile acid levels above 500 mg/L and VS reductions less than 30-40%. Recently new techniques such as Biochemical Methane Potential (BMP) test, which measures the amount of biodegradable material remaining in a sludge sample can be used to measure both the efficiency and degree of digestion by comparing the substrate to digestate (Jeris et al., 1985)(Zheng et al., 2014).

Studies have shown that digestate can still contain a large amount of residual undigested organic matter content and could therefore yield an attractive amount of biogas. Ruile, Schmitz, Mönch-Tegeder, & Oechsner, (2015) investigated the residual methane potential of
whole digestate from 21 full-scale digesters and reported methane yields varying from 24 to 126 NmL CH\textsubscript{4}/g VS (Ruile et al., 2015). Menardo, Gioelli, et al., (2011) reported lower methane potentials for whole digestate, ranging from 3 to 34 NmL CH\textsubscript{4}/g VS. Sambusiti et al., (2015) reported methane yield of untreated digestate of 70 NmL CH\textsubscript{4}/g VS, and an untreated solid-separated digestate of 90 NmL CH\textsubscript{4}/g VS with 33 and 44% degradability.

In addition to raw digestate, WWTPs deal with the handling and treatment of high solid and liquid digestate streams in order to reduce the huge volumes of digestate produced and the corresponding disposal and transportation costs. In full-scale treatment plants, raw digestate goes through dewatering to separate the solid matter and water in the sludge to biosolids with high solids content (cake) and liquid streams. The liquid stream contains fine, low-density solids and high concentration of nutrients (large amounts of nitrogen and potassium), and poor biodegradability with very low residual biogas potential (Sogn et al., 2018; Tampio et al., 2016a). Usually, this stream is returned to the wastewater treatment system or separately treated, although it can be disposed for land application but there can be a risk of nitrogen leaching, groundwater infiltration and pollution of aquatic habitats (Akhiar et al., 2017). The solid fraction, on the other hand, can have solid content ranging from 20 to 40\% depending on the dewatering process (screw press, screening drum press or centrifuge). The digestate solid fraction is usually represented by a great measure of fibers (hemicellulose and cellulose) and lignin, which are responsible for decreasing water permeability and hindering chemical, physical and microbial degradation (Hartmann et al., 2000; Menardo et al., 2011a). The residual fibers then undergo a composting process, and utilized for agricultural purposes for economic and environmental benefits (such as the replacement of commercial fertilisers and contributing to the recycling phosphorus) (Akhiar et al., 2017; Möller and Müller, 2012). However, digestate for land application has certain drawbacks. Since it is produced all year, and the number of biogas plants are increasing, the densification in certain regions could lead to oversupply of digestate at a local level, which could have harmful effects on plants and solids due to impurities (heavy metals, organic contaminants or pathogens) (Alburquerque et al., 2012; Rehl and Müller, 2011; Tampio et al., 2016b). In addition, Rehl and Müller, (2011) estimated when all these factors are taken into account with the inclusion of transportation costs, the value of the digestate could be close to zero. Therefore, digestate are sometimes
stored in uncovered tanks that allow the release of a variety of gases to the atmosphere (Comparetti et al., 2013; Gioelli et al., 2011; Hansen et al., 2006).

There is a need for research on alternative valorization routes for both solid and liquid digestate to reduce the environmental and economic impact of AD plants (Monlau et al., 2015). As found by Monlau et al., (2015) and Akhiar et al., (2017), liquid digestate could be used for microalgae cultivation, while solid digestion valorization pathways such as bio-fuel for domestic furnaces, production of biochar, and post-treatments for methane recovery as emerging options (Akhiar et al., 2017).

2.3.3 TH treatment of digestate
TH treatment of digeste may be an emerging option for enhancing methane productivity. As found by Shana, (2015), in the first stage MAD, the easily biodegradable fraction of sewage sludge feed is expected to be utilized and the part of the non-biodegradable content is expected to be loosened under the influence of the microbial activity. The slackened organic bonds of the digested sludge constituents are then expected to be easily biodegradable after thermal hydrolysis process. The hydrolyzed product can then be expected to be further utilized through biochemical conversion in second MAD, producing additional biogas, and a final biosolid product that is stabilized and able for land disposal.

The optimization of post-treatment conditions studies were carried out in batch anaerobic digestion tests often used different treatment options other than thermal (ozone, alkaline, thermal/alkaline, thermal/acid, ultrasonic, mechanical) to understand the effects of treatment on the digestate composition, biodegradability, and methane potential (Lindner et al., 2015). But just like past experiments for pre-treatment for WAS, the optimal treatment conditions may depend on the composition and structural characteristics that vary with the type of substrate fed to the digester and the AD plant configuration (OLR, HRT). Optimal THP conditions, therefore, found for other substrates may not necessarily apply to all digestates, and studies of how digestate cakes of different origin respond to THP are lacking (Svensson et al., 2018).
Finding the optimum TH conditions is important for energy and economic balance. But unlike WAS, the conditions for digestate have not been concluded to be a certain temperature or time, with some studies defaulting to the use of 165°C/30 min to simulate the industrial norm. In this review few studies investigated the effects of heat treatment on various types of sludge under different conditions were found.

A laboratory-scale thermal treatment by steam explosion on digestate from a wastewater treatment plant, was tested by Ortega-Martinez et al., (2016) using a range of 110-200°C and a time of 0-50 minutes. The authors’ found the optimum setting to be 180 and 200°C at 30 mins, which saw an increased methane yield of raw digestate by 50%. Bjerg-Nielsen et al., (2018) chose a temperature interval of 120, 150, 170, 190 for 30 and 60 minutes, finding the optimum 170°C/30 min to raise the digestate from 52 to 222 L CH₄/kg VS.

Digestate (26 g TS/L) treated at 25, 100 and 180°C and a pH of 2, 4, and 6 for pH with hydrochloric acid for 1 hour was studied by Takashima and Tanaka, (2014). The methane production and particulate organic destruction of anaerobically digested sludge were improved, as the treatment temperature was raised. The cumulative methane production was increased from 0.11 g COD-CH₄/g COD-substrate for control to 0.12-0.18, 0.18-0.25 and 0.3-0.32 g COD-CH₄/g COD-substrate for the acidic thermal treatment temperature of 25, 100, and 180°C. The VSS destruction was also increased to 3.3-6.7, 8.0-11.3 and 24.7-26% for the ascending acidic thermal treatment temperature, against 3.3% for the control.

The methane yield of digested manure fibers (20% TS, 14.8% VS, 1.5 COD/VS ratio) by wet explosion for 145°C/10min, 165°C/10min, 165°C/20min, 165°C/10min O₂, 180°C/10min) was studied by Biswas et al., (2012). The authors found that the highest methane yield (224 mL/g VS) for the digested fibers was achieved after 165°C under the addition of oxygen. However, there was an indication that the addition of oxygen during the WEx treatment may lead to inhibiting compounds. As a secondary option, the authors stated that WEx treatmet at 180°C for 10 minutes without oxygen resulted in the highest increase of methane yield (+136%).
A comprehensive study of different thermal post-treatment conditions (134°C to 175°C from 20 min to 30 min) on the effect of two different digestate cake characteristics was provided by Svensson et al., (2018). In the experiment, 18%TS cake from the Hadeland and Ringerike waste company (HRA), Norway and 21.8%TS cake from the Hampton Roads Sanitation District’s (HRDS) Nansemond Treatment Plant, USA were tested using a small CAMBI steam explosion unit. Results showed that THP temperature and cake origin had significant effect on the solubilization of the digested cake for both cakes for temperatures up to 175°C and treatment time 30 min. The highest solubilization was achieved for both digestates at the 30 min treatment at 175°C (32% and 15%). The differences in solubilization and specific methane yield between HRSD and HRA cakes, were attributed to the use of two different THP pilots and different sludge characteristics (water content, COD:VS ratios, C:N ratios and fraction of fiber). They showed no significant difference in BMP of the two cakes at different THP treatment times or temperatures. The BMP of the treated cake in all cases were lower compared to the untreated cake, and ranged from 41 to 53 mL CH₄/g TS added HRA and 38 to 59 mL CH₄/g TS added (compared to untreated of 75 and 63 mL CH₄/g TS added).

There is some additional literature data that also confirms negative impacts of post-treatments on methane production on digestate (Jagadabhi et al., 2008; Kaparaju and Rintala, 2005; Menardo et al., 2011a; Sambusiti et al., 2015) This may lead to the discussion about which situations, it may be better to instead recycle the centrate rather than treated digestate for improved biogas production.

All studies proved that digestate still contains a considerable amount of undigested organic matter and that holding time has negligible effect on results at higher temperatures. Although the optimum conditions seem to be between 170-180°C for 30 minutes for un-dewatered digestate (10-30% TS), continued experimentation is required as the optimum TH treatment conditions may depend on the effect of organic loading rate, hydraulic retention time and plant feeding for site-specific AD plants.
2.3.3. Literature Review on Intermediate Thermal Hydrolysis
So far, ITHP studies have mainly focused on the proof of the intermediate concept using laboratory-scale studies (see Table 2). As Bjerg-Nielsen, Ward, Møller, & Ottosen, (2018) noted, the limited literature has studied the specific substrate degradation kinetics and dewaterability rather than biogas production potentials. In addition, the consequence of different ITHP setups makes it difficult to compare the results (especially of BMP studies) to others in literature. The reviewed studies are therefore useful for comparisons within their own single assay runs.

Table 2-2: Published papers investigating intermediate hydrolysis process for enhanced anaerobic digestion at a laboratory scale.

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Substrate</th>
<th>Treatment Conditions</th>
<th>AD Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Takashima, 2008)</td>
<td>Sewage sludge (4.5% TS)</td>
<td>120°C for 1h</td>
<td>35°C, 20 HRT</td>
<td>Compared to control VS destruction: 4.5% pre, 6.6% post, 9.9% inter-stage. VSS reduction: Control (48.7%), Pre-(65.8%), Two-stage control (52.7%), Inter-stage (67.6%).</td>
</tr>
<tr>
<td>(Takashima and Tanaka, 2010)</td>
<td>Sewage sludge – mixed primary and WAS (4.3% TS)</td>
<td>Acidoic thermal treatment (ATT) 170°C, pH 5, 1hour</td>
<td>35C, 20 HRT Intermediate (10+10 days)</td>
<td>VSS reduction: Control (48.7%), Pre-(65.8%), Two-stage control (52.7%), Inter-stage (67.6%).</td>
</tr>
<tr>
<td>(Nielsen et al., 2011)</td>
<td>WAS (2.3% TS)</td>
<td>80°C for 10 hr, 130°C (15min), 170°C (15min), 170°C/pH 10 (15min)</td>
<td>37°C, 40 days Intermediate (19-21 days/ 37°C)</td>
<td>Compared to control (methane yield), Pre-treatment, 130°C (+13%), 170°C (+9%), 170°C/pH 10 (+2%); Inter-stage: 130°C (+9%), 170°C (+29%), 170°C/pH 10 (+28%). VS reduction: 55-60% THP, 66-68% ITHP. Biogas Production: 350 m3/TDS CMAD, 387 m3/TDS THP+MAD, 450 m3/TDS ITHP. In ITHP configuration, protein is the main degradation limiting substrate factor. The benefit of ITHP configuration is supported by the rate of carbohydrate, protein, lipids and fibre degradation results.</td>
</tr>
<tr>
<td>(Shana et al., 2012a)</td>
<td>60% primary and 40% SAS (5% DS and 76% VS)</td>
<td>170°C, 8 bar, 15-20 minutes</td>
<td>35°C 18 HRT 16+THP+16</td>
<td>VSr: THP (47%), ITHP (62%). Biogas production: THP (345m3/ TDS), ITHP (478 m3/ TDS) (+38%).</td>
</tr>
<tr>
<td>(Shana et al., 2012b)</td>
<td>60% primary and 40% SAS (5% DS and 76% VS)</td>
<td>170°C, 8 bar, 15-20 minutes</td>
<td>35°C 18 HRT 16+THP+16</td>
<td>VSr: THP (47%), ITHP (62%). Biogas production: THP (345m3/ TDS), ITHP (478 m3/ TDS) (+38%).</td>
</tr>
<tr>
<td>(Shana, 2015)</td>
<td>60% primary and 40% SAS</td>
<td>170°C, 8 bar, 30 minutes</td>
<td>ITHP 38-32 days</td>
<td>VSR: THP (47%), ITHP (62%). Biogas production: THP (345m3/ TDS), ITHP (478 m3/ TDS) (+38%).</td>
</tr>
<tr>
<td>(Ortega-Martinez et al., 2016)</td>
<td>Raw mixed sludge (27.6 g TS/L) and digestate (222 gTS/ kg) both diluted to 12-14%</td>
<td>Thermal/ steam explosion (110-200°C 0,10,30,50 min) 180°C, 30 min best</td>
<td>35°C, 40 days</td>
<td>Biodegradability of mixed sewage sludge rises to 62% from 36% with Inter-stage. ADMI Results: Inter-stage improves methane gen. by 45% and 20% compared to control and pre-treatment.</td>
</tr>
<tr>
<td>(Bjerg-Nielsen et al., 2018)</td>
<td>WAS (4.1%)</td>
<td>Thermal hydrolysis (120-190°C and 30-60 min)</td>
<td>35°C, 15day</td>
<td>170 and 30 min optimum.</td>
</tr>
</tbody>
</table>
Methane yield: Control one-step (52 LCH₄/kg VS), Control two-step (147 L CH₄/ kg VS), ITHP (222 LCH₄/kg VS).

WAS (0.8 %TS, 70% VS) 4% NaOH at 70 and 90°C 35-38°C, 20 day 7-d and 15-d digestate treated

IHP of a 7-day digestate for second-stage AD process, generated 23% and 16% higher methane in comparison to pre-treatments.

VS Destruction: CMAD (44%), THP (59%), SAS only THP (55%), I-THP (65%).

Disposal Volume (m³/ TDS): CMAD (3.3), ITHP (1.8%), SAS only THP (2.1), I-THP (1.5).

Digester Volume for 100 TDS/d, CMAD (46350 m³), IHP (14300 m³), SAS only THP (26250 m³), ITHP (29000 m³).

### 2.3.3.2. HRT Selection

The optimization of the HRT for both the first and second AD reactors is important for understanding the true performance of the ITHP process. But during the review, less emphasis/discussion was placed on HRT selection, as studies were focused on either optimizing TH conditions, or proving the concept validity on a laboratory scale. Generally, the experiment HRT selection followed a pattern when comparing ITHP to CMAD or pre-treatment. For example, if the CMAD had an HRT of 20 days, the semi-continuous IHP would be split into 10 d (A1) and 10 d (A2), or for BMP tests the control would be run for 40 days, and the ITHP for 19-21 days per reactor - although this could have been a function of the experiments organic loading rate. A. D. Shana, (2015), were the only ones to specifically optimize their system for each process. After weeks of digestion, each process was tailored to the following: CMAD (18 d HRT), THP+MAD (16 d HRT), and CMAD+THP+MAD (16 d+16d HRT). As a guide for future experimentation, Chauzy, Kline, et al., (2014) stated for general optimization of the process, the first digester should achieve at least 85-90% VSr compared to CMAD. The authors further estimated that the first digester would typically operate with a HRT of 12-15 days (mesophilic) or 7-9 days (thermophilic), while the second digester would be the usual 15-day HRT.
2.3.3.3. Performance
On a laboratory-scale, the ITHP process performed better in terms of methane yield and volatile solids reduction in comparison to CMAD and pre-treatment configurations. Shana et al., (2013, 2012a) showed that the ITHP configuration produced 20% more biogas compared to pre-treatment configuration with a 66% volatile solids reduction. Similarly, Takashima and Tanaka, (2014) showed that on average, ITHP VSS reduction was 67.6% in comparison to 48.7% for one-stage control, 65.8% for one stage with pre-treatment, and 52% for two-stage without treatment (Campo et al., 2017). In addition, Shana, (2015), results of average volatile solids destruction achieved by ITHP, THP, DMAD, and CMAD were 62%, 47%, 52% and 44%, and a 38% increase in biogas compared to THP.

At a pilot-scale, the ITHP performance was also confirmed by Mills who built on the fundamental science by Shana, and designed and developed a ITHP pilot plant (see Figure 5). After initial running of the ITHP system, the collected data, and with generalized values (that were not site specific or project specific), the performance of three THP variants (CMAD, Conv THP, SAS only THP, and ITHP) were assessed. All were shown to have many advantages over CMAD. THP and WAS only THP were similar in performance although WAS only THP doesn’t require support fuel and had a small reduction in performance. On the other hand, ITHP showed a clear improvement over conventional THP requiring no fuel support and producing 10% more biogas than THP, with a net efficiency of 17%. However, it does require a greater digestion capacity which in many cases may not exist onsite for use.

Figure 2-5: Intermediate THP Pilot from Mills, (2015).
A desk-top study comparison between Cambi and Exelys (Lysis-Digestion (LD) and Digestion-Lysis-Digestion (DLD)) was also completed by Abu-Orf and Goss, (2012) using a 40-dry ton per day biosolids feed. Through collaboration with both THP vendors for system sizing and budget cost estimates, a simulation was made consisting of the following systems: three Exelys LD trains, 2 DLD trains and one Cambi train with three reactors. Exelys systems were found to have a smaller footprint than Cambi for treating the same amount of residuals. Exelys also provided redundancy due to having multiple trains, making it possible to schedule maintenance shutdowns. Cambi, on the other hand, had lower steam consumption due to steam recycling the flash tank. DLD configurations was found to be the most energy efficiency system offered since all the biogas generated could be used for electrical production. However, DLD requires twice the digester volume (512,019 ft$^3$) as Cambi and LD (256,410 ft$^3$). Further investigations are required to determine whether the performance improvement is worth the extra costs.

Table 2-3: Technical performance comparing MAD, THP pre-treatment, SAS only THP, and intermediate THP modified from Mills, (2015).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Conventional MAD</th>
<th>THP</th>
<th>SAS only THP</th>
<th>I-THP</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS Destruction</td>
<td>%</td>
<td>44%</td>
<td>59</td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>DS Destruction</td>
<td>%</td>
<td>34%</td>
<td>45</td>
<td>42</td>
<td>50</td>
</tr>
<tr>
<td>Disposal Volume</td>
<td>m$^3$/TDS</td>
<td>3.3</td>
<td>1.8</td>
<td>2.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Gas Yield</td>
<td>m$^3$/TDS</td>
<td>339</td>
<td>454</td>
<td>421</td>
<td>503</td>
</tr>
<tr>
<td>Gas Yield</td>
<td>MWh/TDS</td>
<td>2.16</td>
<td>2.90</td>
<td>2.69</td>
<td>3.21</td>
</tr>
<tr>
<td>Elec Efficiency (gross)</td>
<td>%</td>
<td>15.4</td>
<td>20.6</td>
<td>19.1</td>
<td>22.7</td>
</tr>
<tr>
<td>Elec Efficiency (net)</td>
<td>%</td>
<td>13.7%</td>
<td>17.8%</td>
<td>16.3</td>
<td>19.1</td>
</tr>
<tr>
<td>Electrical Output</td>
<td>MWh/TDS</td>
<td>0.82</td>
<td>1.10</td>
<td>1.02</td>
<td>1.21</td>
</tr>
<tr>
<td>Support Fuel</td>
<td>MW/TDS</td>
<td>-</td>
<td>0.37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Net Electrical Output</td>
<td>MWh/TDS</td>
<td>0.73</td>
<td>0.95</td>
<td>0.87</td>
<td>1.02</td>
</tr>
<tr>
<td>Digester volume for 100 TDS/d</td>
<td>m$^3$</td>
<td>46350</td>
<td>14300</td>
<td>26250</td>
<td>29000</td>
</tr>
<tr>
<td>Digester vol. efficiency</td>
<td>MWh pa/m$^3$</td>
<td>0.65</td>
<td>2.82</td>
<td>1.36</td>
<td>1.55</td>
</tr>
</tbody>
</table>
2.3.3.4. Economic

From the laboratory and pilot-scale results, it is apparent that incorporating ITHP could provide significant economic benefits due to the potential saving investment costs from a smaller THP unit and operating costs from the reduced amount of material to be treated. For WWTPs looking to retrofit or construct a new anaerobic digester system, project economic and risk simulations/assessments will be needed to help decision makers in choosing the appropriate configuration. Even though the literature is scarce, and current studies are focused on the optimization and design of the process, three economic evaluations were reviewed.

Campo et al., (2017) simulated a full scale WWTP (2,000,000 p.e.) for both pre-treatment and intertreatment, using the results obtained in the laboratory-scale experiment. Results showed that when pre-treatment was used to sustain the system the revenues from electricity sale could increase between 13 and 25% in comparison to the conventional scenario. Intermediate treatments, on the other hand, using the 7-day digestate could gain 26% or 32%, depending on the treatment temperature (70°C or 90°C) (assuming that the heat produced by the digesters and the net produced electricity was sold at 0.217 €/kWh, including the public subsidy, valuing the methane at 0.855 €/Nm³ CH₄).

Mills, (2015) researched the best options for unlocking the full energy potential of sewage sludge, through a comparison with conventional THP, THP with SAS only, ITHP of SAS only, ITHP , THP+drying for fuel, and THP+Drying+Pyrolysis (see Table 4). Based on the performance of laboratory under stable conditions, the use of costs (electricity use, labour, polymer, char disposal, digestion volume, maintenance), revenue (sale of solid fuel, electricity generated, electricity eligible for ROCs) and a combination of CapEX and OpEX, a Net Present Value after 20 years was calculated. ITHP was found to recover the most energy from sewage sludge and the most efficiently (no support fuel) but requires additional CapEx. Regardless, the overall investment (NPV) was superior to all other AD based options.
Table 2-4: Summary of Future Processes Opportunities (information retrieved and modified from Mills, (2015)).

<table>
<thead>
<tr>
<th>Performance</th>
<th>Units</th>
<th>Conv. THP</th>
<th>THP with S.Exp</th>
<th>2nd Gen THP SAS only</th>
<th>2nd Gen THP ITHP</th>
<th>THP+Drying for Fuel</th>
<th>THP+Drying+Pyrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy yield (elec)</td>
<td>KWh/TDS</td>
<td>1,100</td>
<td>1,160</td>
<td>1,020</td>
<td>1,210</td>
<td>950 (+2,380 fuel)</td>
<td>1,830</td>
</tr>
<tr>
<td>Parasitic elec</td>
<td>KWh/TDS</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>200</td>
<td>340</td>
<td>580</td>
</tr>
<tr>
<td>Solids Destruction</td>
<td>%</td>
<td>45</td>
<td>45</td>
<td>42</td>
<td>50</td>
<td>45</td>
<td>77</td>
</tr>
<tr>
<td>Carbon Emissions</td>
<td>kgCO₂/TDS</td>
<td>143</td>
<td>137</td>
<td>154</td>
<td>138</td>
<td>-421</td>
<td>-614</td>
</tr>
<tr>
<td>OpEx</td>
<td>£/TDS</td>
<td>11</td>
<td>19</td>
<td>7</td>
<td>34</td>
<td>48</td>
<td>115</td>
</tr>
<tr>
<td>RE Incentive prop of revenues</td>
<td>%</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>CapEx</td>
<td>£M/100TDS</td>
<td>38.1</td>
<td>37.0</td>
<td>37.6</td>
<td>39.3</td>
<td>41.7</td>
<td>50.8</td>
</tr>
<tr>
<td>IRR</td>
<td>%</td>
<td>12.5</td>
<td>14.7</td>
<td>14.7</td>
<td>16.6</td>
<td>19.0</td>
<td>18.6</td>
</tr>
<tr>
<td>NPV after 20 yrs</td>
<td>£M</td>
<td>13.7</td>
<td>19.1</td>
<td>17.1</td>
<td>20.9</td>
<td>22.7</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Abu-Orf and Goss, (2012) on the other hand, using a 20-year Present Worth (PW) Costs, concluded that ITHP did not appear to a suitable solution for a “green field” plant. With the assumption of inflation and discount factors, and a constant sludge loading over the lifecycle period, the ITHP option (in comparison to Cambi, pre-treatment, and conventional AD) was the most expensive in terms of capital costs. The opinion of cost estimates found that an estimated Exelys DLD option would cost $83,946,000 finding that the savings do not payback with the added expense associated with the additional digester capacity (see Figure 8). The authors concluded that this is only one example case. The benefit of the improved energy balance associated with ITHP options may still be warranted.

2.3.3.5. Alternative Sub-configuration
One alternative sub-configuration is the placement of thermophilic (55°C) as the first digester, a process used at Csepel WWTP DLD (see Figure 5) (Shen et al., 2015). The thermophilic digester (55°C) would allow for faster processing time and a lower HRT, in comparison to a conventional mesophilic reactor. Although thermophilic digesters are not commonly used due to the higher energy costs, lower quality supernatant with large quantities of dissolved solids, its bad odour generation capacity and instability at higher volatile solids loading rates, the inclusion into this process, might be a suitable for its

Figure 2-6: Csepel WWTP Exley™-DLD process (image modified from (Shen et al., 2015)).

In the second alternative configuration, WAS would bypass the first anaerobic digester, where only primary sludge enters the digester before being mixed with untreated WAS for thermal treatment. As shown in Figure 6, this could decrease the first digester in comparison to full DLD process. But, as stated by Chauzy et al., (2014b) the CHP would not be able to produce all the steam alone, and part of the biogas produced would be sent to an additional steam boiler. Chauzy et al., (2014b) also provided an example of where this configuration would be selected over full DLD option. In a case project for the municipality of Ljubljana in Slovenia, this configuration was chosen over Full DLD because the in Full DLD the first MAD would have had too short an HRT (10 days) for proper VS removal. Therefore, the partial DLD was used where only primary sludge was treated which increased the HRT to 15 days for the first stage anaerobic digestion.
2.3.3.6. Recommendations

The Intermediate Hydrolysis Process, from initial laboratory scale results, has the ability to perform better in terms of methane yield and volatile solids reduction than conventional mesophilic anaerobic digester and THP pre-treatment followed by AD. Although research seems to be in the preliminary stage of proving the validity of the concept, there remains a need for more comprehensive studies on the comparison between pre-treatment and two-stage mesophilic digesters. Further continuous flow comparison of ITHP will be needed for both optimizing the process for HRT configuration, recognizing the rate-limiting step, and pinpointing potential operation and design problems (Parkin and Owen, 1986).

2.3.4. Literature Review on Post-Digestion with Recycle Loop Back Into AD
2.3.4.1. Introduction

In the typical post-anaerobic digestion treatment (PAD) setup (currently patented as Digester-Lysis by Veolia), the digestate would be dewatered, and a fraction of the cake would be treated with THP and then recirculated back into the original digester (see Figure 7). Sambusiti et al., (2015) explained the importance of treating digestate before recycling as a fundamental step to this process. As reported by the authors, during the anaerobic digestion process, hemicelluloses are degraded faster than cellulose, which results in an accumulation of cellulose and lignin in the solid digestate. Therefore, applying a pre-treatment technology is fundamental to increasing the availability of cellulose to anaerobic microorganism and
avoiding the build-up of recalcitrant materials. In addition, another factor of major importance to this process is the control of the recycling rate through the THP plant. As stated by Chauzy et al., (2014b), finding the optimum recycling rate through the THP plant will be necessary for maximizing both biogas production and volume reduction.

Figure 2-8: Digester-lysis with recycle loop configuration (retrieved and modified from (Chauzy et al., 2014b)).

Theoretically, the advantages of PAD are: (1) save energy as thermal treatment energy is spent only on recalcitrant compounds; (2) to improve the overall methane yield by recycling thermally treated digestate back to the digester; (3) reduce the digestate volume; (4) reduce GHG emissions emitted from digestate that would have otherwise been stored in outdoor areas; and (5) the possibility of a higher capacity of sludge processing with a fixed reactor size by reducing HRT without decreasing SRT (Thygesen et al., 2014). Monlau et al., (2015) also commented that recirculation could also lower operational costs by reducing nutrient and water addition, when a feedstock has low nutrient contents.

Although, this configuration seems to be advantageous over conventional mesophilic anaerobic digestion, there is scarce literature on fundamental studies focusing on development of post-treatment techniques (in comparison to pre-treatment) (see Table 5). This literature review focused on highlighting the areas that have been investigated so far such as (1) the optimization of post-treatment conditions and the effects on digestate; (2) reactor performance and recycling rate; (3) economic predictions; and (4) investigation of new sub-PAD configurations.
Table 2-5 Published laboratory results in an investigation to prove the effectiveness of anaerobic digestion with post-treatment recycling.

<table>
<thead>
<tr>
<th>Ref</th>
<th>Substrate</th>
<th>Treatment Conditions</th>
<th>AD Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Takashima et al., 1996)</td>
<td>Activated sludge (20.7 g/L TS, 17.8 g/L VS)</td>
<td>Alkaline heat post-treatment (0.1 M NaOH, 175°C, 1hr, with mixing)</td>
<td>35°C, 30 d HRT 50 mL RR Continuous</td>
<td>Particulate solids decomposition: CMAD (61%), Without Post-treatment (100%), Methane production: CMAD (57%), RR (71%).</td>
</tr>
<tr>
<td>(Battimelli et al., 2003)</td>
<td>Waste activated sludge (WAS) 28g COD/L; 27 g SS/L</td>
<td>Partial oxidation through ozonation (0.16 g O3/ g SS)</td>
<td>Semi continuous anaerobic digester, 4L 35°C, Optimum recycling rate 25%</td>
<td>Increases of SS removal and COD removal of 54% and 66% compared to initial conditions. Decrease of hydraulic retention time from 24 to 19 days. No toxic effects. VFA concentrations very low.</td>
</tr>
<tr>
<td>(Jagadabhi et al., 2008)</td>
<td>Co-digestion: Grass (38% TS) Cow manure (6.5% TS)</td>
<td>Alkali-treated (20-60 g NaOH/kg VS) and untreated</td>
<td>5L CSTR anaerobic digester, 35°C 20 d HRT, Semi-continuous</td>
<td>Re-circulation of alkali treated and untreated decreased methane yield 11% and 21%. Problems with scum formation and accumulation of reactor materials and stratification.</td>
</tr>
<tr>
<td>(Biswas et al., 2012)</td>
<td>Filtered cow manure</td>
<td>Wet explosion (WEx) 180°C for 10 min without addition of oxygen</td>
<td>5L, 20 d HRT, Batch, Continuous (180 days)</td>
<td>WEx increase methane yield compared with untreated digested manure 136%. Continuous with recycle showed no inhibition, and overall methane yield 75% higher than control with addition of non-treated FCM.</td>
</tr>
<tr>
<td>(H. Li et al., 2013)</td>
<td>Sewage sludge (80% primary, 20% biofilm) 20 g/L TS</td>
<td>Alkaline post-treatment (0.1 mol/L NaOH)</td>
<td>35°C, 20 d, 10% RR, 15% RR, Continuous</td>
<td>Biogas production: 5% RR (+33% in comparison to control), 10% and 15% and lower increment of biogas yield than 5%, due to excessive inactivation of anaerobic bacteria in digester.</td>
</tr>
<tr>
<td>(Takashima and Tanaka, 2014)</td>
<td>Sewage Sludge (2.5% TS)</td>
<td>ATPT (170°C pH 5-6)</td>
<td>35°C, 20 d HRT, 30% RR Batch, Continuous</td>
<td>With recycle line approximately 755 of VS destruction in ATPT process, 2-2.5 times more than control process, 30-37%. ATPT showed 14-21% more methane production and 22-23% better dewaterability.</td>
</tr>
<tr>
<td>(Sambusiti et al., 2015)</td>
<td>Digestate (DIG) and solid separated digestate (SS-DIG)</td>
<td>Thermal Thermo-chemical enzymatic BMP 35°C, 36 d HRT</td>
<td>Methane yields: Control DIG, Post Thermal (no effect), Post Alkaline (no effect), Enzymatic (+51%).</td>
<td></td>
</tr>
<tr>
<td>(Tian et al., 2016)</td>
<td>Mixed PS and WAS</td>
<td>ultrasonic (ULS), ozone assisted ultrasonic (ULS-Ozone) and alkaline assisted ultrasonic (ULS+ALK) post-treatment</td>
<td>35°C, 30 days, Batch</td>
<td>Methane production increased by 48.3% after ULS-Ozone post-treatment. SMP and HA-like substances were produced as a result of combined post-treatments.</td>
</tr>
<tr>
<td>(Tian and Trzciniski, 2017)</td>
<td>Mixture of primary sludge and thickened waste activated sludge (1:1 15g TS/L)</td>
<td>ultrasonic (ULS), ultrasonic-ozone (ULS-Ozone) and ultrasonic + alkaline (ULS+ALK) post-treatments</td>
<td>35°C, 10 or 20 d HRT Sludge recycle ratio 50 or 100% (Qo/Qin) Continuous</td>
<td>The post-treatment resulted in a decrease in the cellular ATP concentration indicating stress imposed on microorganisms in the reactor. Tested post-treatment methods showed 4-7% decrease in effluent VS.</td>
</tr>
<tr>
<td>(Nuchdang et al., 2018)</td>
<td>Raw algal feedstock</td>
<td>Hydrothermal treatments (WetOx) Hydrothermal Carbonization (HTC) (200°C, initial pressure 0.1, and 0.82 MPa)</td>
<td>35°C, 4L, 3 g VS/L d (OLR), batch</td>
<td>Hydrothermal treatments increased methane yield to 200 LCH4/ kg VS, compared to untreated digestate (66 LCH4/kg VS).</td>
</tr>
</tbody>
</table>
2.3.4.3. Reactor Performance and Recycling Rate

Several studies showed in a continuous feed laboratory-scale digester, the recirculation of the digestate in a biogas plant can be a viable alternative to pre-treatment (Tian et al., 2016). For instance, Biswas et al., (2012) tested the new concept on a laboratory-scale with continuous feeding experiment with recycling, and wet explosion (WEx) (180°C for 10 minutes) applied to residual manure fibers (see Figure 9). They found there was no inhibition, and an increase in the overall methane yield of 75%. Takashima and Tanaka, (2014), also showed that performance enhancement a 30% recycling rate, using two single-stage anaerobic digestion processes (see Figure 8b). The control process showed 30-42%, 30-37% and 31-46% of VS, VSS and COD destruction respectively, while the destruction percentage in the ATPT process was almost double the control (66-67%, 75% and 67-71%).

(b)

Figure 2-9: Laboratory test configurations used by Biswas et al., (2012) and Takashima and Tanaka, (2014).
These first studies are useful for building awareness of the potentials of the configuration, but there is a lack of discussion on the influence of the recycling rate. As Battimelli et al., (2003) and H. Li et al., (2013) indicated, the recycling ratio of the post-treated sludge is an important operational parameter with impacts on the actual solids retention time (SRT) of the anaerobic reactor, as well as the anaerobic digestion performance.

The recycling rate can have an impact on the effluent composition and biogas production. H. Li et al., (2013) showed this by using a RR of 5, 10 and 15% with alkaline as the post-treatment were applied showing an increase in biogas production was increased after the treated sludge was added back into the digester. In the experiment, when the RR was 5%, the accumulative biogas yield increased by 26.5% in a 24-hr cycle and continued to increase for 10% and 15%. Although, the increment of increased biogas yield was lower than when 5% which may be attributed to the excessive inactivation of anaerobic bacterial.

There is the danger of using a recycling ratio that is too high, which can cause the inactivation of anaerobic microorganism, and lead to the deterioration of the anaerobic digester with similar results found by Jagadabhi et al., (2008). In the laboratory experiment, the solids (co-digested grass silage and cow manure) were recirculated (after alkaline treatment) into CSTRs that were operating at an organic loading rate of 2 kg VS/m³ d and a 20 day HRT with semi-continuous feeding. The results showed that the recirculation for both alkaline-treated and untreated forms, caused a decrease in the methane yield by 11% and 21%, with an accumulation of materials and scum formation.

There is also the unexplored issue of when the digested sludge receives the thermal post-treatment more than once with the progress of operation. As noted by Takashima and Tanaka, (2014), in this situation, the non-biodegradable solids may gradually accumulate in the digester. Accordingly, it is presumed that methane production will be decreased, and solids destruction enhanced with time. But the methane recovered was much lower than expected from the solids destruction obtained. This is because, during the thermal treatment, part of organic matters were converted to non-biodegradable compounds, such as soluble coloured ones, and were lost by self-burning at the temperatures higher than 150°C, as shown
by the lower COD recovery (loss during the ATPT to be 22.4 and 13.9 % of the influent COD) (Takashima and Tanaka, 2014).

But for laboratory-scale studies, there is the issue of the batch anaerobic digestion assays, over predicting the performance of the system. As Tian et al., (2016) found, the recycling rate in continuously feed systems can also show lower than expected results than those obtained in batch anaerobic digestion assays. The authors stated that when post-treated digested sludge acted as a substrate in batch assays there was given sufficient time (30 days) for the degradation. But in contrast, the hydraulic residence time (HRT) was much shorter in semi-continuous reactors. For example, in the batch assay ULS-Ozone post-treatment resulted in higher ultimate methane production than the ULS+ALK post-treatment. But this was not the case when the post-treatment was applied in semi-continuous reactors (Tian et al., 2016).

More research on post-treatment recycling rates should be tested, especially in pilot-scale to understand the effects of altered digestate may have on the anaerobic digestion microorganism and in which situations the mixture of feed will decrease the overall performance from a less biodegradable substance. For example, high ammonia concentrations in the digestate can lead to failure of the process and low methane production (Monlau et al., 2015). As stated by Battimelli et al., (2003), information about the anaerobic digestion performance and the stress on microbial communities with post-treatment at different hydraulic retention times (HRTs) and recycle ratios are currently unavailable.

2.3.4.4. Preliminary Economic Predictions
As mentioned above, the treatment of digested sludge in the new treatment concept has the potential to offer significant economic benefits. Sambusiti et al., (2015) stated the configuration has the ability to overcome problems related to disposal, reducing the digestate steam produced and maximizing the economic value of the biomass used. During the literature review, preliminary economic predictions were scarce.
In the discussion of the major differences between pre-treatment and post-treatment, Svensson et al., (2018) emphasized that in comparison to pre-THP, PAD-THP has the ability to improve cake solids during final dewatering which would have a large beneficial impact on economics. The authors cited Hasan et al., (2017) who reported that cake solids after dewatering of a mixed primary and second sludge before pre-THP was around 43%, but decreased to 31% after anaerobic digestion. The results demonstrated that post-treatment could increase the maximum cake solids in digestate cakes. Predicting that the maximum cake solids for HRS could be improved from 34% up to 46%, and 17% up to 43% for HRSD, stating that for HRSD, if PAD-THP was implemented, for every ten trucks need to dispose of digestate cake, only four would be needed if THP was implemented (see Figure 10). The authors also stressed the importance for knowing the wet cake mass before the digestate is treated, as cake that is already dewatered to 30% before PAD-THP will have less potential for reduced wet cake mass, compared to the cake dewatered to 15%.

Sambusiti et al., (2015) quantified the extra electrical production, post-treatment could offer based off experimental batch anaerobic digestion data. In the preliminary energetic balance, three different cases were studied: (A) the recirculation of digestate; (B) the recirculation of enzymatic post-treated digestate, and (C) the recirculation of solid separated digestate. According to authors the energetic balances, the recirculation of DIG and enzymatic post-treated DIG provided a supplementary electrical production of 3182 and 4818 KWh/ day. In the case of recirculation of SS-DIG, an extra electrical production of 3361 KWh/ d was
computed, after subtracting the electrical requirement of the screw mechanical separator. In addition, when government incentive policy for biogas energy in Italy was factored in, the recirculation of digestate and solid digestate offered extra income to farms of 891 and 941 €/day.

Biswas et al., (2012) found that the treatment of the digested fiber faction in the new concept offered two economic benefits: the biogas yield per ton of manure feedstock increases and the costs for treating only the separated digested fiber fraction are significantly lower than for the pre-treatment of the whole reactor feed. In the case of Biokraft’s biogas plant roughly 100 kg of separated digested fibers are leaving the reactor per ton of input. Consequently, the volume to be treated is only 10% compared with pre-treatment of the whole input, reducing the operational costs accordingly. Through the mass balance based on Biokraft’s production data, and the biogas yield by WEx treatment of the digested fibers, the methane yield per ton of feed can be increased from 23 to 28 m$^3$ by the recirculation and to 33 m$^3$ when the biodegradability of the fiber fraction is enhanced from 40 to 75%. This could make the new concept economically viable.

2.3.4.5. Alternative Sub-configurations

Post-treatment with recycling is a flexible configuration that offers multiple ways for efficient operation depending on the specific site. As more research is conducted, it is likely there will be further explorations into the development of more challenging process schemes that can further improve the solid degradation efficiencies. Two reported alternative post-treatment configurations include CambiSolidStream and a “closed loop operation.”

In the CambiSolidStream Process, the digestate would be dewatered, treated with THP and then after THP undergo a subsequent dewatering, where the liquid fraction is recirculated to the anaerobic digester (see Figure 11) (Kjorlaug et al., 2017). From the CambiSolidStream website, it is said this process can increase dewaterability of digested solids to 40-60% DS, with water volume and associated costs reduced by 75%. But, as stated by Svensson et al., (2018), the mechanisms of the technology are not well documented and understood. For example, recirculation of the centrate from the post-treated digestate can results in a
reduction of sludge retention time (SRT), which could reduce the efficiency of the AD process, possible counteracting the benefit effect of post-treatment. Therefore, the microbial community adaptation of centrate should be studied, along with the effects of diluted centrate, in order to avoid high concentrations of propionate and acetate in the reactor (Kjorlaug et al., 2017).

Figure 2-10: Cambi SolidStream concept (Retrieved from Kjorlaug et al., 2017).

Goel et al., (2003) tested a closed loop operation in a laboratory scale study, and found it promising for achieving higher solid reduction efficiencies. In this system, there was no withdrawal of the solids (as shown in Figure 11). The authors tested two configurations: one with pre-ozonation and the other with post-ozonation. It was predicted that for both there would be an accumulation of solids in the reactor, and a possible issue for feasibility. To control the accumulation, the reactor was checked for improvements in substrate biodegradability and organic solid removal. Both systems showed similar performance with solid degradation efficiencies between 81-86%. But post-ozonation was concluded to superior configuration, due to the degradation efficiencies, lower accumulation of volatile solids and lowered ozone dose.
2.3.4.6. Recommendations
Based on the initial laboratory-scale studies reviewed so far, some conclusions were drawn as follows:

1. The optimal THP conditions for other substrates, such as WAS, may not apply to whole, liquid or solid digestate. Studies on how digestates respond to THP, such as dewaterability, are currently lacking (Svensson et al., 2018).

2. More research on post-treatment recycling rates should be tested, especially in pilot-scale to understand the effects of altered digestate may have on the anaerobic digestion microorganism and impacts on the AD system such as energy consumption and effluent/residual generation.

3. Few papers have shown the operation of post-treatment with recycling using long-term continuous processes to prove the effectiveness of the system. This will be necessary for both pinpointing operational difficulties and drawing more precise energetic and economic balances.

2.3.5. Full-scale Configuration Comparison
To understand the potential performance and feasibility of ITHP and post-treatment, studies simulating each process at a pilot and full-scale were reviewed. Exelys patented Digester-Lysis-Digester (DLD) and Digester-Lysis (DL) were the only current full-scale commercial options, therefore the reported information was reviewed to understand the potential feasibility of ITHP and Post-treatment technology.
The only available full-scale comparison of the different configurations was completed by Chauzy et al., (2014b). The study simulated a WWTP producing 100 metric ton of production, using full-scale plant and prototype results. The configurations were compared in relation to biogas production, electricity production and dewatered sludge disposal improvement to conventional anaerobic digestion (see Table 6). Results showed the specific advantages and disadvantages of each configuration, highlighting that choosing the right configuration depends on the needs of the wastewater facility. Pre-treatment is recommended to be used in situations where intensive digestion to improve digester loading capacities, Intermediate could be used in situations where there is a lazy existing digester capacity, a need for class A cake and increased energy production, and Post-treatment could be used in smaller and larger facilities where there is a limitation of digestion capacity and limited room for expansion.

Table 2-6: Modified version of the Summary of the characteristics and main advantages of each configuration in comparison to conventional mesophilic anaerobic digestion (Chauzy et al., 2014b).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Full LD (pre-treatment)</th>
<th>Full DLD (ITHP)</th>
<th>DL (Post-recycling)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class A sludge</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Total Digester Volume</td>
<td>-62%</td>
<td>-15%</td>
<td>+13%</td>
</tr>
<tr>
<td>Overloading Capability</td>
<td>+167%</td>
<td>+18%</td>
<td>-11%</td>
</tr>
<tr>
<td>Size of THP</td>
<td>100%</td>
<td>64%</td>
<td>0 to 66%</td>
</tr>
<tr>
<td>VS removal %</td>
<td>+19</td>
<td>+28</td>
<td>0 to 18%</td>
</tr>
<tr>
<td>Electricity production</td>
<td>-6%</td>
<td>+28%</td>
<td>0-18%</td>
</tr>
<tr>
<td>Final Sludge to Discharge</td>
<td>-40%</td>
<td>-49%</td>
<td>0 to -36%</td>
</tr>
</tbody>
</table>

Viswanathan et al., (n.d.) recommend that an evaluation study should involve: (1) current and future capacity of the plant; (2) current and future energy needs and sources; (3) current and future process efficiency and available treatment options. For example, if the inclusion of a THP satisfies the economics on loading capacity, facility needs, status of current and future infrastructure and drivers such as capacity and regulation along with economic drivers like funding and available rebates, there are three main routes to determine which THP process to
include. Viswanathan et al., (n.d.) explained that if the DTFD of a WWTP is less than 35, and energy cost is not high, a continuous flow THP-LD configuration should be used. If the energy cost is high, a batch flow THP LD configuration should be used. On the other hand, if the DTFD is greater than 35, and the MAD capacity is high, with enough space, a Continuous Flow THP-DLD configuration would be optimal.

2.3.6. Conclusion
In this paper, published journal articles related to the movement from pre-treatment, to post-treatment in intermediate and post-recycling positions of thermal hydrolysis were reviewed. As pre-treatment has been growing in popularity, the literature on the application of post-treatment is limited. As of 2018, although there have been many studies published the potential to increase the methane yield and biodegradability of digestate with thermal treatment, few papers have investigated the enhancement of AD with the continuous flow of sludge through an AD process with intermediate hydrolysis (12 papers related) or post-treatment-recycling (10 related papers).

In this investigation scientists noted that it would be more energy efficient of thermal hydrolysis was applied, instead to influent sludge containing both readily biodegradable and non-biodegradable organic matter but to the effluent to the AD containing non-biodegradable organic matter (Takashima, 2008). In the first stage MAD, the easily biodegradable fraction of sewage sludge feed is expected to be utilized and part of the non-biodegradable content is expected to be loosened. The slackened organic bonds of the digested sludge constituents are then expected to become easily biodegradable after thermal hydrolysis process. The hydrolyzed product can then be expected to be further utilized through biochemical conversion in a second MAD or recycling into the first digester, to produce additional biogas, and a lower volume of final sludge to discharge (Shana, 2015).

On a laboratory-scale, in comparison to CMAD and pre-treatment, ITHP is the most efficient energy recovery AD based option in terms of VS reduction, biogas production, electricity production and the volume of sludge to discharge. But it requires additional capital expense and an additional digester that might not be available on currently operating WWTPs. On the
other hand, Post-THP-recirculation is the most flexible out of the other options due to its ability to be turned off and act like a CMAD. However, the discharged sludge will not be Class A, and the recycling rate will have to be monitored for limiting the washing out of microorganisms.

Using preliminary full-scale simulations by Mills, (2015) and Chauzy et al., (2014b), it was made clear that each configuration (pre-treatment, intermediate, post-) as a tool, have their own sets of advantages and disadvantages, stressing the importance that the selection of the right solution depends on the current and future plans of a WWTP. Pre-treatment is recommended to be used in situations where intensive digestion to improve digester loading capacities. Intermediate could be used in situations where there is a lazy existing digester capacity, class A cake and increased energy production. Post-treatment could be used in smaller and larger facilities where there is a limitation of digestion capacity and room for expansion.

Preliminary economic simulations showed that incorporating ITHP and Post-treatment with recirculation could provide significant economic benefits due to the potential saving investment costs from a smaller scale THP unit and operating costs from the reduced amount of material to be treated. For WWTPs looking to retrofit or construct a new anaerobic digester system, project economic and risk simulations/ assessments will be needed to help decision makers in choosing the appropriate configuration.

Future work is recommended to continue to develop fundamental studies (changes in microbiology of AD process) which are still required to optimize both ITHP and Post-recycling process. The optimal design and operation of the process still requires the recognition of the rate-limiting step and knowing the characteristics of the organic material being digested and creating the proper conditions for obtaining the desired level of organic matter destruction. As Shana, (2015) stated, there is a need to investigate the effect of the ITHP process through targeted changes in digestion process parameters and into the kinetics of the process to understand the mechanisms involved. In addition, pilot and full-scale of each configuration will be needed to confirm laboratory findings.
CHAPTER 3- MATERIALS AND METHODS

3.1. Introduction
Due to the difference in result section, evolving from batch to semi-continuous studies, it is easier for the reader to understand the materials available to the researcher by compiling them into one chapter. Future chapters will have a separate and more specific methodology to express the specifics of each study. These will include experiment objectives, experiment setups, reactor operations, and substrate and inoculum characteristics. For this chapter the general sections to be discussed include (1) feed and seed sludge, (2) reactors, (3) thermal hydrolysis unit, and (4) analytical methodologies for the characterization of sludge properties.

3.2. Feed and Seed
The feed and seed used for this thesis were collected from the Guelph Wastewater Treatment Plant (GWWTP). The GWWTP is located at Guelph, Ontario, Canada and provides treatment of domestic, commercial, institutional and industrial wastewater collected from the community of the Guelph/Eramosa [Wastewater treatment plant annual report]. The Guelph WWTP process consists of preliminary screening and grit removal, primary sedimentation, extended aeration activated sludge treatment, secondary clarifications, rotating biological contactors (RBC) and sand filtration tertiary treatment, and chlorine disinfection. The typical wastewater daily average flow treated by the Guelph WWTP is 50.02±15.6 ML/day, which contained a cBOD5 of 193.4±15.6, TSS of 257.2±27.1, total phosphorus of 5.14±0.38, TKN of 38.5±2.9, and NH3-N of 22.3±1.6 mg/L according to the annual average values from 2011 to 2015, and the recorded removal efficiencies for cBOD5, TSS, TP, TKN, and NH3-N are around 98.8%, 99.2%, 97.0%, 95.9%, and 97.9%, respectively. The raw sludge produced in the Guelph WWTP is thickened in the primary clarifiers and further thickened to a sludge of 4.3% solid content by a rotary drum thickener and sent to the anaerobic digesters. The WWTP plant generated 27,529 m³ of thickened sludge per year to the anaerobic digesters which were operated at a SRT around 15 days.

3.2.1. Feed
Thickened Waste Activated Sludge (TWAS) was collected from two sites for the duration of the experiments: Southern Ontario Water Consortium (SOWC) Guelph Facility and the GWWTP. At the GWWTP, the waste activated sludge from all plants are thickened in a
rotary drum thickener and then sent to one of the primary digesters. The rotary drum thickener is automated to run 24 hrs/ day, provided enough waste activated sludge is available. The unit is used a combination of anionic and cationic polymers at a ratio of approximately 1.10:1 to assist in thickening the waste activated sludge to 4.15% solids. Typical solids capture is 95-98%. Through request sample forms, the wastewater services department coordinated sampling times and on-site operators. The Guelph Wastewater Facility (GWF) is a bench and pilot-scale facility constructed adjacent to the City of Guelph’s municipal WWTP. The facility has a building footprint of 350 m2 and includes a lab and sample preparation areas. The GWF has direct access to the municipal wastewater at flow rates up to 300 m3/day. The GWF has access to various process streams including raw wastewater, primary effluent, secondary effluent, tertiary effluent, raw sludge and waste activated sludge. TWAS was made using a belt filter, thickening the WAS to 3-4% TS. Sludge samples were picked up with coordination with the SOWC Guelph Facility Manager.

3.2.2. Seed
Primary digested sewage sludge from the Guelph Wastewater Treatment Plant was employed as the seed for the batch and semi-continuous reactors. Anaerobic digesters at the GWWTP are high-rate mesophilic, with a working volume of 85-95%. Volatile solids destruction of 40-65%, Solids Residence Time 10-20 days (MOE guideline 15 days). The minimum VS loading is 1.3 kg VS/m3 d and a maximum VS loading of 3.2 kg VS/m3/d. The AD sludge showed a stable TS content of 19.6 ±0.3 g/L over the sampling period, which was very close to the annual average TS 19.5 g/L over the period of 2011 to 2015. The VS/TS and TSS/VSS ratio of the AD sludge were determined to be 0.63±0.14 and 0.70 ± 0.12, respectively. The relative stable TS and VS/TS ratio with the AD sludge suggests that the AD digesters of the WWTP can provide biological consistent inoculum for the sludge BMP tests.

3.3. Reactors
Experiments are typically performed in three different scales: laboratory, pilot and full scale. Full-scale experiments are often not justifiable because: (1) capital costs are high for construction, equipment and instrumentation, (2) operational costs during long evaluation times (several months) in AD research, (3) environmental and sanitary risk, and data quality being affected by noise introduced by factors from the world outside of the experiments (Lüdtke et al., 2017). Laboratory and pilot-scale studies are valuable for designing of an
efficient process and pinpointing operational difficulties. In conducting laboratory and pilot scale studies efficient digestion can be ensured because of maintaining adequate SRT, efficient mixing and the provision of suitable and uniform environment. Therefore, any problems should be associated with feed characteristics. In this regard, a characterization of the feed sludge is imperative (Parkin and Owen, 1986).

In this project, laboratory scale tests were used to keep costs low and experimental accuracy high (Lüdtke et al., 2017; Parkin and Owen, 1986). However, even though experimental noise in many cases can be compensated for by increasing the number of replicates, there will always be a trade-off between resources spent and the expected experimental power. This selection of experimental setup can be meaningless if the transferability to full-scale is low (Lüdtke et al., 2017).

Three different anaerobic digestion reactor configurations were used in this research. Chapter 5 focused on batch reactors, and Chapter 6 on the bench-scale semi-continuous reactors. For details on the organic loading rates and hydraulic retention time of the continuous reactors as well as the incubation period for the batch system, the description can be found in the various chapters. All reactors were incubated at mesophilic temperature, 35°C either in a temperature-controlled room or a water heated tank. Only the details pertaining to the design of the different reactors are described here.

3.3.1. Bottle batch reactor
A laboratory-scale batch test is the most commonly used tool for evaluation of a substrate’s methane yield through biochemical methane potential (BMP) tests and for gathering kinetic degradation data that can be used for modeling and simulations (see Figure 13). Biochemical Methane Potential (BMP) tests are a popular technique to determine the methane potential and biodegradability of wastewater and waste biomass (Remigi and Buckley, 2006). In the test, a substrate is mixed with an anaerobic bacteria culture, normally retrieved from an active digester. The bottles are then stored at a stable temperature of either 35°C or 55°C, and constantly mixed for a period of 30-60 days (Holliger et al., 2016; Strömberg et al., 2014). As the organic material is degraded, methane and carbon dioxide are produced. Since the volume of methane is the gas of interest, the volume of methane is determined by measuring the gas composition. The methane generated from the substrate is then calculated by
subtracting the methane volume from a blank. With the substrate methane isolated, the methane potential can be expressed as per mass of volatile solids added or COD added (Angelidaki and Sanders, n.d.). The biodegradability was calculated by dividing the cumulative methane volume (NmL CH₄ sub/ g COD initial sub) by the theoretical cumulative methane volume, which is obtained from the chemical ratio of 1 g COD= 0.35 mL CH₄ at STP.

\[ \frac{\text{NmL CH}_4 \text{ sub}}{\text{g COD initial sub}} \]

Figure 3-12: BMP serum bottles used for experiments

3.3.2. Semi-continuous reactor

Continuous laboratory scale experiments are applied when batch tests are not sufficient to answer the research question (Lüdtke et al., 2017). In anaerobic digestion research, this relates to topic such as (1) the evaluation of transient behaviour due to large process changes such as operation temperature, (2) the transition from single to multi-stage digestion, (3) influence of mixing and feeding frequency, (4) accumulation/depletion of certain micronutrients, (5) impact of solids retention time manipulation, or (6) the long term adaption of AD to different substrates and inhibitory compounds (Lüdtke et al., 2017).

In this experiment, two semi-continuous laboratory scale anaerobic digesters were used to investigate the performance of the ITHP system. However, there are several disadvantages with only feeding one to three times each day. When fed in batches, the bacteria are alternately starved and flooded with substrate. Since the acid and hydrogen-producing bacteria are much faster growers than the acid and hydrogen utilizing bacteria, batch feeding leads to surges in acid and hydrogen production causing potentially detrimental decreases in pH if not sufficient alkalinity is not present (Parkin and Owen, 1986). Excessively high
concentrations of ammonia-nitrogen may also be released during these surges (Parkin and Owen, 1986). It is recognized, that for long SRTs 30 days, the surges are minimized. The following sections will discuss the setup, operation and monitoring of both reactors.

3.3.2.1. Reactor One (R1)
A 10 L AD manufactured by GE Water and Process Technologies (Oakville, Ontario) was used in this study (see Figuer 14). The reactor was maintained at approximately 35°C using a hot water batch (StableTemp, Cole Parmer, Montreal QC) connected to a hot water jacket surround the mixing tank. The reactor pH was maintained in the range of 6.5-7.5 under batch feeding operation. Mixing was performed using a mechanical mixer at 50 rpm. Biogas flow rate was typically 40 standard cubic feet per minute (SCFM) and was controlled using a 100 standard cubic feet per hour (SCFH) gas flow meter (P5302325C13, Parker, Mississauga ON). Pressure sensors (Cerabar T, Endress Hauser) were installed on the digester tank. Level sensors were used to control the feed pump to maintain a working volume of 10 L in the system. The bioreactor temperature was maintained by recirculating hot water through the water jacket of the reactor. The pH of the reactor was controlled. Biogas production was measured on-line by a mass flow meter (Burkert 8700). A programmable logic controller (PLC) system was used for operation control and data collection.
3.3.2.1. AD preparation
The AD was filled with tap water, and pressure testing after delivery and before being seeded with sludge. Pressure testing was performed with nitrogen gas at an internal pressure of 15 kPa. Leaks were identified using soap and corrected to minimize the amount of oxygen entering and biogas escaping during operation.

3.3.2.1.2. Bioreactor seeding
Approximately 15-20 L of anaerobic sludge with mixed liquor suspended solids (MLSS) of approximately 17 g/L was taken from the Guelph Municipal Wastewater Treatment Plant primary digester. The sludge was sieved through #16 sieve with a nominal pore size of 1.19 mm to remove hair and large particles, which could damage the pumps and clog. The sieved sludge was allowed to settle at room temperature for 24 hours. The recirculation pump calibration cylinder was used to introduce 10 L of AD sludge to fill the bioreactor to the high-level sensor. Nitrogen gas was used to sparge the bioreactor for over one hour to remove oxygen and create an anaerobic environment. The following week periodic gas composition samples were taken to ensure anaerobic conditions.

3.3.2.1.3. Batch feeding
The reactor was fed once per day at an SRT/HRT of 12.5 days (1.6-1.88 g VS/L/ day). The feed was prepared weekly and stored at 4°C until required. The stored feed were sampled twice a week for determination of TS, VS, pH, VFA, and alkalinity to monitor changes in the feed characteristics during storage and digestion. Before the feed was pumped into the reactor, it was heated to 35°C using an incubator.

3.3.2.2. Reactor Two (R2)
A 1 L glass was used to simulate the second reactor (see Figure 15). The reactor was heated using a water batch set to 35°C, and mixed using a magnetic stirrer. To maintain the reactors stability a metal stabilizer was attached to the top plastic lid. The screw seal was drilled a hole through and plastic tube was inserted below the solution level for feed and sampling the digestate using a par pump. The biogas was released through two lines, one connecting to a 60 mL syringe for sampling and the other to a biogas collection bag.
3.3.2.2.1. AD preparation
Before the addition of seed sludge, the reactor was filled with tap water, and pressurized using nitrogen gas. The internal pressure was not monitored. Leaks were identified using soap and corrected. The reactor was left for 3 hours and the headspace gas composition was measured for oxygen. If oxygen was detected, the leak detection procedure was repeated.

3.3.2.2.2. Bioreactor seeding
The reactor was fed once per day at an SRT/HRT of 13.3 days (1.0-1.8 g VS/L/ day). The feed was digestate taken from the 10 L digester then thermally treated. The feed was prepared weekly and stored at 4°C until required. The stored feed was sampled twice a week for determination of TS, VS, pH, VFA, and alkalinity to monitor changes in the feed characteristics during storage and digestion. Before the feed was pumped into the reactor, it was heated to 35°C using an incubator.

3.3.2.3. Digester feeding cycle and Monitoring Schedule
Anaerobic digester monitoring was split into two sections: digestion process and characterizing the feed and digested sludge. The digestion process consisted of parameters that were automatically reported daily, such as feeding and wasting volumes, biogas volume, biogas composition and digester temperature and ORP (see Table 7). Characterizing the feed and digested sludge, on the other hand, required manual measurements and were completed two to three times a week. This schedule was maintained for all three reactors. However, due to the smaller reactors limited daily biogas production and measuring techniques, biogas...
volume was not measured. The use and focus for R2 and R3 was the differences in feed and digested sludge characteristics for thermal treated sludge.

Table 3-7: Anaerobic parameter measuring schedule (Shana, 2015)

<table>
<thead>
<tr>
<th>Anaerobic Digestion Factor</th>
<th>Parameters measured</th>
<th>Frequency of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed and digested sludge</td>
<td>Sludge dry solid and volatile solids</td>
<td>Twice a week</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>Twice a week</td>
</tr>
<tr>
<td></td>
<td>Alkalinity</td>
<td>Twice a week</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>Twice a week</td>
</tr>
<tr>
<td></td>
<td>SCOD</td>
<td>Twice a week</td>
</tr>
<tr>
<td></td>
<td>NH4</td>
<td>Twice a week</td>
</tr>
<tr>
<td>Digestion process</td>
<td>Digester feed and digested sludge volume</td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td>Biogas volume</td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td>Biogas composition</td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td>Digester temperature</td>
<td>Daily</td>
</tr>
</tbody>
</table>

### 3.3.2.4. Start-up period to Achieving steady state
Both digesters were operated at a hydraulic retention time of 12 days for a period of time sufficient to insure the establishment of viable methanogenic culture and to purge the seed sludge solids from the digesters. Then, each digester was subjected to a program of steady-stage operation at several different hydraulic retention times. For each hydraulic retention time studied, the system parameters were measured over one hydraulic retention time. To insure steady state conditions, three HRT were completed before data collection (see Table 8).

Table 3-8: Operating conditions used for controlling the laboratory scale semi-continuous anaerobic reactors, format adapted from (Shana, 2015)

<table>
<thead>
<tr>
<th>Operating condition</th>
<th>Units</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Type</td>
<td>TWAS</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Working reactor volume</td>
<td>L</td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td>HRT</td>
<td>Days</td>
<td>12.5</td>
<td>13</td>
</tr>
<tr>
<td>Feeding Interval</td>
<td>Hours</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Average Dry solids</td>
<td>%</td>
<td>2.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Volatile Solids Loading Rate</td>
<td>kg VS/m3/d</td>
<td>1.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>
3.3.2.5. Operation
To prevent AD failure and because of the low energy yield, low growth rate and extreme sensitivity of methanogens, the operational parameter must be periodically and precisely monitored and maintained within their optimum ranges (Amani et al., 2010). As mentioned previously important operational aspects include temperature, volatile fatty acids, oxidation-reduction potential, organic loading rate, alkalinity, pH, SRT, and mixing. The Table 9 was used as an operational guide for both reactors.

Table 3-9: Optimum and extreme conditions for anaerobic digestion adapted from (Amani et al., 2010)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Optimum</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatile fatty acids</td>
<td>mg/L as acetic acid</td>
<td>50-500</td>
<td>500-2000</td>
</tr>
<tr>
<td>Organic loading rate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesophilic</td>
<td>kg VS/m³/d</td>
<td>0.8-2.0</td>
<td>0.4-6.4</td>
</tr>
<tr>
<td>Thermophilic</td>
<td>kg VS/m³/d</td>
<td>1.5-5.0</td>
<td>1.0-7.5</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>32-37</td>
<td>20-42</td>
</tr>
<tr>
<td>Mesophilic</td>
<td>°C</td>
<td>50-60</td>
<td>45-65</td>
</tr>
<tr>
<td>Thermophilic</td>
<td></td>
<td>6.8-7.4</td>
<td>6.3-7.9</td>
</tr>
<tr>
<td>Oxidation reduction potential</td>
<td>mV</td>
<td>-520 to -530</td>
<td>-490 to -550</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>mg as CaCO₃/L</td>
<td>1300-3000</td>
<td>1000-5000</td>
</tr>
<tr>
<td>Hydraulic retention time</td>
<td>days</td>
<td>12-18</td>
<td>7-30</td>
</tr>
<tr>
<td>Mixing</td>
<td>Vol./d</td>
<td>3-6</td>
<td>8-12</td>
</tr>
<tr>
<td>Biogas composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane</td>
<td>Vol. %</td>
<td>65-70</td>
<td>60-75</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>Vol. %</td>
<td>30-35</td>
<td>25-40</td>
</tr>
</tbody>
</table>

3.3.2.5.1. Volatile Fatty Acids
In a normal and healthy digester, the VFA will be used as the food for methane formers. The typical range for VFAs in a primary digester is between 50 to 300 mg/L. When VFA concentrations climb above 300 mg/L, the digester could be overloaded or experiencing other problems.

3.3.2.5.2. Oxidation-reduction potential (ORP)
The ORP number determines the order or sequence of utilization of the final electron carrier molecules to release or gain electrons (Amani et al., 2010). ORP varies linearly with the logarithm of oxygen concentration. The intrusion of oxygen, even at level beyond the detention limit commercially available oxygen probes can be easily sensed by ORP measurement. The fermentation process in an anaerobic digester does not occur until the ORP is less than -300 mV(Amani et al., 2010).
3.3.2.5.3. Alkalinity
Alkalinity is the buffering capacity of water to neutralize acids. The methane formers in anaerobic digestion are affected by small pH changes, while the acid producers can function. Digestion stability depends on the buffering capacity of the digester contents. To prevent an extreme pH drop in an AD process, an alkalinity of 1000-3000 mg/L as CaCO₃ is required (Amani et al., 2010).

3.3.2.5.4. pH
Methanogens are extremely sensitive to pH, fermentative microorganism generally, are somewhat less sensitive and can function in a wider range of pH between 4.0 and 8.5. There are two strategies for correcting low pH, (1) stopping the feed and allowing the methanogenic population time to reduce the concentration of VFAs, or (2) the addition of bases to raise pH and provide additional buffering capacity (Amani et al., 2010).

3.3.2.5.4. Temperature
Mesophilic digesters should be operated within a temperature range of 32-37°C, while thermophilic digesters should be between 50-60°C. Temperature fluctuations in digesters should be as small as possible. Less than one °C per day for thermophiles and 2-3°C per day for mesophilic.

3.3.2.5.5. Organic Loading Rate
Loading factors is one of the most common methods used to size digesters (Metcalf and Eddy, 2014). Two most favoured factors are based on the mass of volatile solids added per day per unit volume of digester capacity, and 2 the mass of volatile solids added to the digester each day per mass of volatile solids in the digester. The upper limit of volatile solids loading rates is typically determined by the rate of accumulation of toxic materials, particularly ammonia, or washout of methane formers. Excessively low volatile solids loading rates can result in designs that are costly to build and are troublesome to operate. It is important to maintain stable OLR as wide and rapid variations in OLRs can upset the balance between acidogenesis and methanogenesis in anaerobic processes(Amani et al., 2010). As stated by Metcalf & Eddy, (2014), if waste activated sludge is thickened too much, there could be problems with ammonia toxicity.
3.3.2.6. Performance Indicators

Product stability was reported as VFA concentrations and percent VS destroyed. As noted by Parkin & Owen, (1986) VA levels above 500 mg/L and VS reductions less than 30-40 usually indicated incomplete and inefficient digestion and unstable product. (Parkin and Owen, 1986). In order to tell how complete the digestion process actually was, BMP tests were used to measure the products stability. By comparing the amount of biodegradable material remaining in a sludge sample and comparing it with the influent, the efficiency or degree of digestion can be defined. An additional note by Svensson et al., (2018) was made concernening the use of BMP tests measuring the methane potnetial of digestate cakes. The authors stressed that while there may be methane potential left in the digestate cakes, it is not evident that this will be emitted as methane during storage or after land application. It is stressed that BMP data will give the maximum methane yield from a test materail, and therefore the worst-case scenario (Svensson et al., 2018).

3.4. Thermal hydrolysis reactor

3.4.1. Electric versus steam thermal hydrolysis discussion

At laboratory scale, thermal hydrolysis are commonly performed in electric mode, while at an industrial scale, steam mode is generally used. To compare the performances of electric and steam for thermal hydrolysis, Mottet et al., (2009) used a 10 L agitated autoclave to thermal treat waste activated sludge. The authors assessed the impact of heating modes on solubilisation and biodegradation results. Solubilisation of COD, VS and carbohydrates obtained at 165°C in both modes did not show significant differences. It reached around 18, 15 and 15%, respectively in batch modes. On the other hand, protein solubilisation was slightly higher for sludge treated with steam (40.2% against 34.5% in electric mode). The Newman-Keuls test showed that the difference of BMP between treatments at 165°C was not significant Thus, the laboratory thermal hydrolysis was carried out in electric mode as it represents properly industrial thermal hydrolysis carried out with steam injection. For this research, two thermal hydrolysis reactors were used: a 100 mL air-tight stainless-steel column and a 5500 Parr 600 mL compact reactor.

3.4.2. 5500 600 mL HP COMPACT REACTOR

The 600 mL high pressure compact reactor heats the sludge through the metal cylinder through conduction, controlled by a power switch located at the back of the heater (see
Figure 16). The Parr 4848 Reactor Controller brings digital communication to all of the functions to the pressure reactor. The 4848 has the capability to (1) PID programming with Auto-tuning capability for precise temperature control and minimum overshoot, (2) ramp and soak programming, (3) separate heating and cooling control loops, (4) optional solenoid valve module for cooling control, (5) motor speed control, (6) high or low power heater switch, (7) lockout relay and reset for over temperature protection, and (8) optional expansion modules for tachometer, pressure and high temperature alarm. For this experiment of thermal hydrolysis for 30 minutes, the ramp and soak program was used.

To treat the sludge, no more than 300 mL were filled into the metal cylinder. Next, the cylinder head was sealed by tighten the cap screws to 15 ft-lbs then increase to 20-25 ft-lbs tightening should proceed in a crisscross pattern rather than progressively around the circle. The metal cylinder was placed into the mini-reactor. The water cooling lines are connected to the reactor head, the thermocouple is connected to the controller and the mixer is connected to the head. The power cords for both the reactor and controller. The main power switch on the back of both are turned on. To start the program, the run button on the controller was selected. The temperature would rise to the selected value and the water cooling would activate for regulated cooling. After the program had finished, the water line, motor were disconnected. The reactor was then placed in a bucket of cold water to cool down to room temperature. The main power for the heater and controller were turned off. Once the temperature was below 50°C, the gas release valve was opened and the metal cylinder opened. After the liquid had been poured out, the cylinder and cylinder head were cleaned using water and left to dry.
3.5. Analytical Methodologies for Characterisation of Sludge Physical Properties
Analysis included the determination of the concentration of solids, organic carbon, volatile fatty acids and alkalinity, the determination of pH value and composition of biogas.

3.5.1. Physical Characteristics

3.5.1.1. Determination of sludge dry solid content
Dry solid content is the residual remaining after a wastewater sample has been evaporated and dried at a temperature of 105°C (Remigi and Buckley, 2006). To start, an aluminum crucible was placed in a muffle furnace at 550°C for 60 min. The crucible was collected in a desiccator, weighed and stored until used. A sample was transferred to the weighed dish and evaporated in a drying oven at 105°C. The dish was cooled in a desiccator and weighed. The total solids of the sludge sample were determined using Equation 2.

\[ TS = \frac{W_{105} - W_0}{M} \]  

Equation 3-2: Determination of TS

Where \( W_0 \) is the weight (g) of the crucible at tare, \( W_{105} \) is the weight of the crucible after drying in the oven at 105°C.

3.5.1.2. Determination of sludge volatile solid content
Volatile solids that can be volatized and burned off. It is presumed that VS are organic matter, but some organic matter will not burn, and some inorganic solids breakdown at high temperature. The residue from the total solids tests was ignited in a muffle furnace 550°C for 60 min. The dish transferred to a desiccator for final cooling. The dish is weighed once completely cooled. The volatile solids for a sludge sample were calculated using Equation 3.

\[ VS = \frac{W_{105} - W_{550}}{M} \]  

Equation 3-3: Determination of VS

Where \( W_{105} \) is the weight of the crucible after 105°C during the total solids test, \( W_{550} \) is the weight of the crucible after ignition in a furnace at 550°C, and \( M \) is the mass or volume of the sludge sample initially added onto the crucible.
3.5.1.3. Total and volatile suspended solids

Suspended solids is the portion of the TS retained on a filter with a specified pore size measured after being dried out at a temperature of 105°C. The filter used was a Whatman glass fiber filter, with a pore size of 1.58 um. It is important to note, Metcalf and Eddy, (2014) stated that TSS test itself has no fundamental significance because (1) the measured values of TSS are dependent on the type and pore size of the filter used in the analysis, (2) depending on the sample size used for the determination of TSS, where suspended solids have that have been intercepted by the filter also serve as a filter can occur. Auto filtration will capture smaller particles than otherwise possible and cause an apparent increase in the measured TSS value over the actual value. (3) depending on the characteristics of the particulate matter, small particles may be removed by adsorption to material already retained by the filter, (4) because the number and size distribution of the particles that comprise the measured value is unknown, TSS is a lumped parameter (Metcalf and Eddy, 2014).

\[
TSS = \frac{SSW_{105} - SSW_0}{SSM}
\]

Equation 3-4: Determination of TSS

Where SSW0 is the weight (g) of the crucible and filter at tare, SSW105 is the weight of the crucible and filter after drying in the oven at 105°C, and SSM is the mass or volume of the initial sludge sample added.

3.5.1.4. Volatile suspended solids

VSS are those solids that can be volatized and burned off when the TSS are ignited at 550°C (see Equation 5).

\[
VSS = \frac{SSW_{105} - SSW_{550}}{SSM}
\]

Equation 3-5: Determination of VSS

Where SSW105 is the weight of the crucible and filter after 105°C during the total suspended solids test, SSW550 is the weight of the crucible after ignition in a furnace at 550°C, and SSM is the mass or volume of the sludge sample initially added onto the crucible.
3.5.2. Inorganic chemical characteristics
Inorganic chemical constituents of concern include nutrients, non-metallic constituents, metals and gases. Non-metallic inorganic constituents include pH, nitrogen, phosphorus, alkalinity, chlorides, sulfur and other inorganic constituents and odours. The parameters measured in this experiment included pH, alkalinity and volatile acids and nitrogen.

3.5.2.1. Sludge pH
pH is used to measure the acidity or basicity of an aqueous solution (Metcalf and Eddy, 2014). Measurement of pH is done using a pH electrode, manually immersed into the sample of sludge or effluent. Care must be taken in ensuring that the pH determination of a sample is a true measure of the pH in the system. If the sample is allowed to stand exposed to air, carbon dioxide will escape causing the pH to rise. Similarly, stirring the sample while measuring the pH, favours the stripping of carbon dioxide and alters the pH value (Remigi and Buckley, 2006). The pH probe was calibrated using pH coloured solutions of 4, 7 and 10.

3.5.2.2. Sludge Alkalinity
Alkalinity in wastewater results from the presence of the hydroxides, carbonates and bicarbonates of elements such as calcium, magnesium, sodium, potassium and ammonia (Metcalf and Eddy, 2014). Alkalinity in wastewater helps to resist changes in pH caused by the addition of acids. Alkalinity was determined by titrating against a standard acid 0.1 N NaOH, and the results were expressed as calcium carbonate (mg/L CaCO₃) (see Equation 6).

\[
\text{Alkalinity as CaCO}_3 = \frac{\text{meq}}{L} \times \frac{50 \text{ mg CaCO}_3}{\text{meq CaCO}_3}
\]

Equation 3-6: Determination of Alkalinity

3.5.2.3. Determination of volatile fatty acids contents
TNTplus 872 Hach test kits were used to measure the volatile acids for digested sludge using DR 28000 spectrophotometer. Samples were measured in triplicate. If the readings were over the measuring range, the same was adjusted using dilutions. The following procedure was used to measure concentration of acetic acid:

1. Sludge samples were centrifuged for 10 minutes at 10000 rpm
2. The centrate was filtered through 0.45 um filter using a syringe
3. The DRB200 reactor was set to a temperature of 100°C.
4. 0.4 mL of Solution A was pipetted into the test vial
5. 0.4 mL of the sample was added into the test vial.
6. The vial was capped and inverted 2-3 times and placed into the DRB200 reactor for 10 minutes
7. After 10 minutes, the vials were removed from the reactor and cooled to room temperature.
8. 0.4 mL of Solution B was pipetted into the vial.
9. The vial was capped and inverted 2-3 times
10. 0.4 mL of Solution C was pipetted into the vial
11. The vial was capped and inverted 2-3 times
12. 2.0 mL of Solution B was pipetted into the vial.
13. After 3 minutes the vial was cleaned and inserted into the DR5000 for reading.

Results were reported as mg/L acetic acid.

3.5.2.4. Ammonia

Hach TNTplus 832 test kits were used to measure the ammonia concentration of digested sludge centrate. The following steps were followed for the test procedures:

1. Sludge samples were centrifuged for 10 minutes at 10000 rpm
2. The centrate was filtered through 0.45 um filter using a syringe
3. The lid from the DosiCap was removed from the test vial
4. 0.2 mL of sample was pipetted into the test vial.
5. The DosiCap Zip was tightened on the vial.
6. The vial was shaken 2-3 times to dissolve the reagent in the cap.
7. After 15 minutes, the vial was cleaned and inserted into the cell holder. The results were reported as NH₃-N.

3.5.3. Determination of organic chemical characteristics

Organic compounds are normally composed of a combination of carbon, hydrogen, and oxygen, and in some cases nitrogen. Because of the complex nature of wastewater the organic characteristics of interest in wastewater are classified as aggregate and individual. Aggregate organic constituents are comprised of a number of individual compounds that can not be distinguished separately (Metcalf and Eddy, 2014). In general, the analyses used to
measure aggregate organic material may by divided into those used to measure gross concentrations of organic matter grater than 1 mg/L. Laboratory methods commonly used to measure gross amounts of organic matter in wastewater include (biochemical oxygen demand (BOD), chemical oxygen demand and total organic carbon. In this experiment, COD was used to substitute for BOD.

3.5.3.1. Chemical Oxygen Demand
Chemical oxygen demand is used to determine the amount of oxygen needed to stabilise organic compounds (Metcalf and Eddy, 2014). The principle of the tests is the observation that most organic matter is destroyed by a boiling solution of chromic and sulphuric acid. The amount of oxidizable organic matter is proportional to the dichromate consumed.

Problems associated with COD measurements, as stated by Remigi and Buckley, (2006), are: (1) halogen can be oxidised, (2) aromatic carbohydrates and some aromatic heterocyclic compounds are not oxidised, (3) volatile straight-chain aliphatic compounds are not oxidised to any appreciable degree, (4) reduced inorganic compounds. In comparison to the BOD test, COD tests can be completed in 2.5 hours, compared to 5 or more days for the BOD test.

Hach high range COD (HR) 20 to 1500 mg/L test vials were used to determine the COD content of the wastewater. The following steps were taken for the test procedure:

1. Samples were mixed well and homogenized
2. The DRB200 Reactor was preheated to 150°C.
3. Blank was prepared by adding 2.00 mL of deionized water to the vial.
4. Vials were closed tightly and inverted several times to mix.
5. The vials were placed into the preheated DRB200 reactor.
6. The vials were heated for 2 hours
7. Afterwards, the vials were cooled to 120°C and inverted several times fro mixing.
8. The vials were then cooled to room temperature.
9. The blank sample was cleaned and inserted int the cell holder.
10. The display was zeroed.
11. The sample vial was cleaned and inserted into the cell holder.
12. The results were reported as mg/L COD. If a dilution was used, the results were multiplied by the dilution factor.
3.5.3.2. Proteins and humic acid

The modified Lowry method against the interference of divalent cations in soluble protein measurements was used to measure the soluble protein content (Shen et al., 2013). Unlike the original modified Lowry method proposed by Frolund et al., (1995), this method eliminated interference from calcium and magnesium which lead to underestimation of protein concentration and low experiment reproducibility (see Table 10) (Lowry et al., 1951):

\[
A_{\text{total}} = A_{\text{protein}} + A_{\text{humic}}
\]

Equation 3-7: Determination of Total Absorbance

\[
A_{\text{blind}} = 0.2A_{\text{protein}} + A_{\text{humic}}
\]

Equation 3-8: Determination of Blind Absorbance

\[
A_{\text{protein}} = 1.25(A_{\text{total}} - A_{\text{blind}})
\]

Equation 3-9: Determination of Correct Protein Absorbance

The original Lowry procedure was modified by to correct the interference of humic substances. In their method, the protein absorbance was calculated using the Equation (9) by combining equation (7) and (8). Each test was carried out with triplicate samples.

Table 3-10: Lowry procedure used for measuring humic and protein substances.

<table>
<thead>
<tr>
<th>Reagent name</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.7 g/L NaOH+28.6 g/L Na₂CO₃</td>
</tr>
<tr>
<td>B</td>
<td>14.3 g/L CuSO₄ · 5H₂O</td>
</tr>
<tr>
<td>C</td>
<td>28.5 g/L Na₂C₃H₆O₄ · 2H₂O</td>
</tr>
<tr>
<td>Lowry reagent I (with CuSO₄)</td>
<td>100 mL A+ 1 mL B+ 1 mL C</td>
</tr>
<tr>
<td>Lowry reagent II (without CuSO₄)</td>
<td>100 mL A+ 1 mL water+ 1mL C</td>
</tr>
<tr>
<td>Folin-Ciocalteu reagent</td>
<td>Modified Lowry Protein Assay Kit, 1.0 mol/L</td>
</tr>
</tbody>
</table>
To start the chemical reagents in Figure were collected and formulated. After reagent A, B, and C were collected, two different Lowry reagents were made: Lowry reagent 1 with CuSO4 and Lowry reagent 1 without CuSO4. Two samples were go through the same procedure, the only difference being the A total would have the addition of 0.7 Lowry reagent 1 and A blind would have the addition of 0.7 mL Lowry reagent 2. The procedure outline can be observed in Figure 17.

![Diagram of the modified Lowry method](image)

**Figure 3-16: Protein determination procedures of the modified Lowry method**

3.5.3.3. Carbohydrates Analysis in EPS/ SMP Sample (Modified Dubois et al. 1951 method for lower concentration samples)

1. 5% Phenol solution was prepared using 5 g of saturated Phenol (Fisher Scientific, BP1750-400) in 100 ml distilled water).

2. 3 mL of sample were added into 10 mL HACH vials

3. 1mL of the phenol solution and immediately afterwards 5 mL of pure sulfuric acid (96%) was added on the liquid surface.

4. The vials were settled for 10 minutes

5. The vials were incubated in a water bath at 30 C for 20 minutes

6. The vials were cooled down for 10 minutes at room temperature

7. Using the HACH DR 5000 spectrophotometer, the carbohydrate absorbance was set to 490 nm.
8. The instrument was zeroed using distilled water
9. The carbohydrate absorbance of the blank was subtracted from the absorbance of the unknown samples.
10. The protein concentration for each unknown sample was determined using a standard curve for glucose at different concentrations.

3.5.4. Biogas Analysis
Biogas composition was determined by the GC (HP6890, Agilent Technologies, US) with a thermal conductivity detector (TCD) and a HP-PLOT Molesieve GC column (30 m×0.530 mm, Agilent Technologies, US). Argon was used as the carrier gas. Injector and detector were maintained at 200 °C and 150 °C, respectively. The oven temperature was held at 35 °C for 7.5 min, and then increased from 35 °C to 206 °C at a rate of 24 °C/min, and held at 206 °C for 1 min. The peaks were identified and quantified by comparing with biogas mix standards including methane, carbon dioxide, nitrogen, oxygen, and hydrogen at various concentrations (Praxair Inc., Canada) (see Figure 18). The 20 mL or 50 mL glass syringes lubricated with distilled water were utilized to measure the generating biogas volumes and take biogas samples from the headspace. It was assumed that the gas composition is the collected syringe sample collected and gas remaining in the headspace were homogeneously mixed, meaning the gas composition results were the same for each volume.

![Figure 3-17 Standard Chromatogram of a Mixture of Gases](image-url)
CHAPTER 4: BIOCHEMICAL METHANE POTENTIAL (BMP) ASSAY METHOD FOR ANAEROBIC DIGESTION RESEARCH

Abstract: Biochemical methane potential (BMP) tests are widely used for characterizing a substrate’s influence on the anaerobic digestion process. As of 2018, there continues to be a lack of standardization of units and techniques, which impacts the comparability and validity of BMP results. However, BMP methods continue to evolve, and key aspects are studied to further eliminate systematic errors. This paper aims to update these key aspects with the latest research progress both to introduce the importance of each variable to those new to BMP measurements and to show the complexity required to design an accurate BMP test.

Keywords: Anaerobic digestion, biochemical methane potential, energy recovery, sludge treatment
4.1. Introduction
Anaerobic digestion (AD) has been used for its emphasis on energy conservation and recovery and desire to obtain beneficial use of organic waste (Feodorov, 2016; Jain et al., 2015; Tchobanoglous et al., 1991). Acting through a series of complex microbiological processes, diverse types of bacteria work in an assembly line fashion going through four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Gonzalez-Fernandez et al., 2015). These bacteria are sensitive to environmental conditions, and it is important to balance a range of factors to maximize the chances for achieving optimum design and efficient operation (Mao et al., 2015; Shana, 2015). The approach often involves the recognition of the rate-limiting step, which is linked to knowing the characteristics of the organic material being digested. Therefore, the feed characteristics such as toxicity and biodegradability have been found to be major factors for affecting system design and performance (Parkin and Owen, 1986).

Biochemical Methane Potential (BMP) tests are a popular technique to determine the methane potential and biodegradability of wastewater and waste biomass (Remigi and Buckley, 2006). In the test, a substrate is mixed with an anaerobic bacteria culture, normally retrieved from an active digester. The bottles are then stored at a stable temperature of either 35°C or 55°C, and constantly mixed for a period of 30-60 days (Holliger et al., 2016; Strömberg et al., 2014). Methane and carbon dioxide are produced during the testing period due to the anaerobic degradation of organic contents of the substrate. The methane generated from the substrate is then measured and the methane potential of the substrate which is expressed as per mass of volatile solids added or COD added can be calculated by subtracting the methane volume from a blank. In addition, the substrate may be expressed as in terms of biodegradability by dividing the cumulative methane volume by the theoretical cumulative methane volume, which is obtained from the chemical ratio of 1 g COD= 0.35 mL CH₄ at STP (Angelidaki and Sanders, 2004).

Since the popular methodology of Owen et al., (1979) was published, BMP test have been used to characterize a wide variety of substrates and have become important tools for investigating possible pre and post digestion treatment options. As computer models and the
complexity of mathematical expressions to describe the anaerobic digestion process improved, the information from batch experiments have been found to produce reasonable predictions of full-scale behaviour. The BMPs of the substrates to be digested and their specific organic loads could be used to design different components of full-scale anaerobic digestion plants such as the size of the digesters and possibilities of exploiting the produced biogas. For example, Holliger et al., (2017) compared the volume of methane predicted by BMP data with the methane volume measured on-site from a full-scale installation over a period of 7 to 9 months. The authors found that the BMP weekly methane production rates were similar and followed the same pattern. In addition, Li et al., (2017) found that information obtained from BMP degradation rates could also be used as a practical tool for evaluating process performance in full-scale biogas processes.

Currently, the central issue with BMP tests is the lack of standardized procedures and information required for reporting. Many international and national procedures have been proposed, each using different serum bottles, test inoculum, food to microorganism ratios, nutrients and methane measurement devices. As stated by Pham et al., (2013), the most popular methods are the German standard procedure VDI 4630, (Möller and Müller, (2012), Hansen et al., (2004) and Angelidaki et al., (2009). But because of a lack of standardized protocol, there have been serious drawbacks impacting the industry user. As the reliability of generated information could be under question due to laboratory specific experimental, operation conditions, and data presentation, limiting the comparability of published results.

In addition, there is the issue of a lack of clear instructions for new operators to start BMP tests. Most BMP methodologies provide general guidelines to accommodate all substrates. As a result, it is difficult for a new operator to design a test with accuracy and confidence due to increased room for variation and misinterpretation. It might be useful to provide methodologies specific to certain groups of substrates. There could be increased confidence in the transferability of the methodology to other labs investigating similar substrates, such as the biodegradability of sludge in the range of 0.5% to 6% solid content. In addition, what is missing from other methodologies is transparency of experimental setups. By being simple and clear through providing an example test setup data such as liquid volumes (which are
never shown in other papers), COD mass balance, or the number of bottles used, this would be useful for new labs as it can serve as a model for comparison. Even if labs find there are many areas in need of improvement or obvious sections contributing to inherent inaccuracy, their method could be improved faster because areas in need of development can be more easily pinpointed for their specific lab setup and wastewater sample.

The objective of this paper was to (1) review recent studies that completed experiments to provide insight into key factors such as inoculum, substrate, experimental conditions, operational conditions and data analysis/reporting, as at the time of most protocols, no previous research had been carried out to study the influence of several key factors on anaerobic biodegradability in batch mode, (2) outline an easy to understand BMP serum bottle syringe method for new operators using primary and secondary sludge from a WWTP as a case example, and (3) provide the reader with perspective on work investigating future areas of BMP development.

4.2. Review of BMP Variability Factors

To understand the BMP method, it is important to provide background information discussing each of the required components of a test. The following section goes through the required serum bottle sets, the environmental conditions needed for healthy digestion, the test components quality for wastewater characterization, and techniques used to monitor the progress and health of the anaerobic digestion process during incubation.

4.2.1. Set-up of BMP bottle test
BMP tests are usually carried out in a volume range depending on the substrate homogeneity. Smaller volumes (125-500 ml) should be used for homogenous substrates, while large volumes (500 to 2000 mL) are more appropriate for heterogeneous substrates (Holliger et al., 2016; Pearse et al., 2018). Smaller bottles may not ensure realistic operation conditions due to the smaller microbial consortia and reduced VFA compared to large scale reactors where higher concentrations of microorganism exist (Pearse et al., 2018). Pearse et al., (2018) recommended that even though larger bottles, due to increased concentrations of microorganism accelerate hydrolysis and VFA build up in the system, they will provide more realistic predictions of gas generation.
BMP tests require a blank, control and substrate. All groups should be performed in triplicates for reproducibility of the tests and statistical analysis. The substrate bottle is filled with inoculum, the substrate, and added nutrients if needed. The blank is filled with the inoculum, a medium or water, but no substrate to provide the background methane generation from the organic material in the inoculum. The control assesses the accuracy of the BMP test using a substrate with a known theoretical methane yield.

The control bottles are filled with inoculum, the control substrate, and added nutrients if needed. To calculate the theoretical reference methane yield value for the selected control substrate, the Buswell formula is commonly used for substrates with known chemical composition (carbon, hydrogen and oxygen)(Jingura and Kamusoko, 2017; Rodriguez-Chiang and Dahl, 2015). Microcrystalline cellulose, is the most common choice for a control substrate because, as stated by Koch et al., (2017) it is relatively easy to calculate the theoretical BMP, its degradation involves all steps in AD, it is cheap, and in high-quality and purity (theoretical methane potential of 415 mL CH₄/g VS at STP) (Wang et al., 2014). However, results are rarely 100% accurate when calculating the methane yield of the positive control. There is agreement that during AD, 10% of the substrate is for biomass growth and transformation into heat (Wang et al., 2014). This is reflected in the VDI 4630 guideline recommends that when cellulose is digested in a BMP test it should produce a biogas yield of at least 80% of its theoretical maximum yield (332 kg CH₄/ VS)(Himanshu et al., 2017). Similarly, Holliger et al., (2016) stated the positive control should achieve at least 85% of the theoretical BMP. Although controls are necessary to provide verification of the accuracy of a BMP method, they are uncommon in BMP papers (Raposo et al., 2012).

4.2.2. BMP bottle environment

It is important to maintain consistent environmental conditions for the microbiology and biochemistry for anaerobic digestion to maximize the chances for achieving optimum performance (Mao et al., 2015; Shana, 2015). As stated by Parkin and Owen, (1986), to ensure efficient digester operation, a balance between the acid-forming and hydrogen-forming bacteria and the methane producers must be maintained. In situations where
environmental conditions are non-uniform or unstable the final BMP value can be significantly underestimated. For BMP tests there must be (1) a temperature-controlled environment, (2) proper mixing, and (3) sufficient incubation time for the degradation of biodegradable material.

4.2.2.1. Temperature
Temperature influences the growth rate and metabolism of micro-organism and the population dynamics in the anaerobic reactor, but also effects factors such as gas transfer rates and settling characteristics of biological sludge. Most anaerobic digesters are operated in either mesophilic (30-38°C) or thermophilic (50-58°C) temperature ranges. Thermophilic digestion is faster than mesophilic digestion since the biochemical reaction rates increase with increasing temperature. Additional advantages are increased solids reduction, improved dewatering, and increased destruction of pathogenic organisms (Metcalf and Eddy, 2014). But the use of thermophilic temperatures has a higher energy requirement, a lower quality supernatant with large quantities of dissolved solids, a higher odour potential and much poorer process stability (Appels et al., 2008). It is preferred that the temperature of the BMP bottles is the same as the inoculum originating digester. The majority of data in experiments performed at mesophilic temperature, with only some at thermophilic (Raposo et al., 2012). BMP vessels should be incubated in a temperature-controlled environment with maximum variations of +/- 2°C (Holliger et al., 2016).

4.2.2.2. Mixing
Mixing influences the distribution of microorganism, nutrients, substrate, alkalinity and the release of gas bubbles trapped in the digester content and prevention of sedimentation of a particulate material and evening out temperature distribution in the digester (Lindmark et al., 2014; Parkin and Owen, 1986; Stroot et al., 2001; Wang, 2016; Wang et al., 2016). In the case where there is inadequate mixing, inhibition can arise due to the accumulation of toxic metabolic by-productions (Ghanimeh et al., 2018; Stroot et al., 2001). So far, there remains no optimum mixing pattern for BMP test (Ghanimeh et al., 2018). Wang et al., (2016) studied the influences of no mixing, shaking in a water bath, manually shaking once per day, automated unidirectional and bidirectional mixing for BMP tests. In the experiment, results were found to be dependent on the sludge rheology. When the sludge has a viscous content (12-22 Pa s at 20 l/s), the highest methane potential and highest maximal daily specific
methane production was obtained at the highest mixing intensity (Wang et al., 2016). On the other hand, slight stirring or natural movement by the biogas may be enough to avoid inhibition by-productions for sludge with low total solids. The authors further reported that no mixing or manually shaking once per day may be sufficient if the digester content is dilute or easily degraded (Wang et al., 2016). However, as a general observation that mixing lacks precision, the mixing condition with BMP tests should try and replicate the basic fluid dynamics of large-scale reactors. Most full-scale reactors are mixed to some extent to reduce SRT and to release entrained methane.

4.2.2.3. Incubation Time
Solid retention time (SRT) is regarded as the most important parameter for anaerobic digester design and operation (Parkin and Owen, 1986). SRT accurately defines the relationship between the bacterial system and digester operation conditions. Hydrolysis, fermentation and methanogenesis are directly related to the SRT, where an increase or decrease in SRT results in an increase or decrease in the extent of each reaction. As the objective is to determine the maximum volume of methane to be generated from a substrate, the longer the SRT the higher the overall methane production and reduction of biodegradable material. The challenge for the operator has often been selecting an optimal SRT for a substrate that is long enough to ensure efficient conversion of complex organic matter to methane and carbon dioxide, but under time restrictions. In literature reported incubation times range from 30 to over 100 days (Raposo et al., 2012). These recommendations should only be used as guides. If the daily methane production over three consecutive days is <1% of the cumulated methane production, the test could be finished sooner (Zaman, 2010).

4.2.3. BMP bottle contents
4.2.3.1. Inoculum
Inoculum supplies the microorganism to the anaerobic digestion process, and is one of the most important BMP factors with origin, time of sampling and concentration having the ability to significantly influence results (Raposo et al., 2012, 2011; Rozzi and Remigi, 2004). Throughout literature there is great variability in the inoculum used in BMP tests, originating from sources such as sewage sludge digesters, agricultural biogas plants and biowaste treatment plants (De la Rubia et al., 2018; Gu et al., 2014; Moreno-Andrade and Buitrón, 2004; Raposo et al., 2011). Recently, there have been comprehensive studies on the effects of
the selection of different inoculums. Most protocol studies state that differently sourced inoculum can lead to different substrate biodegradabilities and flawed data, due to different bacterial population, substrate adaption, and initial microorganism activities (Elbeshbishy et al., 2012; Y. Li et al., 2013; Moreno-Andrade and Buitrón, 2004; Pearse et al., 2018). There seems to be a collective conclusion that when selecting inoculum, priority should be the source already adapted to the substrate. The most commonly recommended being the anaerobic digestate from wastewater treatment plants due to the full range of diverse and active microorganisms (Pearse et al., 2018; Raposo et al., 2012).

Part of the standardization of inoculum involves a quality check to indicate whether the operational parameters of the digester are of good quality (see Table 11). The most common recommendation is to pre-incubate the inoculum for 1 to 5 days at 35 °C to degas and reduce the impact of its methane production. Elbeshbishy et al., (2012) studied the influence of inoculum pre-incubation and found no significant difference in methane yield or biodegradability compared to non-incubated inoculum, except for higher maximum methane production rates using fresh inoculum at all SIR ratios. Holliger et al., (2016) stated that the decision should be based on whether the inoculum has a low endogenous methane yield (~50 NmL CH₄/g VS). In cases where the total methane production from the blank contributes more than 20% of the total methane production, pre-incubation for exhausting the inoculum might be needed (Holliger et al., 2016).

### Table 4-11: Recommended inoculum conditions for BMP tests

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Recommended Range</th>
<th>Units</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin Source</td>
<td>Active digester treating municipal wastewater sludge</td>
<td>---</td>
<td>[Chynoweth et al., 1993; Elbeshbishy et al., 2012; Holliger et al., 2016; Pearse et al., 2018; Rozzi and Remigi, 2004]</td>
</tr>
<tr>
<td>pH</td>
<td>7≤x≤8.5</td>
<td>---</td>
<td>[Holliger et al., 2016]</td>
</tr>
<tr>
<td>VFA</td>
<td>&lt;1</td>
<td>g CH₃COOH/L</td>
<td>[Holliger et al., 2016]</td>
</tr>
<tr>
<td>NH₄</td>
<td>&lt;2.5</td>
<td>g NH₄/L</td>
<td>[Holliger et al., 2016]</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>&gt;1.5</td>
<td>g CaCO₃/L</td>
<td>[Holliger et al., 2016]</td>
</tr>
<tr>
<td>Concentration</td>
<td>15 to 20</td>
<td>g VS/L</td>
<td>[Holliger et al., 2016]</td>
</tr>
<tr>
<td>Storage</td>
<td>1 to 5 day at 25C</td>
<td>---</td>
<td>[Elbeshbishy et al., 2012]</td>
</tr>
<tr>
<td>Methane Yield</td>
<td>~ 50</td>
<td>NmL CH₄/g VS</td>
<td>[Holliger et al., 2016]</td>
</tr>
</tbody>
</table>
4.2.3.2. Substrate
Due to the unpredictable diversity of acceptable substrates and their origins, there are few exact chemical and physical property requirements (see Table 12). Wang et al., (2015) recommended that samples should have particle sizes less than 10 mm in any dimension. Substrates should also be analyzed for TS, VS, pH, VFA, TKN, ammonium and alkalinity to design the tests and eliminate potential inhibition problems. In addition, the German standard (VD1 4630) recommended that substrate concentrations should be around 10 g VS/L, when inoculum concentrations are between 1.5 and 2% to achieve inoculum to substrate ratio of 2 (Raposo et al., 2012).

Wang et al., (2015) found the measured methane yield might vary with substrate concentration. In the case of substrates with high concentrations, there is the possibility of overloading the digester, leading to inhibition due to the accumulation of intermediate production. Wang et al., (2015) proposed two solutions to minimize the effect of high substrate concentration. One involves lowering the SIR to a more realistic relationship between the sample and the microbial population, as might be found in a full-scale anaerobic digester (hydraulic loading rate/organic loading rate). Option two, requires the dilution of the substrate. Although as shown in Wang et al., (2015), the dilution of inoculum or a substrate should be avoided as it might induce underestimations of the methane potential. In Wang et al., (2015) experiment the authors used microcrystalline cellulose as the substrate (96.1% VS) and anaerobic inoculum from a mesophilic sewage treatment plant. The BMP of the substrate was then evaluated at increasing VS loads, from 1g VS (2.5 g VS substrate/L) to 6 g VS (15 g VS substrate/L). For each substrate load, three samples were run, one with a dilution using distilled water, a dilution using nutrient/buffer solution, and no dilution. Results showed that the methane potential (NmL/gVS) increased with the VS load. The authors noted that if the substrate concentration is too low, there is a possibility of low quantities of gas production due to the low metabolic activity of the microorganism resulting in low methane yield.
4.2.3.3. Nutrients
Optimal operation of biogas digesters requires balanced concentrations of C:N:P:S (~600:15:5:1), macronutrients (K, Na, Ca and Mg), trace metals (Fe, Zn, Mn, B, Co, Ni, Cu, Mo, Se, Al, W and V) and vitamins to support microbial growth (Brulé et al., 2013). In BMP tests, any lack can have inhibitory effects (Angelidaki et al., 2009; Speece, 1983). Examples of BMP nutrients solutions can be found at Rozzi and Remigi, (2004), Owen et al., (1979), and Angelidaki et al., (2009).

In most cases it is not clear whether BMP tests will have sufficient nutrients available from the sludge and substrate or if additional supplements are necessary. In some cases, nutrients supplementation can be avoided when the seed is suspected of having enough nutrients and the seed volume can prevent reactor acidification (Zaman, 2010). Wang et al., (2015) studied the impact of a BMP set using no dilution, distilled water and nutrient/buffer solution on methane yield and degradation rate. Positive effects on degradation rate was found when nutrients were added, but regarding the final methane yield calculation there were minor differences in comparison to the strong effects of the choice of substrate concentration. In the situation where, digested sewage sludge is the inoculum, nutrient supplementation could be exempted. As stated by Shelton and Tiedje, (1984)), digestate is likely to have all mineral and metal nutrients in amply supply (except for potassium, ammonium and cobalt), and the addition of excessive nutrients could be inhibitory.

### Table 4-12: Recommended substrate conditions for BMP tests

<table>
<thead>
<tr>
<th>Factors</th>
<th>Recommendation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size</td>
<td>&lt;10 mm</td>
<td>[[Angelidaki et al., 2009]]</td>
</tr>
<tr>
<td>Concentration</td>
<td>10 VS g/L</td>
<td>[[Rozzi and Remigi, 2004]]</td>
</tr>
<tr>
<td>Compulsory Parameters</td>
<td>TS, VS, pH, VFA, TKN, NH4, ALK, COD</td>
<td>[[Holliger et al., 2016]]</td>
</tr>
</tbody>
</table>
4.2.4. BMP testing monitoring

4.2.4.1. Biogas Monitoring

As the organic material in the substrate is degraded through a series of complex microbiological processes, biogas is continually produced during incubation until there is no biodegradable material left. Since biogas production is the key factor to determine the methane potential and biodegradability of a substrate, it is important for the BMP method to both collect the biogas without significant losses or error and apply correction factors to convert the observed methane potential to standard temperature and pressure conditions for standardized results (Walker et al., 2009). Techniques for measuring the rate and volume of biogas produced from anaerobic biodegradability assays include: lubricated syringes, volume displacement devices, pressure manometers or transducers, manometer assisted syringes, or low flow pressure.

4.2.4.1.1. Syringe method
In the case of syringe method, a glass syringe is inverted straight into the lid of the reactor. The overpressure inside the reactor pushes the piston until there is balanced in the pressure buildup to atmospheric pressure (Remigi and Buckley, 2006). The volume of biogas can be read off the syringe. The gas can be injected back into the bottle or wasted. An added advantage of venting the biogas produced is that headspace pressures and the carbon dioxide solubility in the bioreactor vessel can be kept to a minimum.

However, this method, due to its manual operation has potential areas for human error. In most cases, the incubated bottles are removed from the temperature-controlled environment during the measurement of gas. This change of temperature can easily affect the equilibrium between the gas and liquid phase which can result in the change in headspace gas concentration and microbiology of anaerobic digestion (Parajuli, 2011).

4.2.4.1.2. Liquid Displacement
In the volumetric methods, the produced biogas can move into an external collection system that measures the volume. In liquid displacement, a vessel is filled with a barrier solution and inverted in a reservoir. As biogas is produced, it passes through the liquid vessel and
displaces an equivalent liquid volume. A prevent issue with this method is the dissolution of CO₂ into the barrier solution. Different setups use different liquids such as tap water, oil, acidified water and carbonated water, but each need to use different correction factors (Parajuli, 2011). Gas solubility errors can be eliminated by collecting gas in a gas bag and measuring the gas volume with liquid column meters. Zaman, (2010) recommended using a suitable barrier solution such as highly acidic or saline to avoid CO₂ diffusion. The use of displacement gasometers requires that measurements taken directly from the gas column (liquid levels, pressure) are used to calculate gas volumes. As well as adjusting to STP, it is also necessary to consider the vapour content and correct for any hydrostatic pressure on the gas (Pham et al., 2013).

Pham et al., (2013) compared the intermittent measurements with syringe (1000 mL), intermittent measurements with liquid replacement system (LRS), and continuous measurements with liquid replacement (CLRS). All three techniques were used for the VD1 batch fermentation method of pig manure, cow manure, cellulose and inoculum samples. In the case of cellulose, CLRS, LRS, and the syringe determined the methane yield to be 537.79±9.10 NL/kg VS, 571.36±10.24 NL/kg VS, and 583.76±5.94 NL/kg VS. The results showed that the liquid replacement system had a tendency for higher gas volume measurements than the syringe and CLRS methods. The reason could be that the syringe plunger was not withdrawn far enough to get the total production in each test and left a higher pressure in the headspace, or that in the case of the CLRS method there were small leaks in the setup, as the biogas is contained not only in the digester but also through the whole water replacement system. However, the difference in the gas volumes obtained using three different measurement techniques were much less than the differences caused by different fermentation procedures and gas measurement techniques (Pham et al., 2013). Therefore, the authors concluded that the differences between the tested methods were not significant.
4.2.4.1.3. Manometric
Manometric methods using the pressure transducer require the pressure to build up inside the reactor. This method is easier to perform than the liquid displacement but requires more effort in the test setup, and depending on the gas to liquid ratio, accuracy can be sensitive to the gas non-ideal behaviour, change in gas space volume during the test, dissolution of methane and CO$_2$ in the liquid (Rozzi and Remigi, 2004). Zaman, (2010) stated that the main drawback of the manometric approach is that variation in the pressure of the headspace gases alters the quantity dissolved in the liquid phase, especially carbon dioxide.

Manometric and volumetric biogas measurement techniques were compared by Raposo et al., (2011) in an inter-laboratory study on methane produced by cellulose. In the inter-laboratory study 19 laboratories participated. Volumetric methods were used most (63%), followed by manometric methods (26.3%) and by GC methods (10.5%). Laboratories using manometric method reported lower methane yield for cellulose than those using volumetric BMP methods (Himanshu et al., 2017). Similar results were found Himanshu et al., (2017) in a review of Logan et al., (2002) who reported a lower biogas yield with a manometric method compared to a variation of the volumetric method (Himanshu et al., 2017). Although the measurement of the biogas production using a pressure transducer as the detector is easier and more reliable than the liquid displacement, errors related to CO$_2$ solubility in the bioreactor liquid can still lead to underestimation of biogas production if not accounted for (Guwy, 2004).

4.2.4.1.4. Biogas composition monitoring
Methane production, as a process performance indicator is one of the most sensitive since it is directly related to organic matter destruction. Typical values of percent methane for digesters operating on municipal wastewater sludge are 60-75%. During system imbalance, methane production and total gas production will decrease, while the percent CO$_2$ will increase (Parkin and Owen, 1986). Gas chromatography (GC) is often used for its high resolution, high sensitivity and quantitative results, to measure the content of methane and carbon dioxide in a biogas sample (Angelidaki and Sanders, 2004). However, as found by Parajuli, (2011), varying temperatures and water vapour content in the biogas sample can
cause measurement errors. Parajuli, (2011) studied three potential temperature difference between the sampled biogas and standard calibration gas. The samples were humidified in comparison to dry standard. In the temperature and dry and humidified biogas measurement tests, a synthetic gas of 50% CH\(_4\) and CO\(_2\) were measured at temperature 5 °C, 25 °C, 55 °C and 70 °C using a calibration standard at 23 °C. At 35 °C, the concentration of 50% CO\(_2\) and CH\(_4\) was 52.1% for dry gas and 53.4%, respectively. At 55 °C the results were 56.9% and 58.8%. Ideally the best solution would be to use a vapour saturated standard, but this would be laborious and time consuming. The author found that rapid injection of samples without delay and the use of insulated syringe would give more precise results.

An alternative to the GC to measure CH\(_4\) in biogas can be determined by the liquid replacement method (Angelidaki and Sanders, 2004; Pham et al., 2013). Pham et al., (2013) compared the measured CH\(_4\) concentration using liquid replacement method and a GC. The LRM generally had higher CH\(_4\) concentrations (68%) in comparison to the GC (64.94%). The authors stated that due to the very low differences, the LRM could be a viable alternative for measuring biogas content for laboratories with limited access to expensive equipment.

4.2.4.2. Liquid Monitoring
Measurement of the physical and chemical composition of the digested liquid can be carried out regularly to monitor the digester environment and performance. Imbalanced digestion can be triggered by changes in organic or hydraulic loading, changes in organic feed characteristics, changes in temperature, or introduction of toxic substances. During imbalanced digestion, typically volatile acid concentrations increase while bicarbonate alkalinity, pH, gas production, percent methane, and the destruction of organic matter all decrease. Careful monitoring of these variables should allow operations personnel to observe the onset of stress and take appropriate remedial measures to prevent system failure (Parkin and Owen, 1986).

To monitor the changes in concentrations of these liquid phase parameters, it is necessary to set up a certain number of “sacrifice bottles” in BMP tests. These extra bottles (identical to the blank and substrate sets) can be sacrificed by being periodically opened throughout the experiment period to provide samples for the liquid phase parameter measurement. For
instance, due to the nature of the methane yield first order curve, the start-up period often slows/ends after 10-12 days before plateauing. Therefore, the operator might place extra bottles could be taken out at the initial start, after 5, 10, 15, and 30 days for analysis. Monitoring liquid phase parameters (TCOD, sCOD, VFA, total NH₃ nitrogen, TKN, TN, TP, PO₄-P, pH, alkalinity, TS, VS, TSS, VSS, EPS components of proteins, carbohydrates, and humic acids, etc) could allow the operator to assess the performance of the anaerobic digestion process, determine reaction stability, and identify potential inhibitory factors (Parkin and Owen, 1986).

4.2.4.2.1. pH
A narrow operating range of 6.5-7.6 is often recommended, since pH influences the microorganism enzymes and can change their configurations and influence the kinetics of the reactions (Parkin and Owen, 1986; Remigi and Buckley, 2006). A low pH can bring about an accumulation of VFA, which inhibits digestion (Parkin and Owen, 1986; Pearse et al., 2018). This can occur when a substrate with sufficient inhibiting substances (NH₃, H₂S and heavy metals) is added into the serum bottle, or when bottles are exposed to transient temperature changes (Jingura and Kamusoko, 2017; Remigi and Buckley, 2006). As a result, unstable operations can develop as the VFA production rate exceeds the methanogenic VFA utilization rate. As the pH lowers, the VFA utilization kinetics and methanogenic activity decreases: advancing VFA accumulation, inhibiting methane production and resulting in a process failure (Remigi and Buckley, 2006). Both methane and carbon dioxide content can be used as indicators of an upset. Typically, the methane content of biogas is in the range from 60 to 75% with carbon dioxide comprising the remainder. Large decreases outside this range could indicate a failing test. A high pH, on the other hand, can be inhibiting due to concentrations of free ammonia and ammonium ions. FA has been suggested to play a major role in inhibition because it can freely pass through the membrane of the microorganisms and diffuse into the cell, leading to proton imbalance and potassium deficiency (Barber, 2016; Wang, 2016). Ammonia concentration (NH₃-N) of less than 200 mg/L are beneficial for the AD process as it is an essential nutrient (Wang, 2016). According to Parkin and Owen, (1986), researchers suggest that FA concentrations above 100 mg/L can cause toxicity.
Normally, an alkalinity in the range of 2000 to 3500 mg/L as CaCO₃ is needed to maintain the pH at neutral. In the BMP tests, the production of VFA will reduce the alkalinity while the production of NH₃ from protein and amino acid deamination will increase the solution alkalinity. For the materials that have a high protein contents, there will be less likely to see a significant drop in pH in BMP.

4.2.4.2.2. Monitoring Solid Concentration Reductions
Sacrifice bottles can also provide insight into the kinetics of the reaction process by observing the reduction in solid concentrations (see Figure 19). The destruction of organic matter is the primary objective of anaerobic digestion. Therefore, COD and VS must be measured to determine the overall process efficiency. Monitoring physical properties of wastewater is important to assess the reusability of the wastewater and determine the most suitable type of process for its treatment. As shown in Figure 1, TS, VS, TSS and VSS measured over 30 days for both blank and test bottles could provide insight in to which parameter has the greatest reduction.

But as an indicator of imbalanced digestion, organic matter destruction is not a sensitive measurement of process imbalance. It will only confirm what trends VA, pH, TALK and methane production have already shown. Frequent monitoring of influent COD and VS levels may help determine if system imbalance was caused by increased organic loading (reduction in effective SRT) and may help to predict and minimize detrimental effects if the monitoring is frequent enough (Parkin and Owen, 1986).

![Figure 4-18: Example of solids reduction measurements during 30-day BMP test](image)
4.2.4.2.3. Mass balance
COD mass balances can assist in validating results and making them comparable (Kinyua, 2013). COD mass balances can be carried out because COD is not destroyed but re-disturbed in anaerobic digestion. Theoretically, the COD in the influent is equal to the COD leaving the system, which occurs through effluent, methane generation or incorporated into new bacterial mass (Lier et al., 2008).

\[
COD_{\text{influent}} = COD_{\text{effluent}} + COD_{\text{gas}} + COD_{\text{sludge}}
\]

Equation 4-10: COD Balance

The methane COD can be calculated using the empirical relationship, where 1 kg COD can be converted into 0.35 m³ CH₄, and the COD difference between the COD influent and COD effluent (Lier et al., 2008) (see Equation 10 and 11). However, the COD mass balances of a reactor will not be 100%. If the liquid COD measurements are accurate, the gap between could provide insight into the amount of newly grown and entrapped biomass (Kinyua, 2013). But to complete a perfect COD mass balance is difficult in accounting for fates of COD in the anaerobic digestion process and potential errors in measuring the COD in the anaerobic liquid.

\[
V \left( \frac{m^3}{d} \right) = 0.35 \frac{m^3 CH_4}{kg COD} \times (COD_i - COD_e) \left( \frac{kg}{m^3} \right) \times Q
\]

Equation 4-11: Volume of Predicted Methane Generation

There are various fractions in the anaerobic digestion process contributing to a gap in the COD balance. Lier et al., (2008) reported the relative importance of the indicated COD fraction in influent, effluent, sludge, and biogas in terms of soluble organic/inorganic, suspended organic/inorganic, absorbed, entrapped CH₄, H₂, H₂S, N₂ and newly grown biomass. The authors discussed two frequently cited causes for the COD gap. One occurs when there is a “loss of electrons” to oxidise anions like SO₄²⁻ and NO₃⁻, and the other is when COD is entrapped or accumulates in the sludge bed. The latter situation occurs when
the wastewater being treated had a high fat or LCFA content. In these situations, the combination of high measured COD removal efficiency but low methane production rates could lead to large gaps in the COD balance, indicating long-term operational problems (Lier et al., 2008).

Moreover, there remains a question of the accuracy of COD measurements for solid and liquid samples with high suspended solid content in anaerobic research. Raposo et al., (2011) stated, directly measuring COD is thought to produce erroneous results. Angelidaki and Sanders, (2004) listed possible reasons that might cause problems during COD measurements as (1) volatile straight-chain aliphatic compounds are not oxidized to any appreciable degree, (2) aromatic carbohydrates, and some aromatic heterocyclic compounds are not oxidized, (3) NO₂⁻ exerts a COD of 1.1 mg/mg NO₂⁻N, and (4) reduced inorganic compounds such as ferrous iron, sulphide, managanese are oxidized quantitatively under the species (Angelidaki and Sanders, 2004). In 2008, the first Proficiency Test (PT) of COD was completed with twenty-six labs from sixteen countries to measure the COD of two solid samples and two high concentrated suspended solid samples (F. Raposo et al., 2009). All participants used potassium dichromate as the oxidant reagent but with different experimental procedures. Out of the total participants reporting data (twenty-six labs), 36% of results were satisfactory, 9% doubtful, and 5% unacceptable. Only two labs (8% of participants) reported the four samples adequately. The short-term conclusion was that solid samples and liquid samples with high solid concentration could not be analyzed accurately. A second PT was carried out in 2009. In comparison with the previous results, the overall performance improved by 30%, respectively (Raposo et al., 2010). Raposo et al., (2010) interpreted it as a sign of general improvement, and possible to accurately measure the COD of difficult samples with acceptable quality. Despite the sensitivity of obtaining perfect mass balance results, COD mass balances should be developed. They can still be useful trouble shooting tools for new laboratories starting conventional BMP tests.
4.2.5. Data Quality and Reporting

4.2.5.1. Complexity of Methane correction

Standardized accumulated methane volume measurements are important for reliable and comparable BMP and rate constant values. But corrections of methane volumes to standard conditions are often poorly communicated in published experiments. This often involves uncertainty due to the missing information about emerging factors such as temperature, pressure, water vapour and headspace composition. According to Strömberg et al., (2014), most scientific papers in the field of anaerobic digestion simply quote gas production volumes without mentioning any corrections applied to standard conditions. Strömberg et al., (2014) completed a short literature study on gas normalisation of 23 papers (exclusively on the digestion of cattle manure). One out of the 23 correctly accounted for temperature, pressure and water vapour. Eight reported a correction for temperature and pressure but not water vapour, and seven were missing correction information.

The main confusion for researchers converting methane volumes could be focused on two factors: (1) confusion about which standard reference conditions to adopt, and (2) confusion about which correction equation to use depending on the type of biogas monitoring technique. As stated by Parajuli, (2011), there is an issue when there are different standard reference conditions. For example, the National Institute of Standards and Technology uses 101.325 kPA and 20°C, while the International Union of Pure and Applied Chemistry uses 100 kPA and 0°C (Parajuli, 2011). But also selecting from the variety of correction equations reported in literature for syringe (Equation 12), liquid displacement (Equation 13) and manometer (Equation 14). The major difference between each technique being the decision to adjust for water vapour, include overestimate correction factors, or which order in which equation to adjust for temperature and pressure. Until there is clarity about a single method, or clarity of conversion for each of the three methods, there may always be some question about the validity of methane corrections. It is estimated that in the future, this area will be central for the standardized method.
Syringe Method (see Trzcinski and Stuckey, 2012): \( V_{CH_4,t} = V_{biogas,t} \frac{\%CH_4,t}{100} + V_{headspace} \frac{\%CH_4,t-\%CH_4,t-1}{100} \)

Liquid Displacement Method (see Strömberg et al., 2014): \( V_{acc,i} = V_{acc,i-1} + (VM - VOE,i) \left( 1 - \frac{P_{vap,i}}{P_{gas,i}} \right) \left( \frac{T_{STP}}{T_{gas,i}} \right) \)

Manometer Method (see Valero et al., 2016):
\[
V_{CH_4,t} = \left( V_{headspace} \frac{T_{STP}}{T} + V_{biogas,STP} \right) \left( \frac{\%CH_4 \text{ dry, current}}{100} \right) - \left( V_{headspace} \frac{T_{STP}}{T} \right) \left( \frac{\%CH_4 \text{ dry, previous}}{100} \right)
\]

Where \( V_{CH_4,t} \) is the volume of methane produced in the measurement interval (mL), \( V_{headspace} \) is the volume of the headspace within the serum bottle (mL), \( V_{biogas,STP} \) is the volume of biogas measured during the sampling period converted to STP (mL), \( VOE,i \) is the over-estimated volume at the sampling point (see Strömberg et al., (2014)), \( V_{acc,i} \) is the accumulated volumes, \( V_{acc,i-1} \) is the accumulated volume calculated from the previous sampling point, \( VM \) is the volume for each measurement point (see Strömberg et al., (2014)) \( \%CH_4 \text{ dry, current} \) is the current methane percentage of the generated biogas determined by GC, \( \%CH_4 \text{ dry, previous} \) is the methane percentage of generated biogas in the last sampling time point, \( T_{STP} \) is the standard temperature (273.15K), \( T \) is the incubation temperature (K) for the BMP test, \( P_{vap,i} \) is the water vapor pressure (5.626 kPa) at the 35°C, \( P_{gas,i} \) is the pressure of the measured gas (101.325 kPa).

4.2.5.2. Methane Curve Interpretation

Understanding the meaning of the methane yield curve could provide the operator insight into the rate limiting step of the test material during anaerobic digestion (see Figure 20). As stated by Remigi and Buckley, (2006), there are four possible interpretations of a methane yield curve of the test material. Curve 1, the test material is readily biodegradable. Biogas and methane are immediately produced, and the methane yield curve quickly levels off.

Curve 2, the test material is biodegradable after a lag phase. A lag phase could indicate hydrolysis as the rate limiting step in the anaerobic digestion process. Curve 3, the test material is inhibitory in the initial phase of incubation. In this case, the test material contains toxic substances that are inhibiting the microorganisms, causing the test material methane
production to be lower than the blank. For this reason, when the methane is subtracted from the blank, the methane yield becomes negative. Curve 4, the test material is inhibitory throughout the entire period of incubation. The test material contains toxic substances inhibiting methane bacteria and hydrogen producing and consuming bacteria. No methane is produced in comparison to the blank which is slowly producing methane. Therefore, as the test continues and the substrate bottles continue to no produced biogas, the methane yield becomes increasingly negative.

![Graph showing different methane yield curves for four different test materials.](image)

Figure 4-19: Example of different methane yield curves for four different test materials.

### 4.2.5.3. Kinetics

BMP kinetic rate constant (k) provides useful information of degradation kinetics of materials to achieve optimal design and operation of anaerobic digesters. But finding the correct value is difficult to achieve, as it is more sensitive to the experimental conditions than the methane yield (Jensen et al., 2011; Q. Li et al., 2015). In published literature, many kinetic models have been used to describe the methane production of BMP tests (First order rate model, Monod type model, Modified Gompertz model, a combination of two first order rate models, Chen and Hashimoto model) (see Equations 15,16,17,18,19).

First order rate model: $BMP(t) = BMP(\infty) \times (1 - \exp(k \times t))$  

Equation 4-15: First Order Model

Monod Type Model $BMP(t) = \frac{BMP(\infty)k(t-\theta)}{k(t-\theta)+1}$  

Equation 4-16: Monod Type Model
Modified Gompertz model: \( BMP(t) = BMP(\infty) \times \exp\left\{ \frac{-\theta_1 \times \exp(-k_1 \times t)}{k_1} - \frac{\theta_2 \times \exp(-k_2 \times t)}{k_2} \right\} \)

Equation 4-17: Modified Gompertz Model

A combination of two first order rate models:
\( BMP(t) = BMP(\infty)(1 - X \times \exp(-k_1 \times t) - (1 - X) \times \exp(-k_2 \times t)) \)

Equation 4-18: Two First Order Model

Chen and Hashimoto Model: \( BMP(t) = BMP(\infty)(1 - \frac{K_{CH}}{HRT \times \mu_m + K_{CH} - 1}) \)

Equation 4-19: Chen and Hashimoto Model

Where \( BMP(t) \) is the BMP value at time \( t \) (day), \( BMP(\infty) \) is the ultimate BMP (NmL/g VS) \( k_1 \) and \( k_2 \) are rate constants, \( X \) is the fraction of readily degradable material, \( \theta \) (1/day) describe the lag period (Strömberg et al., 2015). HRT is the digestion time (days), \( K_{CH} \) is the Chen and Hashimoto kinetic constant, \( \mu_m \) is the maximum specific growth rate of microorganism (1/day)(Kafle and Chen, 2016).

4.2.5.3.1 Determination of Kinetic Constant

Currently, there is no standardized model to apply to all BMP results. The variability of the selection of the model is based on the substrates used. There are some common models that are more accurate and applicable than others (Mohamed et al., 2018). Kafle and Chen [60] compared the first order model, to the modified Gompertz model and Chen and Hashimoto model. The first order model showed better fit than the modified Gompertz, but when a lag phase was reported the modified Gompertz model better predicted the BMP compared to the first order. Strömberg et al., (2015) evaluated of six different kinetic models (first order rate model, a first order rate model with variable order of time dependency, a combination of two first order rate models, a Monod type model, a quadratic Monod type model and a modified Gompertz model) in predicting the final BMP and test time. The Monod type, quadratic model and first order had positive effects on BMP predictions. While the first order, two combined first order and the modified Gompertz had negative impacts. C. Li et al., (2015) compared the first order model, modified first-order model and the Gompertz model to fit the
BMP curve of wheat straw, separated stem. The modified first-order model had the highest simulation precision, while the first order model had the lowest precision. The maximum BMP value simulated by the Gompertz was the closest among the three models. As a generalization, the first order model is used for fast and abruptly stopping degradation, Monod model better describes the slowly declining gas production at the end of the process, the combination of two first order equations are used when a substrate has two separate degradation profiles, the modified Gompertz equation can be used when a lag phase is present.

It is difficult to compare kinetic constants due to the complex nature of each individual experimental setup (particle size, origin of inoculum, mixing rate and temperature). One of the most studied aspects of influence being the SIR. Multiple studies have shown the variability of kinetic constants of BMP methane production with the substrate to inoculum ratio Hashimoto, (1989) with ball-milled straw, F Raposo et al., (2009) with sunflower oil cake and Moset et al., (2015) for maize. In most cases, high hydrolysis rates were reached in anaerobic biodegradability tests with a low SIR, showing a degree of dependence of hydrolysis to inoculum concentration and activity. As stated above, an experiment that choses one SIR from recommended may not be the optimal ratio for a specific substrate, possibly underestimating the value.

4.2.5.4. Data Rejection and Data Reporting

Holliger et al., (2016) stressed the often-unaddressed area of quality control, stating that data must pass some quality criteria for use such as than the test results must be rejected:

1. if the Relative Standard Deviation (RSD) of the blank or the positive control is >5%, even after applying a statistical test to eliminate a single outlier
2. if the RSD of a homogenous substrate is >5%, even after applying a statistical test to eliminate a single outlier.
3. BMP of the positive control <85% or >100% of theoretical BMP

The analysis and presentation of BMP data are one area often left partially addressed in most standard procedures, specifically the type of equipment and applied experiment set-up, which
many times are self-developed and specific for each laboratory. Details that should be accounted for in the final report were combined from Angelidaki et al., (2009) and Holliger et al., (2016). The following details should be presented in the final report:

1. Inoculum and substrate physiochemical characteristics
2. Test Conditions and Setup
3. Graphs of gross methane production of the substrate batches
4. Positive Controls and blanks

4.2.6. Summary

As of 2018, there continues to be a lack of standardization/ universal BMP testing procedure, limiting the comparability of results. However, BMP methods continue to evolve, and key aspects studied to further the elimination of systematic errors. In this paper, key aspects of proposed BMP methods were reviewed and summarized with the latest research progress to inform a simplified serum bottle method. Updating these recommendations may increase the probability of obtaining validated and reproducible BMP.

4.3. BMP Serum Bottle Syringe Method for Wastewater Sludge Anaerobic Digestion Studies

The BMP serum bottle method was outlined in the following section to determine the key steps and parameters of the BMP test to characterize methane production potential and biodegradability of WWTP primary and secondary sludge (see Figure 21). Serum bottle syringe method was chosen for its flexibility, quick set up and ease of use. The objective was to structure the following sections for a new operator to increase the ease of starting new tests to provide insight into their anaerobic digester system. This BMP serum bottle method procedure has four main components: (1) test preparation, (2) test start up and operation, (3) data analysis, (4) data presentation.

![Flow diagram for BMP procedure](image-url)

**Figure 4-20:** Flow diagram for BMP procedure.
4.3.1. Materials
The materials required for the BMP serum bottle syringe method include:
1. Batch anaerobic digester containers: 160 mL Wheaton serum bottles; American Scientific Products, McGraw Park III
2. Temperature controlled environment: New Brunswick Scientific C25 Incubator
   Shaker Classic was used as the incubator.
3. Flush gas: Pure nitrogen
4. Biogas Production Measurement Device: 10 – 50 mL glass syringes (Popper and Sons Inc.)
5. Gas Composition Analysis: Agilent 6890 GC (gas chromatography) system (Agilent Technologies, USA) with TCD. Argon was used as the carrier, with an inlet temperature of 200 °C.
6. Characterization of inoculum and substrates: Apparatus for the determination of COD, solids, alkalinity, and VFA

4.3.2. Test Preparation
Anaerobic digester, primary and secondary sludge were collected from the Guelph, Wastewater Treatment Plant (WWTP). The Guelph WWTP is located at Guelph, Ontario, Canada and provides treatment of domestic, commercial, institutional and industrial wastewater collected from the community of the Guelph/Eramosa [Wastewater treatment plant annual report]. The Guelph WWTP process consists of preliminary screening and grit removal, primary sedimentation, extended aeration activated sludge treatment, secondary clarifications, rotating biological contactors (RBC) and sand filtration tertiary treatment, and chlorine disinfection. The typical wastewater daily average flow treated by the Guelph WWTP is 50.02±15.6 ML/, which contained a cBOD5 of 193.4±15.6, TSS of 257.2±27.1, total phosphorus of 5.14±0.38, TkN of 38.5±2.9, and NH3-N of 22.3±1.6 mg/L according to the annual average values from 2011 to 2015, and the recorded removal efficiencies for cBOD5, TSS, TP, TKN, and NH3-N are around 98.8%, 99.2%, 97.0%, 95.9%, and 97.9%, respectively. The raw sludge produced in the Guelph WWTP is thickened in the primary clarifiers and further thickened to a sludge of 4.3% solid content by a rotary drum thickener and send to the anaerobic digesters. The WWTP plant generated 27,529 m³ of thicken sludge per year to the anaerobic digesters which were operated at a SRT around 15 days.
Total solids (TS), volatile solids (VS), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were determined by standard methods (Method 2540-1997, and EPA Method 160.4). Chemical oxygen demand (COD) and volatile fatty acids (VFA) of tested samples were determined using Hach test vials (Hach, Canada). Raw or pretreated secondary sludge was centrifuged at 10,000 rpm, 4 °C for 15 min, and the supernatant was filtered through a 0.45 um syringe filter, and the pH of filtered sample was determined by TitraLab® 870 titration workstation (Hach, Canada).

Table 13 summarises the main characteristics of AD sludge of the Guelph WWTP for different sampling times. The AD sludge showed a stable TS content of 19.6 ±0.3 g/L over the sampling period, which was very close to the annual average TS 19.5 g/L over the period of 2011 to 2015. The VS/TS and VSS/TSS ratio of the AD sludge were determined to be 0.63±0.14 and 0.70 ± 0.12, respectively. The relative stable TS and VS/TS ratio with the AD sludge suggests that the AD digesters of the WWTP can provide biological consistent inoculum for the sludge BMP tests. The inoculum AD sludge was stored in 2L sealed plastic bottles with the headspace flushed with 100% nitrogen, and kept in the incubator at 35°C for 1 to 5 days to degas and reduce the impact of its methane production (Angelidaki et al., 2009; Raposo et al., 2011).

Table 4-13: Inoculum characteristics.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD</td>
<td>mg/L</td>
<td>17160±350</td>
<td>18460±221</td>
<td>17060±222</td>
<td>19000±240</td>
</tr>
<tr>
<td>SCOD</td>
<td>mg/L</td>
<td>250±9</td>
<td>525±0.33</td>
<td>546±34</td>
<td>647±20</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>19.08±0.02</td>
<td>19.28±0.16</td>
<td>19.74±0.10</td>
<td>19.74±0.25</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>16.05±0.07</td>
<td>11.07±0.16</td>
<td>10.92±0.03</td>
<td>10.92±0.12</td>
</tr>
<tr>
<td>TSS</td>
<td>g/L</td>
<td>11.05±0.39</td>
<td>18.14±0.28</td>
<td>18.40±0.19</td>
<td>18.40±0.48</td>
</tr>
<tr>
<td>VSS</td>
<td>g/L</td>
<td>9.71±0.28</td>
<td>11.06±0.18</td>
<td>12.17±0.04</td>
<td>12.17±0.38</td>
</tr>
<tr>
<td>ALK</td>
<td>mgCaCO₃/L</td>
<td>4825±19</td>
<td>5867±95</td>
<td>5187±173</td>
<td>4772±160</td>
</tr>
<tr>
<td>pH</td>
<td>---</td>
<td>7.7±0.1</td>
<td>7.6±0.1</td>
<td>7.4±0.1</td>
<td>7.6±0.1</td>
</tr>
</tbody>
</table>

Primary and secondary sludge were passed through a 4.75 mm sieve to remove any large particles and analyzed to determine total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), Chemical Oxygen Demand (COD) according Standard method (Method 2540-1997, and EPA Method 160.4). The characteristic
parameters of the primary and secondary sludge are shown in Table 14. Compared to the secondary sludge, the primary sludge had a much higher TCOD, SCOD, TS, and VS contents and VS/TS ratio. The alkalinity of primary sludge was also significantly higher than the secondary sludge.

Table 4-14: Substrate characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Primary Sludge</th>
<th>Secondary Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCOD</td>
<td>mg/L</td>
<td>47055±2991</td>
<td>10670±254</td>
</tr>
<tr>
<td>SCOD</td>
<td>mg/L</td>
<td>1945±83</td>
<td>58±6</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>36.02±0.22</td>
<td>10.36±0.25</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>26.09±0.24</td>
<td>6.63±0.12</td>
</tr>
<tr>
<td>TSS</td>
<td>g/L</td>
<td>33.57±0.24</td>
<td>9.32±0.48</td>
</tr>
<tr>
<td>VSS</td>
<td>g/L</td>
<td>25.03±0.17</td>
<td>6.55±0.38</td>
</tr>
<tr>
<td>ALK</td>
<td>mgCaCO_3/L</td>
<td>2333±175</td>
<td>83.6±0</td>
</tr>
<tr>
<td>pH</td>
<td>---</td>
<td>6.5</td>
<td>7.36</td>
</tr>
</tbody>
</table>

4.3.3. Design Calculations
To make sure the BMP is carried out in conditions that are not limiting or inhibiting of the anaerobic digestion process, each BMP test will be designed differently depending on the inoculum and substrate concentrations. This involves adjusting both the inoculum and test material volumes until the (1) estimated gas production, (2) substrate to inoculum ratio, (3) reactor VFA/Alkalinity Ratio, and (4) headspace to total solution volume, are all balanced within their recommended parameter ranges (see Table 15).

4.3.3.1. Substrate to Inoculum Ratio (SIR)
In order to find the maximum methane potential and methane production rate, the right balance between the substrate and microorganisms are needed (Chen and Hashimoto, 1996; Chudoba et al., 1992; Neves et al., 2004; Yoon et al., 2014). As stated by Raposo et al., (2012) theoretically, the methane yield should be independent of the SIR, and the SIR only affects the kinetics of the methane production. However experimental data shows that SIR can have an influence on both, due to the strong evidence that the ratio directly affects the growth patterns of microorganisms (Liu, 1996; Raposo et al., 2012, 2006). As a baseline, Owen et al., (1979) first proposed, that 1 g VS substrate/ g VS should be used (Chynoweth et al., 1993; Owen et al., 1979; Raposo et al., 2011). The German standard, VDI 4630 recommended a SIR of less than 0.5 (Wang, 2016). Although this provides a useful guideline for the selection of SIR, different substrates may react differently. As stated by Elbeshbishy
et al., (2012), there is a wide range of optimum SIR depending on the substrate and inoculum.

The authors investigated the influence of SIR ratios on the methane yields and kinetic constants of the primary and secondary sludge by varying the SIR from 0.1, 0.5, 1, 1.5, and 3 g substrate COD/g inoculum VS. In these tests, the total working volumes were set to 55 mL and 60 mL for the primary and secondary sludge BMP tests, respectively, while the volumes of the substrate and inoculum were varied to achieve the desired SIRs. For the primary sludge tests the substrate/inoculum volumes were 2mL/53mL, 7 mL/48 mL, 12 mL/43 mL, 15 mL/40 mL, 23 mL/32 mL and for secondary sludge 5 mL/55 mL, 20 mL/40 mL, 30 mL/30 mL, 25 mL/35 mL, 45 mL/15 mL. The blanks were used for each condition by replacing the substrate with the same volume of DI water. Triplicates of BMP bottles were used for every testing condition. Figure 22 depicts the methane production increased with increasing SIR at a linear fashion. Based on these results, the differences in methane production were due to the increase of organic matter added into the serum bottles.

![Graph](image)

Figure 4-21 Left: Primary sludge ratio test: cumulative substrate methane production, right: secondary sludge ratio test: cumulative substrate methane production (where 0.1, 0.5, 1, 1.5 and 3 are the g substrate COD/g inoculum VS ratios).

Figure 23 shows the methane yield results for primary and secondary sludge. The methane yield in the primary test was found to be 481±1, 470±1, 495±1, 482±1, and 470±1 NmL CH₄/g VS, and corresponding biodegradability (%) of 60±1, 59±1, 62±1, 60±1, and 59±1. These results were similar to those in literature. As stated by Parkin and Owen, (1986), primary sludge from the primary clarifier is comprised of natural fibers, fats and other solids.
and has a high biodegradability (69%), reporting typical values in literature of 40-60% reduction in COD and 40-70% reduction in VS (Parkin and Owen, 1986). The methane yield of the secondary sludge was 45±1, 166±1, 218±2, 230±2, and 218±1 NmL CH4/g VS, and corresponding biodegradability (%) of 8±0, 29±1, 39±2, 41±2, and 39±2, for SIR 0.1, 0.5, 1, 1.5, and 3. In literature secondary sludge or waste activated sludge (WAS) is reported to be half as digestible as primary sludge with biodegradability ranging from 30-50% due to the microbial cells that are often hardly biodegradable causing the degradation kinetics to act slowly (Parkin and Owen, 1986). It is important to note the reduction in accuracy as the SIR decreased below 1.0, which is underestimation due to a combination of factors. One would be due to the small volume of secondary sludge added into each serum bottle. As the volume of the substrate was lowered, the secondary sludge had very little to offer the microorganism, and from having a high headspace volume in relation to the liquid volume lower gas flows and more influence of the initial head space gas (Strömberg et al., 2014; Wang et al., 2015). As stated by Elbeshbishy et al., (2012), having too low SIR may prevent induction of the enzyme necessary for biodegradation. In addition, there is the measurement inaccuracy due to little amount of biogas produced, which would affect the conversion and calculation of the methane yield resulting in significant underestimation. This was observed when the total methane produced by the test bottles for 0.1 and 0.5 generated 2 and 16mL of CH4 after the blank was subtracted. In comparison, test bottles for primary sludge at 0.1 and 0.5 with the blank subtracted produced 21.1±0.6 and 71±2 mL CH4.

Figure 4.22: Left: Primary and secondary sludge ratio test methane yield results. Right: primary and secondary sludge biodegradability results for different SIR.
Although the most common trend reported was an overestimation of BMP values as the SIR decreased, the substrates used in the experiments appeared to be high in organic content. In the comparison, an underestimation of BMP values could be the case for substrates with very low COD and solid content, therefore requiring higher SIR ratios to be used than wastewater with high organic contents. It is recommended that for substrates with low organic content, with a history of being difficult to digest, SIR should be designed at higher ranges compared to substrates with high organic content and readily biodegradable. In this study, SIR above 1g COD/g VS should be used to determine the BMP values for secondary sludge, while SIR for primary sludge can be lower than 1:1, but it is not recommended. A minimum of three different substrate to inoculum ratios be tested for every new substrate. Additional tests are required to observe the accuracy of BMP values at higher range of F/M values (>3) to observe overloading effects.

To observe the possible impacts the SIR can have on measured kinetic constants, the methane yield curves for primary and secondary were analyzed. The methane production rate constant for a BMP serum bottle experiment was calculated using the following equation, where k is the first order kinetic constant (per day), t is the digestion time (days), and BMP (∞) is the ultimate methane production at the end of the test (Elbeshbishy et al., 2012).

\[
BMP(t) = BMP(\infty) \times (1 - \exp(k \times t))
\]

MATLAB was used to find the value of k by minimizing the sum of squared differences between the experimental and calculated values. Figure 24 shows the kinetic modelling of the primary and secondary sludge ratio tests. Figure 25 shows there were significant variations between the kinetic constant values between primary and secondary tests. Both experiments k values decreased as the loading rates increased. Primary sludge kinetic values ranged from 0.21 to 0.51, while secondary sludge ranged from 15.2 to 0.151. The kinetic constants for the secondary sludge tests below 1:1, had greater variation because the substrates were added in small volumes to the inoculum, and were quickly converted to methane. As a result, the accuracy of modelling 0.1 and 0.5 SIR methane yield curves decreased, with \( R^2 \) values of
0.49 and 0.96. Kinetic values found in BMP tests should be used with caution, in predicting the kinetic behaviour of continuous digesters. There is the possibility that basic kinetic models over-simplify the dynamics of rate-limiting step, not considering the various conditions in a continues digester operation such as wastewater characteristics, hydraulic loading (Strömberg et al., 2015).

There are two general rules for narrowing down the SIR selections. One is the recommendation that for easily biodegradable substrates where rapid accumulation of fermentation intermediates such as VFA could inhibit anaerobic digestion, the inoculum volume should be greater than the substrate or a SIR less than or equal to 0.5 should be applied to minimize the possibility of acidification or inhibition problems (for instance SIR of 0.5 or 0.25) (Liu, 1996). The second rule is that for substrates that have a high content of non-readily biodegradable organics, a SIR higher than 0.5 should be applied. But, regardless of these rules of thumb, a series of SIR for a new substrate should be tested in order to obtain a reliable BMP values (Chynoweth et al., 1993; Neves et al., 2004; Raposo et al., 2006; Yoon et al., 2014).

Figure 4-23: Left: Primary sludge ratio test kinetic modelling of methane yield, right: secondary sludge ratio tests kinetic modelling.
4.3.3.2. Managing Potential Biogas Production

During the period between two subsequent re-equilibrations (gas measurements and gas wasting), the serum bottles are pressurised from gas production. As shown in Figure 26, depending on the organic content of the substrate, a test with highly biodegradable substrate may require more frequent re-equilibrium/gas releases than a non-biodegradable substrate. For operation purposes it is helpful to predict the estimated volumes of generate biogas for scheduling inspects. The COD to methane conversion ratio, allows for the prediction of the volume of generated biogas. Using 1 g COD=0.395 L CH₄ for conditions at 35°C, it is important to balance the liquid volumes of each solution added to avoid the total biogas per day exceeding the headspace volume – leading to over pressurization and requiring increased gas releases. It is recommended that at least 100-200 mL CH₄ or 250-400 mL biogas (assuming 60% CH₄) be produced, allowing for a volume of 10 to 60 mL of biogas to be collected per extraction time. This is important for accurate manual syringe readings and acquiring enough biogas to be processed by the GC.

4.3.3.3. Headspace to Total Solution Volume

The headspace is defined as the non-liquid volume in the serum bottle after filling with testing materials and inoculum. Ratios of the headspace to total bottle volume (160 mL) range from 30 to 70% in reported BMP tests. Normally, the headspace should be larger than
the expected maximum produced biogas volume in the first day as it is important to avoid the bottle becoming over pressurized due to the production of biogas and increased temperatures.

Figure 4-25: Example of headspace pressure releases for a highly biodegradable and non-biodegradable substrate.

4.3.3.4. Reactor VFA/Alkalinity Ratio

The VFA/alkalinity, as stated by Feng et al., (2013) has three critical levels to assess the stability of anaerobic digestion, where (1) < 0.4 stable; (2) 0.4-0.8, some instability will occur, (3) >0.8 significant instability (Feng et al., 2013). Therefore, during planning stage, it is recommended that the operator adjust the inoculum and substrate volumes for the final solution to be below the first critical level.

4.3.3.5. Guideline Recommendations

As discussed in the above sections, accurate BMP tests need proper design of the testing parameters to achieve balanced acidification and methanogenesis reactions so that the BMP results can reflect the ultimate methane yield and biodegradability of the substrates. Table 15 shows an example of the design of key BMP parameters used for testing the primary and secondary sludge sampled from the Guelph WWTP. Since the primary and secondary sludges had different properties, the liquid volume of the seed and substrate were different for both tests in order to meet the desired SIR. In order to determine a proper SIR for given sludge properties, as discussed in section 3.3.1, a series of BMP tests needs to be conducted to assess the effect of SIRs on the methane production. The determination of the total and
substrate volumes should consider the total biogas production (3.3.2), headspace to total solution volume (3.3.2), and GC measurement requirement (2.4.1.4). The total solution alkalinity of the mixed solution or VFA/alkalinity ratio is important to maintain a stable pH condition. For the anaerobic digestion of wastewater sludge, alkalinity is produced by breakdown of proteins to \( \text{NH}_3 \) which reacts with \( \text{CO}_2 \) to form \( \text{NH}_4^+ \) and \( \text{HCO}_3^- \). The accumulation of VFA in the BMP bottles, which will consume alkalinity and cause pH to drop, could inhibit methanogenesis reactions. As stated in 3.3.4, it is recommended that the mixed solution have an alkalinity equal to or higher than 3 g \( \text{CaCO}_3 \)/L or the VFA/Alk be less than 0.4. It should be kept in mind that the values shown in table 5 are only examples we determined for the primary and secondary sludge from the Guelph WWTP. These values may not be suitable for other substrates. The optimal SIR, substrate/inoculum volumes, and predicted biogas production will depend on the characteristics of the organic content of substrate.

Table 4-15: BMP test design for primary and secondary sludge from the GWWTP

<table>
<thead>
<tr>
<th>Section</th>
<th>Parameter</th>
<th>Recommendations</th>
<th>Primary Sludge Test</th>
<th>Secondary Sludge Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>Sludge</td>
<td>mL</td>
<td>43</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>mL</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Substrate</td>
<td>mL</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Total Volume</td>
<td>mL</td>
<td>50-120</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Headspace</td>
<td>%</td>
<td>30-70</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Sub COD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIR</td>
<td>g/AD VS g g VS/ g VS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Predicted Methane</td>
<td>mL</td>
<td>165</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Biogas Gen. Max</td>
<td>mL</td>
<td>300-500</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>Biogas Production</td>
<td>mL</td>
<td>55</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Per 5 Days Max</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Alkalinity</td>
<td>g ( \text{CaCO}_3 )/L</td>
<td>3.87</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td>VFA/ALK</td>
<td></td>
<td>&lt;0.4</td>
<td>0.028</td>
</tr>
</tbody>
</table>

It should be kept in mind that the values shown in table 5 are only examples we determined for the primary and secondary sludge from the Guelph WWTP. These values may not be suitable for other substrates. The optimal SIR, substrate/inoculum volumes, and predicted biogas production will depend on the characteristics of the organic content of substrate.
4.3.4. Execution of BMP syringe test

The experimental procedure is split into three sections: (1) start-up, (2) biogas monitoring, and (3) final testing. The start-up stage starts by heating up the seed and substrate to the working temperature. The solution volumes determined from the test design are then added into triplicate groups of serum bottles. The headspace of each bottle is then flushed with nitrogen, and immediately capped with rubber stoppers and sealed with aluminum crimps to make sure the stoppers do not fall out. The bottle are placed in the incubator and after 1 to 2 hours, the gas composition of each bottle are measured to ensure the absence of oxygen.

Biogas monitoring stage consists of repeated gas sampling, gas composition measurements and gas wasting periodically until the methane production had leveled off. This stage usually lasts longer than 30 days. Final testing stage is the opening of the bottles and measuring the contents for insights into the solution health, solids concentration reduction and mass balance. Table 16 outlines the key steps and the times estimates for each stage.

Table 4-16: Execution stages and steps for manual operation of BMP serum bottle test

<table>
<thead>
<tr>
<th>Stage</th>
<th>Step</th>
<th>Time</th>
<th>Process</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start-up</td>
<td>1</td>
<td>1-2 hrs</td>
<td>Warm liquids to 35°C</td>
<td>Active inoculum, test material, control solution.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.1 hrs</td>
<td>Pour contents into vials</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.1 hrs</td>
<td>N₂ Flushing</td>
<td>Every bottle’s headspace was immediately flushed with 100% nitrogen gas to remove any oxygen.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.1 hrs</td>
<td>Seal bottles and Store at 35°C</td>
<td>Cap bottles with rubber stoppers and sealed with an aluminum crimp. Place bottles in the incubator at 35°C.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.1 hrs</td>
<td>Re-equilibrate</td>
<td>After 1 to 2 hours and measure the gas composition.</td>
</tr>
<tr>
<td>Biogas</td>
<td>6</td>
<td>&gt;30 days</td>
<td>Biogas sampling</td>
<td>Shake each serum bottle before the gas is vented.</td>
</tr>
<tr>
<td>Monitoring</td>
<td></td>
<td></td>
<td></td>
<td>Using a gas tight syringe (equipped with a valve between the needle and opening) collect biogas from at least two of the triplicate bottles. At least 10 mL of biogas are needed for accurate GC measurements. Inject the sample into the GC.</td>
</tr>
<tr>
<td>Final</td>
<td>7</td>
<td>0.5-1 day</td>
<td>Open bottles and measure contents</td>
<td>Stop test when the methane production has levelled off and the gas composition is constant.</td>
</tr>
<tr>
<td>Testing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3.5. Data Analysis

After the methane volumes were recorded and the test had ended, the methane volumes were converted into standard conditions (see Table 17). To standardize the BMP results, the as-measured volumes must be converted to standard conditions (0°C at 1 atm). This involves compensating for both volume occupied by water vapour (generates over-estimations of 2-8% in the gas volume at ambient temperature range) and thermal expansion effects (Richards et al., 1991; Strömberg et al., 2014). Normally, volumes are measured at one atmosphere, so no pressure correction is required (Richards et al., 1991). The biogas sampling intervals was calculated using the following equation:

\[
V_{\text{CH}_4,n} (\text{mL}) = ((V_{\text{biogas},n} + V_{\text{headspace}}) \times \frac{\%_{\text{CH}_4,n}}{100}) - (V_{\text{headspace}} \times \frac{\%_{\text{CH}_4,n-1}}{100})
\]

\[\times \left(\frac{T_{\text{STP}}}{T_{\text{gas}}}\right) \times \left(1 - \frac{P_{\text{vap}}}{P_{\text{gas}}}\right)\]

Equation 4-21: Volume of Methane Per Sampling Interval

\[P_{\text{vap}} = 10^{8.1962 - (\frac{1730.63}{T_{\text{gas}} - 273.15})}\]

Equation 4-22: Pressure Correction

Where \(V_{\text{CH}_4,n}\) is the methane generation volume (mL) of the mixed liquor; \(V_{\text{biogas},n}\) is the biogas generation volume (mL) of mixed liquor; \(V_{h}\) is the headspace volume (mL) of each BMP bottle; \(\%_{\text{CH}_4,n}\) is the current methane percentage of the generated biogas determined by GC; \(\%_{\text{CH}_4,n-1}\) is the methane percentage of generated biogas in last sampling time point; \(T_{\text{STP}}\) is the standard temperature (273.15 K); \(T_{\text{gas}}\) is the incubation temperature (K) for the BMP test; \(P_{\text{vap}}\) is the water vapour pressure (kPa); \(P_{\text{gas}}\) is the pressure of the measured gas (101.325 kPa).

The methane yield curves were generated by dividing the normalised methane volumes by the mass of volatile solids from the substrate added into the serum bottles. Next, the data when through a screen process to determine if the test was accuracy. As suggested by Holliger et al., (2016), the positive control (cellulose) had a relative standard deviation below 5% and produced a methane yield that was within 85 to 100% of its theoretical value. Now
that the test had been checked and passed data rejection stage, the final data was organized for presentation.

Table 4-17: Data analysis stages and steps for BMP

<table>
<thead>
<tr>
<th>Stage</th>
<th>Step</th>
<th>Process</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data analysis</td>
<td>1</td>
<td>Calculate volume of methane of gas produced</td>
<td>Calculate the volume of methane of gas produced in the interval and then calculate the cumulative net volume of the methane produced over the test period</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calculate the methane yield and biodegradability of test material</td>
<td>Dividing the STP methane volume by the mass of the substrate’s solids added into the serum bottle.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Calculate kinetic rate constant</td>
<td>MATLAB was used to find the value of k by minimizing the sum of squared differences between the experimental and calculated values</td>
</tr>
<tr>
<td>Data Rejection</td>
<td>4</td>
<td>Check data for data rejection</td>
<td>If the Relative Standard Deviation (RSD) of the blank or the positive control is &gt;5%, even after applying a statistical test to eliminate a single outlier</td>
</tr>
</tbody>
</table>

4.3.6. Data Report

Data reporting followed the recommendations of Angelidaki et al., (2009). The authors stated that the goal for data reporting is to present a clear description of the inoculum source, substrate, test conditions, and graphics of the specific methane production for the blank, control and substrate. Table 18 and Figure 27 provides an example used for the characterization of primary and secondary sludge using a substrate to inoculum ratio of 1.0. In addition, the inclusion of the kinetic constant can be included as it may provide insight into the time required to generate the ultimate methane potential Angelidaki et al., (2009).
Table 4-18: Example final data information for primary and secondary WWTP sludge

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Parameter</th>
<th>Inoculum (Blank)</th>
<th>Control (Cellulose)</th>
<th>Primary Sludge</th>
<th>Secondary Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCOD (mg/L)</td>
<td>19000±240</td>
<td>17400±165</td>
<td>47055±2991</td>
<td>10670±254</td>
</tr>
<tr>
<td></td>
<td>SCOD (mg/L)</td>
<td>647±20</td>
<td>—</td>
<td>1945±83</td>
<td>58±6</td>
</tr>
<tr>
<td>Physical and</td>
<td>TS (g/L)</td>
<td>19.74±0.25</td>
<td>—</td>
<td>36.02±0.22</td>
<td>10.36±0.25</td>
</tr>
<tr>
<td>Chemical</td>
<td>VS (g/L)</td>
<td>10.92±0.12</td>
<td>14.00±0.01</td>
<td>26.09±0.24</td>
<td>6.63±0.12</td>
</tr>
<tr>
<td>Characteristics</td>
<td>TSS (g/L)</td>
<td>18.40±0.48</td>
<td>—</td>
<td>33.57±0.24</td>
<td>9.32±0.48</td>
</tr>
<tr>
<td></td>
<td>VSS (g/L)</td>
<td>12.17±0.38</td>
<td>—</td>
<td>25.03±0.17</td>
<td>6.55±0.38</td>
</tr>
<tr>
<td></td>
<td>ALK (mg CaCO3/L)</td>
<td>4772±160</td>
<td>—</td>
<td>2333±175</td>
<td>83.6±0</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>7.6±0.1</td>
<td>7.4±0.1</td>
<td>6.5±0.1</td>
<td>7.1±0.1</td>
</tr>
<tr>
<td>Biochemical</td>
<td>Methane Yield</td>
<td>83</td>
<td>375</td>
<td>495</td>
<td>218</td>
</tr>
<tr>
<td>Methane Potential</td>
<td>Biodegradability</td>
<td>10%</td>
<td>85%</td>
<td>65%</td>
<td>38%</td>
</tr>
<tr>
<td>Data</td>
<td>Kinetic First order</td>
<td>0.0855</td>
<td>0.2296</td>
<td>0.3568 (0.981)</td>
<td>0.1406 (0.98)</td>
</tr>
<tr>
<td>Temp: 35°C</td>
<td>constant (R²)</td>
<td>(0.994)</td>
<td>(0.998)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixing: 100 rpm</td>
<td>Duration: 30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIR: 1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4-26: Example final report methane yield graph of primary and secondary sludge from the GWWTP.

4.4. Future Aspects

To provide perspective, recent research areas being conducted to progress the conventional BMP test were reviewed. So far, these areas include (1) investigations into faster methods to predict the BMP of a substrate, (2) the move towards automated BMP products for standardization, (3) creation of standardized inoculum, and (4) the use of online methane yield data base for data harmonization. The following section will provide a brief description and discussion for each topic.
4.4.1. Early prediction of BMP using kinetic modelling

Long test durations are a major drawback of the BMP test. As stated by Da Silva et al., (2018) this factor limits the applicability for waste utilities, consulting companies and plant operations how decision-making cannot wait 30 to 100 days. Scientists are researching methods to achieve faster BMP values, such as theoretical determinations and near-infrared spectroscopy. However these methods are limited in providing information about the toxicology and loading rate of the substrate due to the absence of a biological anaerobic degradation process (Strömberg et al., 2015). In addition, these approaches are time independent measurements and offer no information on kinetic degradation. Therefore, early prediction models based on the methane curves. So far Strömberg et al., (2015) and Da Silva et al., (2018) are the most comprehensive studies to provide laboratory test evidence that shorter test durations could be made using BMP tests.

Strömberg et al., (2015), built off the study by Ponsá et al., (2011) who showed it was possible to statistically predict the final BMP of a sample with a large enough database. The authors compiled a data base of 138 BMP tests of various substrate types, and 61 different algorithms, to predict the final BMP and required degradation time. The results from the factorial design experiments showed that the Monod-type, quadratic Monod-type and the first-order model with variable time dependency were able to predict the ultimate methane yield. The statistical prediction estimated the final BMP values and test times by cross-referencing the registered BMP profiles. In a comparison between experimental and predicted results, BMP values after 1, 5, 10, and 15 days made clear that the model predictions improve as the experiment progresses. In addition, by combining the best algorithms, the BMP was predicted with a relative root mean squared error of less than 10% after 6 days of experiment duration. The authors noted that this experiment used only one type of inoculum at mesophilic temperatures, and extensive testing remains at a larger scale (ie >138 samples).

Da Silva et al., (2018) focused on the first order model to describe the methane yield and kinetic constant rate for easy adaptability. In the study, a threshold time (minimum testing time) was determined using an estimation of the rate constant (using Matlab ‘fitnlm’
function). Where early parameter estimation is correlated to the k value, slowly biodegradable substrates (k<0.1 1/day) should have a minimum testing time greater than 15 days, and rapidly biodegradable substrates (k≥0.2 1/day) have testing times lower than 7 days. In the experiment, three regression results were compared: traditional regression (based on all experimental points from day 0 to day 30 above), threshold regression (all experimental points up to minimum testing time) and balanced threshold regression (using three data points of the initial, threshold and average time between them). The balanced threshold regression improved the quality of parameter estimation, in comparison to threshold regression due to the reduced effect of the initial experimental data. Using the mesophilic BMP test described by Angelidaki et al., (2009), common anaerobic digestion substrates such as sewage sludge, primary sludge, pig manure, paunch, blood, and sewage sludge and glycerol mixture, were tested. Although comparing kinetic constant rates to those of other studies is highly variable, the values obtained were within the literature ranges. In comparison to Strömberg et al., (2015), Da Silva et al., (2018) commented that the minimum testing times for Strömberg et al., (2015) were lower. Where Strömberg et al., (2015) was able to use a minimum testing time of 4 days for sewage sludge and the authors using 8 and 10 days respectively. The authors stated that threshold time could be reduce if the R² criterion was increased from 0.8 to 0.9, however this would increase the occurrence of inaccurate predictions.

Continued research to optimize early prediction methods are recommended. This has significant potential to increase the practicality of BMP tests for waste and water utilities, consulting companies and AD plant operators. In addition, the development may become accurate, with the increased use of automatic methane potential tests as they standardized results.

4.4.2. Automated BMP

The automated methane potential test system II, developed by Bioprocess Control, that has been gaining popularity in anaerobic biodegradability studies for its ability to provide automatic and real-time measurements, recording and reporting of biogas production (Badshah et al., 2012; Bellaton et al., 2016; Control, 2016; Himanshu et al., 2017; Holliger et
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al., 2017; Koch et al., 2017; Li et al., 2017; Nazari et al., 2017; Strömberg et al., 2015, 2014; Wang, 2016; Wang et al., 2016, 2015). This tool allows up to 15 test vials (500 mL) in a 35°C for kinetic information, specific methogenic activity assays and residual gas potential analyses. Using volumetric gas measurement technique, produced biogas is led through flexible tubing to a scrubbing reactor (Himanshu et al., 2017). The acid gases are trapped by an alkali solution and the remaining biogas is passed into the measuring cell containing a gas counter based on the liquid displacement. As the gas bubbles generate impulsions, the volume is recorded and translated into NmL CH₄/g VS by AMPTS v5 software (Bellaton et al., 2016).

In comparison to conventional gas measuring systems such as manometer, water column or gas bag, the automatic system is time and labour saving. Wang et al., (2014) compared the workload of each BMP test by different experiment setup. By separating a BMP test into three work stages (1) time for inoculum and substrate addition, (2) time for experimental follow up gas volume and composition analysis during incubation, (3) data management and interpretation. The total workload (min/sample) was 540, 220, 220, and 40 for the manometer, water column, gas bag and AMPTS II. In comparison to manual manometric method is simpler to operate. It is predicted that in the future standardization of the experimental setup, it is inevitable that the BMP method will be automated system due to minimized human error and workload demand, analytical precision, and a standardization of data interpretation (Strömberg et al., 2014; Wang et al., 2014).

4.4.3. Standardized Inoculum

The development of a method to produce standardized inoculum for a range of substrate in anaerobic digestion batch tests are estimated to increase reproductivity (Heerenklage et al., 2017). As of 2017, the Hamburg University of Technology (TUHH) are researching long term preservation methods for anaerobic inocula. Testing results showed that inocula resuspended after preservation high recovery of expected methane production. But, the authors reported a 7-10 day lag phase of methane production possibly due to the damage of microorganism by the preservation method. Further investigations on the optimize preservation process are required (Heerenklage et al., 2017).
4.4.4. Online Methane Yield Database and meta-analysis

Online infrastructure is the next step for published BMP results data harmonization, integration and analysis of overlapping data. Murovec et al., (2015) developed an online community supported methane yield database. This hierarchically organized, curated and community supported collection of reported methane yields represented the largest publicly available collection with 1164 methane yield entries (15,749 data points) by 71 parameters and 42 substrate categories. During analysis of submitted methane data, the authors reported significant issues due of no variation of due to the variety of data reporting in published literature, highlighting the importance of standardized methods and reporting for results comparability. Out of all the data information entries, only 5.6% contained all required data and 80% of methane yield entries had uninformative metadata. 17.2% of entries had to be removed because entry data were ambiguous data. In order to improve the methane yield database to be valuable, the authors had three recommendations: (1) there needs to be adjustments made by both scientists and the industrial community in the reporting, (2) the methane yield data sever needs to expand the available data categories and parameters, and (3) there needs to be and advancement of data analysis tools for developing the ability to exchange data in both directions (Murovec et al., 2015).

4.5. Conclusion

The objective of this paper was to outline an accessible and simplified serum bottle method. Unlike previous methods proposed, this is a very simple laboratory technique, and allows a large number of replicates at different conditions that can be carried out simultaneously to provide comprehensive reproducible results. As of 2018, there continues to be a lack of standardized/ universal BMP testing procedure resulting in a lack of comparable BMP values due to the differences in equipment, experimental conditions and procedures. However, the BMP methods continue to evolve and further the elimination of systematic errors. In this study, BMP protocols guidelines and recommendations were reviewed and summarized, which rendered the following conclusions:

1. The substrate to inoculum ratio has been found to be an important design parameter for achieving accurate BMP serum bottle results. In addition, the accuracy of BMP tests could also be significantly affected by selection of blank and control bottles,
head spacing flushing, mixing, pH control, and methane production monitoring and correction methods.

2. Kinetic models could be used to predict the methane production based on BMP tests with a reduced testing period but the selection of the model and kinetics could varied widely with different testing conditions and need to be carefully verified.

3. Future aspects of BMP test include further research into time saving techniques, the use of online database for accessibility, standardized inoculum, and increased use of automated BMP systems for time saving, standardized results and reductions in human error.
CHAPTER 5: METHANE YIELD TRENDS OF THERMAL TREATED ANAEROBIC DIGESTATE FROM A MUNICIPAL WASTEWATER TREATMENT PLANT FOR ENHANCING METHANE PRODUCTIVITY

Abstract
The methane yield of thermally treated anaerobic digestate from a municipal wastewater treatment plant was investigated for opportunities to enhance methane productivity. In this study, biochemical methane potential (BMP) tests were used to assess the methane yield of untreated and treated digestate. Four main conclusions were drawn from this study: (1) thermal treatment was confirmed to be more efficiently applied to anaerobic digestate in comparison to primary sludge or thickened waste-activated sludge for methane yield enhancement, (2) a substrate’s digestion time before thermal treatment will impact the methane yield of the process, (3) the optimization of thermal treatment conditions requires more information than treatment temperature and time, and (4) digestion of thermally treated digestate centrate could produce significant quantities of methane and is an area recommended for further investigation.

Keywords: anaerobic digestion; intermediate thermal hydrolysis; biochemical methane potential; methane productivity
5.1. Introduction

Anaerobic digestion (AD) is one of the oldest processes for sludge stabilization and remains a major treatment process for municipalities due to its emphasis on energy conservation and recovery of wastewater biosolids (Tchobanoglous et al., 1991). Although AD is a slow process often limited by long retention times (20-30 days) and an overall degradation efficiency of wastewater at 20-50% (Parkin and Owen, 1986). In many situations digesters operating at a suboptimal performance are influenced by the rate and extent of hydrolysis of the specific substrate. As stated by Carlsson et al., (2012), some biodegradable compounds may be less bioavailable than others due to the differences in complex hardly biodegradable structures and compounds of large particles with limited surface areas.

In wastewater treatment plants, AD is mainly fed either primary, waste-activated or combined sludge. Primary sludge (PS), from the primary clarifier is often comprised of natural fibers, fats and other solids and has a high biodegradability around 69% (Parkin and Owen, 1986). Waste-activated sludge (WAS) contains nonbiodegradable debris from dead bacteria and refractory organics. As a result, WAS is inherently less biodegradable than primary sludge, where long retention times will not reduce COD or cause the VS reduction to exceed 50% or raise WAS biodegradability to the level of primary sludge. Only through pretreatment of WAS can it be made as degradable as primary sludge (Parkin and Owen, 1986).

The goal of pre-treatment is to disrupt the EPS matrix and cell wall to make the organic matter more accessible to microbes to speed up the conversion of organic solids. As a result there is higher biogas production, reduced retention times, and a reduction in the quantity of biosolids. In the past three decades AD pre-treatment in the form of chemical, biological, physical, electrical and thermal have been gaining attention in the scientific community (Zhen et al., 2017). Out of these options, thermal hydrolysis (TH) has been the most frequently applied pre-treatment for secondary and combined sludge for enhanced AD performance.

Applying thermal treatment to anaerobic digestate is a configuration currently under investigation due to the evolution of the THP technology. The goal of this configuration is to be more energy efficient than pre-treatment. Instead of treating both digestible and non-
digestible material ahead of digestion, energy should be focused on the material resilient to digestion (Takashima, 2008). But currently, literature on intermediate thermal hydrolysis remains limited, with only a few acknowledgements in contemporary review papers (Monlau et al., 2015; Raheem et al., 2018; Shen et al., 2015). Thus, there is a need for studies investigating the behaviour of the system to illuminate optimal treatment conditions for different sludge properties to further the enhancement of energy recovery.

In this present study a series of batch biochemical methane potential tests were conducted to investigate four major aspects of the AD$_1$+TH+AD$_2$ configuration. These studies include: (1) a comparison of TH on different digester feeds (primary sludge, secondary sludge, and anaerobic digestate, (2) how the first anaerobic digester retention time influences the post digestion methane yield, (3) how TH temperature and holding time influences digestate methane yield, and (4) how the TH influences the behaviour of liquid and solid fractions of anaerobic digestate. It is the aim of the authors that the data from this study will provide the readers insight into potential alternative system conditions for further investigation.

5.2. Materials and methods

5.2.1. Sludge collection
Municipal sludge samples were taken from the Guelph Wastewater Treatment Plant (GWWTP) (Ontario, Canada). The wastewater treatment process at the Guelph WWTP consists of preliminary screening and grit removal, primary sedimentation, extended aeration activated sludge treatment, secondary clarification, rotating biological contactors, and sand filtration tertiary treatment. According to the annual average wastewater characteristics from 2011 to 2015, the average daily wastewater flow treated by the Guelph WWTP was 50.02 ± 15.6 million liter per day.

Anaerobic digesters at the GWWTP were operated at high-rate solid retention times of 10 to 20 days, and a minimum VS loading of 1.3 kg VS m$^3$/day and maximum VS loading of 3.2 kg VS/ m$^3$/ day. The AD sludge over the course of the experiment showed a stable TS content of 19.6 ±0.3 g/L over the sampling period, which was very close to the annual
average TS 19.5 g/L over the period of 2011 to 2015. The VS/TS and TSS/VSS ratio of the 
AD sludge were determined to be 0.63±0.14 and 0.70 ± 0.12, respectively. The relative stable 
TS and VS/TS ratio with the AD sludge suggests that the AD digesters of the WWTP can 
provide biological consistent inoculum for the sludge BMP tests.

Primary and secondary sludge were taken respectively from the wasting line of the primary 
and secondary clarifiers. The sludge samples were transported to the lab about half an hour 
after sampling. The secondary and primary sludge samples were filtered through a standard 
sieve with the average opening size of 4.76 mm. The physical and chemical characteristic 
details of each sample are reported for each study in the following sections.

5.2.2. Bench-Scale Sludge Hydrolysis Pre-treatment
Thermal hydrolysis of TWAS and anaerobically digested sludge was conducted using a 600 
mL 5500 HP Compact Reactor (Parr Instrument Company: Moline, Illinois, USA). The 
thermal reactor consisted of a high pressure compact reactor, a heating coil, mixer, 
thermocouple, water cooling line and a control unit. After selecting the desired temperature 
and time program on the controller, the temperature would rise to the selected value and the 
water cooling would be activated for regulated cooling. Once the treatment was completed, 
the reactor was placed into a bucket of cold water to cool down to room temperature and the 
treated sludge was collected for further analysis and digestion.

5.2.3. Sludge Analysis
Total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended 
solids (VSS) were determined by standard methods (Method 2540-1997 and EPA Method 
160.4). Chemical oxygen demand (COD) and total volatile fatty acids (VFA) were 
determined using Hach test vials (Hach, Canada). The pH and alkalinity of sludge samples 
was determined using the filtrate of sludge samples by Titra Lab 870 titration workstation 
(Hach, Canada). The filtered samples were prepared by centrifuging the sludge at 10,000 rpm 
and 4°C for 15 min and then filtering the collected supernatant through a 0.45 um syringe 
filter. The biogas analysis was performed using an Agilent 6890 gas chromatography (GC) 
system (Agilent Technologies, USA) with a thermal conductivity detector (TCD). Argon was 
used as the carrier at an inlet temperature of 200°C. The oven temperature was held at 35°C 
for 7.5 min, and then increased from 35 to 206 °C at a rate of 24°C/min, and held at 206°C
for 1 min. The peaks were identified and quantified by comparing with biogas mix standards including methane, carbon dioxide, nitrogen, oxygen, and hydrogen at various concentrations.

5.2.4. Biochemical Methane Potential (BMP) assay Method

The effect of TH on the biogas production of anaerobic digestate was assessed using batch BMP tests. The BMP serum bottle method described as the following was adapted from a combination of recommendations by Remigi and Buckley (2006), Hansen et al. (2004), Owen et al. (1979), Angelidaki et al. (2009), Raposo et al. (2011), Holliger et al. (2016), Strömberg et al. (2014), Möller et al. (2004), and Filer (2019). In this study, anaerobic methane production was assessed at 35°C using 160 ml capacity serum bottles (American Scientific Products, McGraw Park III). The total volume of substrate and inoculum mixture in each BMP bottle was adjusted to achieve a desired substrate and inoculum ratio (SIR) and total bottle volume to headspace ratio. The SIR was defined as the ratio of gram of substrate COD added to the gram of inoculum VS added. For each condition, triplicate testing and blank bottles were used. The blank BMP bottles only contained DI water and inoculum.

Once the substrate and inoculum mixture were filled into the BMP bottles, the BMP bottle was immediately flushed using nitrogen gas for 15 seconds at a low flow rate. The bottles were caped with rubber stoppers, sealed with an aluminum crimp, and placed upside down in the incubator (New Brunswick Scientific C25 Incubator Shaker) with constant shaking at 100 rpm at 35°C. In the first 1 to 2 hours after the BMP tests were initialized, each bottle had their biogas production measured using 10 mL or 50 mL glass syringes equipped with a luer valve (Popper and Sons Inc.) to re-equilibrate the headspace, relieving the expansion of gas caused by the increased temperature. During the BMP test period, at least two of the triplicate testing bottles were sampled for biogas measurement. In general, around 10 mL of biogas is needed for an accurate GC measurement and the sample was injected into the GC using a 0.45 μm filter to avoid contamination of the columns. The GC analysis determined the concentrations of oxygen, nitrogen, methane and carbon dioxide of the biogas.

To standardize the BMP results, the as-measured methane volumes was converted to standard conditions (0°C at 1 atm) using the following equation (Richards et al., 1991):
\[ V_{CH_4,n}(\text{mL}) = \left[ (V_{\text{biogas,n}} + V_h) \times \frac{\% CH_4,n}{100} - (V_h \times \frac{\% CH_4,n-1}{100}) \right] \times \frac{T_{\text{STP}}}{T_{\text{test}}} \times \left(1 - \frac{P_{\text{vap}}}{P_{\text{gas}}} \right) \]

Equation 5-23: Volume of Methane Determined for BMP test

where \( V_{CH_4,n} \) is the methane generation volume (mL) of the mixed liquor; \( V_{\text{biogas,n}} \) is the biogas generation volume (mL) of mixed liquor; \( V_h \) is the headspace volume (mL) of each BMP bottle; \( \% CH_4,n \) is the current methane percentage of the generated biogas determined by GC; \( \% CH_4,n-1 \) is the methane percentage of generated biogas in last sampling time point; \( T_{\text{STP}} \) is the standard temperature (273.15 K); \( T_{\text{gas}} \) is the incubation temperature (K) for the BMP test; \( P_{\text{vap}} \) is the water vapor pressure (5.626 kPa) at the TMP temperature (35 °C); \( P_{\text{gas}} \) is the pressure of the measured gas (101.325 kPa).

The methane generated from the substrate was calculated by subtracting the methane volume from the blank. With the substrate methane isolated, the methane yield was calculated by dividing the STP methane volume by the mass of the VS solids or the TCOD of the substrate added into the serum bottle (reported as NmLCH\(_4\)/g VS or NmLCH\(_4\)/gCOD). The biodegradability was calculated by dividing the measured methane yield (NmLCH\(_4\)/gCOD) by the theoretical methane yield at STP (0.35 NmLCH\(_4\)/gCOD).

5.2.5. Experiments

5.2.5.1. Study 1: TH treatment of primary sludge, thickened waste activated sludge and anaerobic digestate.

In Study I, the effect of thermal hydrolysis at the industrial norm (165°C, 30 min) on AD was compared with primary sludge (PS) and TWAS. PS and TWAS have been compared in previous investigations, but it would useful to use them as references to observe the influence of TH on digestate. Each substrate methane yield was carried out using their own BMP set and blank. The substrate to inoculum ratio used was 1g VS/ 1 g VS. The properties of inoculums are given next: Primary sludge: TS= 28.14 g/L, VS= 20.54 g/L, TCOD= 36.84 g/L, SCOD= 1374 mg/L, VFA= 744 mg/L. TWAS: TS= 22.78 g/L, VS= 16.95 g/L, TCOD=...
24.85 g/L, SCOD= 550 mg/L, VFA=52 mg/L. Anaerobic Digestate: TS= 24.38 g/L, VS= 13.26 g/L, TCOD= 22.16 g/L, SCOD= 330 mg/L, VFA=52 mg/L.

5.2.5.2. Study 2: TH treatment of sludge digested at different retention times
In Study 2, the AD1+TH+AD2 process included digesting the wasted activated sludge from the Guelph WWTP at 35 °C for 5 and 8.5 days (AD1), treating the AD1 digestate by TH at 165 °C for 30 minutes (TH), and digesting the digestate treated by TH again at 35 °C (AD2). The total digestion time of AD1 and AD2 was 30 days. Both AD1 and AD2 digestion were carried out using BMP testing method described in section 2.1.2 with a SIR of 1 g COD/g VS used in the tests. A total of 15 BMP bottles that contained 70 mL of Guelph WWTP secondary sludge (substrate) and 25 mL of anaerobic digester sludge (inoculum) were placed in the incubator with constant shaking at 100 rpm at 35°C. Five BMP bottles were removed from the incubator on day 5 and day 8.5, respectively, for sludge property characterization and TH treatment, while those remained in the incubator were used as the controls. The sludge sampled on day 5 and day 8.5 were treated at 165 °C for 30 minutes using the TH reactor described in section 2.2. The TH treated sludge (60 mL) were cooled down to 35 °C, analyzed and mixed with fresh inoculum (35 mL) and, then, placed in the incubator to resume the BMP test for the AD2 treatment. The properties of inoculum used for AD1 were TS = 24.3, VS = 3.4, TCOD = 22.16g /L, SCOD = 289 mg/L, VFA = 42 mg/L; for AD2 treating 5-day TH digestate were TS = 23.2 g/L, VS = 12.8g/L, TCOD = 21.1 g/L, SCOD = 192 mg/L, VFA = 11 mg/L; for AD2 treating 8.5- day TH digestate were TS = 23.6 g/L, VS = 14.2g/L, TCOD = 22.6 g/L, SCOD = 192 mg/L, VFA = 34 mg/L.

5.2.5.3. Study 3: Thermal treatment at increasing temperatures
In Study 3, the effect of thermal hydrolysis temperature and time (140°C - 200°C for 30 and 60 minutes) was investigated to observe the impact on digestate methane yield. In these experiments, thermal treatment was performed in a 600 mL stirred batch reactor (Parr 600 mL 5500 Compact Mini Bench Top Reactor and 4848 Reactor Controller). The same BMP test procedures described in 1.1 was used but with slightly different volumes of inoculum and substrate. For experiments of this stage, 70 mL of inoculum were added into 24 (eight sets of triplicate bottles) 160 mL serum bottles. 20 mL of the substrates were added to each set, with
the blank having only 70 mL of inoculum and 20 mL of distilled water, and the control with 90 mL of inoculum (digested sludge). The ratio between the sludge and inoculum was the same for all test vials (0.5 g COD substrate/ g VS inoculum). The properties of inoculums are given next: digestate: TS = 23.2 g/L, VS = 13.6g/L, TCOD = 19.36g/L, SCOD = 52 mg/L, VFA = 52 mg/L.

5.2.5.4. Study 4: Methane Enhancement of AD unseparated, centrate and solid cake
In Study 4, we separated the untreated and thermally hydrolyzed digestate (165°C, 30 min) into centrate and solid fractions and examined the methane yields of the centrate and solid fraction of the digestate before and after the TH treatment. The digestate samples were separated into centrate and solid fractions using a centrifuge at 10,000 rpm for 10 minutes. BMP tests were designed to assess the methane production by the digestate treated by TH, the TH centrate and solid cake separated from the digestate treated by TH, the digestate without TH treatment, the AD centrate and solid cake separated from the digestate without TH treatment, cellulose solution (control), and a blank sample. For the liquid substrate (digestate and centrate), 75 mL of inoculum and 25 mL of substrates were added to 60 mL serum bottles, while for the BMP with the solid cake substrate, 5 g of cake were added to 75 mL of inoculum and mixed. The blank had only 75 mL of inoculum and 25 mL of distilled water, and the control with 75 mL inoculum and 25 mL of cellulose solution. All the samples had triplicate bottles used in the BMP tests. The main characteristics of the substrates are given next: digestate: TS = 22.45 g/L, VS = 12.58 g/L, TCOD = 22.38 g/L, VFA = 57 mg/L. AD centrate: TS = 2.1 g/L, VS = 0.4 g/L, TCOD = 600 mg/L VFA=50mg/L, AD solids: TS = 85.4 g/L, VS = 54.6 g/L, TH centrate: TS = 4.8 g/L, VS = 3.5g/L, TCOD = 5220 mg/L, TH solids: TS = 133.7 g/L, VS = 71.4 g/L. ach set.

5.3. Results
5.3.1. Study 1: TH treatment of primary sludge, thickened waste activated sludge and anaerobic digestate.
Figure 28a shows the percent changes of TS, VS, TSS, VSS and TCOD for PS, TWAS and anaerobic digestate after thermal treatment. The change in TS, VS and TCOD caused by TH treatment were negligible as no gas was released during treatment. TH instead impacted TSS
and VSS content of the sludge. The results showed that the reduction of suspended solids to dissolved solids for all sludge samples behaved similarly corresponding to TSS reduction of 21, 16, 19%, respectively.

Figure 28b shows the effects of TH on sCOD and VFA contents. Generally the concentration of soluble COD provides insight into the hydrolytic efficiency of the treatment process (Wilson and Novak, 2009). Anaerobic digestate had the highest percent increase in sCOD and VFA raising the sCOD from 297 to 7035 mg/L and the VFA from 42 to 589 mg/L. In comparison, PS sCOD and VFA concentrations were raised from 1374 to 9670 mg/L and 744 to 1145 mg/L and TWAS sCOD and VFA concentrations from 550 to 9750 mg/L and 1190 to 1540 mg/L. These results corroborate with previous investigations of PS and TWAS. As reported by Wilson and Novak, (2009), the breakdown of proteins, lipids and polysaccharides for both WAS and PS responded similarly to THP.

Figure 5-27: (a) percent reduction in solid content, (b) percent increase in concentrations of SCOD, VFA and ammonia after thermal treatment of PS, TWAS, and AD.
The effects of TH treatment on methane production were also assessed using biochemical methane potential. Figure 29 shows the VS based methane yield over the operational period of 30 days. In all cases there were no signs of inhibition or a lag period. The 30 day methane yield at STP for untreated PS, TWAS and AD were 377, 313, 87 NmL CH₄/g VS, and for treated TH-PS, TH-TWAS, and TH-AD were 390, 405, and 236 NmL CH₄/g VS, respectively. As shown TH-AD had the biggest impact on raising the methane yield by 2.71 times, followed by TWAS 1.29 and TH-PS by 1.03. These results are similar to the insight provided by Pinnekamp, (1989), who found that the lower the volatile solids concentration the higher the percentage increase in gas yield attainable by thermal treatment, with the effectiveness of THP inversely related to the initial biodegradability of the raw sludge.

Based on sludge samples collected from the GWWTP the results support the assessment for applying thermal treatment to anaerobic digestate instead of PS and TWAS. As reported by Pérez-Elvira and Fdz-Polanco, (2012) several studies have been published recommending the thermal treatment of TWAS over PS, as the effectiveness of appears to TH rely on the substrates composition related to bioavailability and biodegradability (Carlsson et al., 2012). In the TWAS and PS discussion, the structure and organization of an organic compound within a bacterial cell has been found to have a profound effect on its biodegradability (R. Cano et al., 2014; Carlsson et al., 2012). Researchers have found that decreased biodegradability appears to be due to a lack of accessible actives sites in which hydrolytic exocellular enzymes can attach and leave complex macromolecules to simpler and more biodegradable constituents. In the case of PS, TH has not shown remarkable effects in substrates rich in lipids or with high content of easily degradable carbohydrates (Pérez-Elvira and Fdz-Polanco, 2012). It is for this the energy to treat PS is too high compared with the subsequent little increase in biogas. For this reason TH of secondary sludge is often recommended. As stated by R. Cano et al., (2014) substrates with high content of fiber or microbial cells are more susceptible to treatment in order to improve its degradation capacity. Perhaps this theory can be extended to the treatment of AD over TWAS.
5.3.2. Study 2: TH treatment of sludge digested with different retention times

When anaerobic digestion is coupled with an intermediate thermal hydrolysis process (iTHP), the first anaerobic digester retention time is a major factor for rendering cost-effective conversion of complex organic material to methane and reducing volatile solids (Parkin and Owen, 1986). However, within the limited research of iTHP, few discussions on the selection of AD retention times have been made, as most studies focused on either optimizing iTHP conditions or proving the concept’s validity on a laboratory scale. In this work we tested the retention times of 5 and 8.5 days for the first stage treatment. This selection of such a short digestion time under the recommended minimum 10 day SRT would allow the first stage AD to focus on the destruction of readily biodegradable organics but leave the non-readily biodegradable facet to be treated by intermediate TH for a secondary anaerobic digestion stage.

Figure 30 shows the methane yield profiles of the control (30 day digestate without thermal treatment) and the sludge thermal treated after 5 and 8.5 day digestion. A sharp increase in
the methane yields were observed at after 5 and 8.5 digestion were thermal treated, with methane production reaching a stable condition within 20 days. The calculation of the methane yields as the end of the 30 day digestion showed that the intermediate TH treatment nearly doubled the methane production from 165 ± 3 NmLCH₄/g VS to 308 ± 7 NmLCH₄/g VS and 294 ± 12 NmLCH₄/g VS, respectively, for the sludge treated by TH at day 5 and day 8.5.

It was noticed that the methane production from sludge treated by the initial digestion varied to some extent with digestion time prior to TH treatment. In this study, as the retention time of AD1 increased from 5 to 8.5 days, the methane yield of the digestate treated by TH decreased by around 4.5% because the sludge with a longer AD1 retention time produced more methane prior to the TH treatment. Campo et al., (2017) showed a similar trend in the methane production from digestate that was treated at lower thermal temperatures (70 °C, NaOH 4%, and 90 °C, NaOH 4%), followed anaerobic digestion at the hydraulic retention times of 7 and 15 days. Perhaps by holding the digestate longer for more organic material to be converted to methane in the first stage digestion, TH will have less of an impact in raising the total methane yield. Further research is required to assess the impact of the first digester solid retention time on a two stage anaerobic digestion system total methane productivity at a continuously fed at pilot and full-scale configuration.

Figure 5-29: Methane yield in BMP assay for non-TH treated sludge and sludge treated by TH at AD of day 5 and day 8.5.
5.3.3. Study 3: Thermal treatment of digestate at different temperatures

The optimum TH conditions, in the past have been selected for WAS by increasing the temperature up to a point where there is a decrease in sludge anaerobic digestibility and a significant increase in the production of refractory material (Barber, 2016). As stated by Pilli et al. (Pilli et al., 2015), the most common temperature and time conditions for WAS to obtain maximum biodegradability and biogas production occurs at 165-170 °C for 30 minutes. However limited studies have determined the optimum condition for anaerobic digestate.

Figure 31 a shows the cumulative methane yield for the anaerobic digestate after thermal treatment at 140 °C, 160 °C, 170 °C, 180 °C, 190 °C and 200 °C. All conditions had a positive effect on methane production and improved the methane yield from 93±5 NmLCH₄/g VS for raw digestate to 263±12, 295±13, 309±14, 319±15, 328±14, 348±19 for sludge treated by 140 °C, 160 °C, 170 °C, 180 °C, 190 °C and 200°C for 30 minutes. The extent of degradation increased by the release or exposure of organic material that was originally inaccessible to microorganism or the transformation of material that was originally non-biodegradable (Barber, 2016; R Cano et al., 2014; Mills, 2015). In addition, the rate of degradation increased, as seen through the first order reaction rate constant due to the increase solubilisation and particle size reduction or organic matter that would have been slowly hydrolyses (Haug et al., 1978; Hii et al., 2013).

The results did not follow the common trend where biogas production decreases at temperatures greater than 190 °C. As reported by Pilli et al., (2015) at temperatures greater than 190 °C biogas production is lowered due to the formation of toxic refractory materials. At temperatures above 190 °C, biodegradation decreases compared to lower temperatures and can even be lower than the control (Mottet et al., 2009; Stuckey and McCarty, 1984). In this study the methane yield of 190 °C and 200 °C continued to increase. Therefore, no optimum temperature point for the TH treatment of digestate was found where solubility increased while the methane production receded.
Treatment time was studied to understand the variable’s significance, as past studies have shown time may or may not be significant depending on the temperature (Pilli et al., 2015). To understand the influence of TH time on digestate methane yield, a comparison between 30 and 60 minutes were made for temperatures between 140 °C and 200 °C (see Figure 4 b). In all cases above 140 °C, the SCOD and methane production increased with time and temperature up to 200°C/60 minutes. As the time increased from 30 to 60 minutes, the methane yield increased to the next 20°C temperature for 30 minutes. For example, the increase in treatment time from 30 to 60 minutes at 160°C raised the methane yield from 233 to 314 meeting the 180°C/30min methane yield for 298 NmL CH₄/g VS. This was also true for 180°C/60 meeting 200°C/30 minutes. Therefore, it is possible that treatment time at temperatures above 140°C can have an equal effect as raising the temperature 20°C.
This continuous rise in methane yield above 175°C and 60 minutes was different from those few who also experimented on digestate. Ortega-Martinez et al., (2016) used thermal treatment steam explosion at a laboratory scale on digestate from a wastewater treatment plant, using temperatures in a range of 110-200°C and a time of 0-50 minutes. Ortega-Martinez et al., (2016) found the optimum setting to be 180 and 200 °C at 30 mins. Bjerg-Nielsen et al., (2018) tested TH temperatures of 120 °C, 150 °C, 170 °C, 190 °C for 30 and 60 minutes. They found a decrease in methane yield at 190°C for 30 minute and concluded 170 °C/30 min to be the optimal condition. But for this experiment there was no optimum treatment conditions were found, as the solubilisation and methane yield of the digestate continued to rise.

To find the optimum operation conditions for TH requires more information than temperature and time. Both variables were found the increase both digestate solubilisation and methane production, and no point of diminishing returns was found. Therefore, the results for the optimization of TH for WAS share the same conclusion made by Sapkaite et al., (2017). The authors stated that an exhaustive control of thermal treatment conditions appears to be unnecessary. What is necessary is knowing how the selection of operation conditions will play on the process economics. For proper optimization of TH, all factors such as total solids, thermal treatment time, temperature, net energy, and sludge dewaterability are required (Pilli et al., 2015).

5.3.4. Study 4: TH treatment influence on methane yield of whole, centrate and cake digestate fractions

In an anaerobic digestion configuration integrated with post thermal hydrolysis, after digestate is hydrolyzed, there are a few options for re-digestion. For example, the thermally hydrolyzed digestate could enter directly into the secondary digester, or it can pass through a dewatering stage where solid and liquid fractions are separated. In the latter situation, there are two commonly proposed situations: (1) the centrate is re-digested into the first digester and the solid content is disposed or (2) the centrate is recycled to the plant influent and the solid content enters the second digester. To optimize the iTHP system for maximum methane productivity, the influence of each of these three stages on the final digestate should be investigated. This requires determining the methane yield profiles for each anaerobic digestate fraction.
Figure 32 shows the methane yields for untreated and treated anaerobic digestate fractions. The methane yields for both unseparated and centrate fractions were significantly increased by TH. The 30 day methane yields were 102, 0, and 206 NmLCH$_4$/g VS for the untreated AD fractions of unseparated, centrate and solids, respectively, and for the treated AD fractions of unseparated, centrate and solids were 374, 652 and 144 NmLCH$_4$/g VS, respectively. The centrate after TH had the highest methane yield and percent increase of the group. The cumulative methane production of the TH-centrate during the 30-day period was similar to the unseparated TH-AD although its VS content was only 3.5 g VS/L in comparison to 12.58 g VS/L for the latter. The solid fraction after TH, in comparison, showed much lower methane yield than that of the TH-centrate. These results support the interest into intermediate and post-treatment with redigestion for enhanced methane production. It is recommended that further research investigate systems for either the recycle of TH unseparated or centrate into the main anaerobic reactor.

Figure 5-31: Methane yield of unseparated, liquid and solid digestate fractions before and after thermal hydrolysis.
To further explore the potential for enhancing the methane production of an anaerobic digestion system by digesting thermal treated liquid and solid digestate fractions, a mass balance was used to predict a second full-scale anaerobic digester’s behaviour (Table 2). As shown by Holliger et al., (2017), the BMP of a substrate and their organic loads could be used to design different components of a full-scale anaerobic digester. For this experiment, the size of the digesters and possibilities for exploiting produced biogas were simulated by following the method used by Pérez-Elvira et al., (2016), who studied the digestion of liquid and solid fractions of thermally pre-treated secondary sludge using BMP data.

Table 19 shows that thermal hydrolysis increased the volatile solids content of the digestate liquid fraction from 2.4% to 28.0%, while the solid fraction decreased from carrying 97.6% to 71.4% of the digestate’s volatile content. Pérez-Elvira et al., (2016) had similar results where the liquid phase fraction of thermally pre-treated secondary sludge increased from 4% for the untreated to 74% for the treated sludge, while the solid content decreased from 96% to 26% for the control solids and TH solids. These results could be visually confirmed by the phase separation of the centrate and pellet after being centrifuged. This transfer of organic material to the liquid fraction could also be observed through the increase and decrease of THAD liquid methane yield and THAD solid content methane yield in comparison to the control.

Table 5-19: Comparison of untreated and treated AD liquid and solid fraction mass balance

<table>
<thead>
<tr>
<th>Solution</th>
<th>Liquid Fraction Volume</th>
<th>g VS</th>
<th>Solid Fraction Percentage</th>
<th>Measured Values</th>
<th>Mass Balance Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Methane Yield (mL CH₄/g VS fed)</td>
<td>First Order Constant (1/d)</td>
</tr>
<tr>
<td>AD</td>
<td>100.0%</td>
<td>12.6</td>
<td>100%</td>
<td>120</td>
<td>0.121</td>
</tr>
<tr>
<td>AD-Liquid</td>
<td>77.5%</td>
<td>0.3</td>
<td>2.4%</td>
<td>20</td>
<td>0.000</td>
</tr>
<tr>
<td>AD-Solid</td>
<td>22.5%</td>
<td>12.3</td>
<td>97.6%</td>
<td>206</td>
<td>0.096</td>
</tr>
<tr>
<td>THAD</td>
<td>100.0%</td>
<td>11.0</td>
<td>100.0%</td>
<td>374</td>
<td>0.269</td>
</tr>
<tr>
<td>THAD-Liquid</td>
<td>88.9%</td>
<td>3.1</td>
<td>28.0%</td>
<td>652</td>
<td>0.343</td>
</tr>
<tr>
<td>THAD-Solid</td>
<td>11.0%</td>
<td>7.8</td>
<td>71.4%</td>
<td>144</td>
<td>0.213</td>
</tr>
</tbody>
</table>

The mass balance assumed 1 kg of volatile solids within the unseparated AD sludge was leaving the first digester. To estimate the methane productivity and digester volumes required for each configuration. The selection of each HRT was based on the time required for each substrate to reach their individual ultimate methane yields. TH-AD unseparated, THAD
centrate and THAD solids all produced at least 90% of their ultimate methane yields within 15 days, and AD unseparated and AD solids required the full 30-day incubation period. Based on the methane productivity the three top configurations were (1) the re-digestion of unseparated THAD (+522% increase), (2) the digestion of THAD liquid and disposal of THAD solids (+360% increase), and (3) the re-digestion of AD solid (+67.5% increase) (see Figure 6). But if the priority were given to the reduction of the secondary digester size, the order changes to the digestion of (1) THAD solids (78.9% reduction), (2) AD- solids (77.7% reduction) and (3) THAD- liquid (49.6% reduction).

However, to select the most efficient configuration requires more information than methane productivity and digester sizing. As reported by Li et al., 2019, there are a large amounts of strategies from lab-scale studies showing high potential for alternative anaerobic digestion schemes to achieve enhanced methane production, but whether they are economically feasible for consuming more energy than that of the increased methane production is often left unaddressed (Li et al., 2019a). Some studies focus on energy production schemes to address the feasibility of the proposed anaerobic digestion scheme, but as stated Pinnekamp, (1989) the question for feasibility should not be focused on energy budget because if one is willing to spend enough money on heat exchange equipment and reactor insulation all thermal energy could be recaptured. The more important question is whether the cost of methane production is decreased through employment of a thermal treatment process. The most economically feasible approach should not only decrease more solid waste but also increase methane yield and the net cost price of extra methane production (Li et al., 2019a).

For example, while the digestion of unseparated THAD may have the highest methane productivity, it may not be the best for reducing the biosolids for further processing. This configuration might have the highest costs for trucking sludge and biosolids and require supplemental bulking agents. In the situation of digesting either untreated or treated solid content, while these two configurations may have the lowest secondary digester volumes, they may require a secondary dewatering process post-secondary digester. This would further complicate the system. Perhaps the simplest option is to dispose of the Class A disinfected biosolids (THAD solids) after dewatering and focus on the resource recovery of the TH liquid. The TH liquid could be sent to an anaerobic membrane bioreactor to take advantage
of the methane generation potential or sent back to the plant influent. In order to conclude a recommended configuration for further investigation a proper economic evaluation should be completed.

5.4. Conclusion
This study provided insights into the methane yield behaviour of thermally treated anaerobic digestate from a municipal wastewater treatment plant. The main conclusions drawn from this study were as follows:

1. Thermal treating anaerobic digestate results in a higher percentage increase in methane yield in comparison to primary sludge and TWAS, supporting recent interest into intermediate thermal hydrolysis anaerobic digestion configuration.

2. In an anaerobic digestion configuration integrated with intermediate thermal hydrolysis, the influence of the longer first digester solid retention time could decrease the methane yield of thermally treated digstate. More experiments are required to test a wider range of solid retention times (15, 20, and 30 days).

3. Thermal hydrolysis temperature and time were found to increase the digestate methane yield from 140°C/30 minutes to 200°C for 60 minutes. Unlike previous thermal studies on waste activated sludge, the AD methane yield did not decrease at temperature above 180 °C. Therefore to find the optimum operation conditions for TH requires more information than temperature and time.

4. TH increased the dewaterability of the AD and transferred organic material from the solid fraction to the liquid fraction. This was observed through an increase in methane yield of AD liquid fraction from 20 to 652 NmL CH₄/ g VS, while the AD solid fraction decreased in methane yield from 206 to 144 NmL CH₄/ g VS.

5. A mass balance using the AD liquid and solid fractions predicted that a secondary digester after thermal hydrolysis should treat either (1) unseparated THAD, (2) THAD liquid, or (3) the re-digestion of AD solids for enhanced methane production. A proper economic evaluation of each alternative proposed configuration is required.
CHAPTER 6: PERFORMANCE OF LAB-SCALE ANAEROBIC DIGESTION WITH INTERMEDIATE THERMAL TREATMENT FOR THICKENED WASTE ACTIVATED SLUDGE STABILIZATION

Abstract: In this study, the degree of stabilization of thickened waste activated sludge (TWAS) was measured during anaerobic digestion using intermediate thermal treatment. Biochemical methane potential (BMP) tests were set up to evaluate the methane production of sludge samples for the laboratory scale digestion system. As the first digester solid retention time increased from 0, 5, 9, 12 and 30 days, the percent increase of methane yield for thermal treated sludge (165°C/30min) increased by 29, 92, 65, 109 and 215%, respectively. An energy balance evaluation of the anaerobic digestion coupled with an intermediate thermal hydrolysis process (ITHP) was completed using a mass balance and the collected laboratory performance data. The total system performance in terms of methane yield and VS reduction were similar for all ITHP systems regardless of the section of the first digester HRT. Feasibility was discussed and futures studies are needed to address whether the cost of extra methane production will decreased through the use of intermediate thermal treatment.

Keywords: Anaerobic Digestion, Methane Productivity, Volatile Solid Destruction. Biochemical Methane Potential, Thermal Treatment, Solid Retention Time, Intermediate Thermal Treatment
6.1. Introduction
Anaerobic digestion (AD) is one of the oldest process for sludge stabilization, and currently plays a key role in the recovery of renewable energy from waste materials (Carlsson, 2015; Metcalf and Eddy, 2014). Pretreatment of AD substrates results in higher biogas production, reduced retention times and a reduction in the quantity of bio solids for disposal (Carlsson, 2015). In the past decades, AD pretreatments in the form of chemical, biological, electrical and thermal have been gaining attention in the scientific community (Carlsson et al., 2012). Out of these options, thermal hydrolysis (TH) has been the most frequently applied pretreatment the secondary and combined sludge of wastewater treatment plants (WWTP) (Barber, 2016).

Applying intermediate thermal hydrolysis between two anaerobic digesters (AD1-TH-AD2) is a new configuration currently under investigation for the evolution of the TH technology (Barber, 2016; Mills, 2015; Shana, 2015). The goal of this configuration is to be more energy efficient than the thermal pre-treatment. Instead of applying energy to both readily digestible and non-digestible materials ahead of anaerobic digestion, energy should be focused on the material resilient to digestion (Takashima, 2008). But so far literature on ITHP remains limited. Thus, there is a need to investigate the behavior of the system to illuminate optimal treatment conditions to further enhance energy recovery and feasibility.

The influence of the solid retention time of the first digester in an ITHP AD process on the degree of sludge stabilization may have on the total system performance of methane productivity and VS removal is an area yet to be studied. Solid retention time (SRT) is regarded as the most important parameter for the anaerobic digestion design and operation (Parkin & Owen, 1986). SRT has been found to accurately define the relationship between the bacterial system and digester operation conditions. The challenge for the engineer or operator is selecting the optimal SRT for a substrate to ensure efficient conversion of complex organic matter to methane and carbon dioxide. A value chosen closer to the minimum SRT required for the sludge degradation and methane generation could result in a smaller digester size and increased rate of methane production, but a lower degree of sludge stabilization and increased risk of digester failure. On the other hand, a longer SRT digestion could oversize the digester with little extra improvement in the digester performance but a
massive increase in Capex and Opex costs. The most common SRT for full-scale plants anaerobic digester design is between 15 and 20 days.

This study focused on the influence of a first digester retention time (0, 5, 9, 12 and 30 days) in a ITHP AD system (AD1-TH-AD2) using a lab-scale semi-continuous system for the treatment of TWAS. To verify the stabilization of TWAS through the three-stage process, BMP tests were used to measure sludge stability to provide a direct measurement about the extent of completion of digestion of biodegradable organic matter. The results presented are based on two scale of investigation: (1) substrate level impact and (2) local AD system performance. It is the aim of the authors to observe and find general trends of TWAS methane yield through the AD1-TH-AD2 configuration for future studies to use as inputs to more comprehensive investigations. Based on the experimental results, an energy balance was conducted to evaluate the potential conditions to to achieve an energy balance with ITPH AD for the treatment of wastewater sludge.

6.2. Materials and Methods

6.2.1. Sludge Sample Collection and Preparation
Thickened waste activated sludge (TWAS) was collected from the Southern Ontario Water Consortium Guelph Facility (GWF) for days 0 to 117 and from the Guelph Wastewater Treatment Plant (GWWT) from days 118 to 210. The GWF is a bench and pilot-scale facility constructed adjacent to the GWWTP. The GWF has access to various process streams including raw wastewater, primary effluent, secondary effluent, tertiary effluent, raw sludge and waste activated sludge. TWAS was made using a belt filter, thickening the WAS to 3-4 %TS. At the GWWTP waste activated sludge from all plants are thickened in a rotary drum thickener and then sent to one of the primary anaerobic digesters. Through request samples forms, the wastewater services department coordinated sampling times with on-site operators.

Anaerobically digested sewage sludge from the GWWTP was employed as the seed for the batch and semi-continuous reactors. Anaerobic digesters at the GWWTP are operated at high-rate mesophilic temperatures, with working volumes of 85-95%, volatile solids destruction of 40-65%, and solid residence times of 10-20 days. The AD sludge showed a stable TS content of 20.1±0.6 g/L over the sampling period, which was very close to the
annual average TS 19.5 g/L over the period of 2011 to 2015. The relative stable TS with the AD sludge suggests that the AD digester of the WWTP can provide biological consistent inoculum for the sludge BMP tests.

6.2.2. Bench-Scale Sludge Treatment
Thermal treatment (165°C/30 min) of anaerobically digested sludge was conducted by filling 300 mL of sludge into a 600 mL 5500 HP Compact Reactor (Parr Instrument Company: Moline, Illinois, USA). The thermal reactor consisted of a high pressure compact reactor, a heating coil, mixer, thermocouple, water cooling line, and a control unit. The sludge was heated to the desired hydrolysis temperatures under mixing condition. After selecting the desired temperature and time program on the controller, the temperature would rise to the selected value and the water cooling would activate for regulated cooling. Once the treatment was completed, the reactor was placed into a bucket of cold water to cool down to room temperature and the treated sludge was collected for further treatment.

6.2.3. Semi-continuous anaerobic digesters experiment
Figure 1 shows the laboratory-scale anaerobic digestion configuration with intermediate thermal treatment. Reactor one was a 12 L digester manufactured by GE Water and Process Technologies. The reactor was maintained at approximately 35°C using a hot water bath connected to a hot water jack surrounding the tank. Mixing was performed using a mechanical mixer at 50 rpm. Biogas flow rate was controlled using a 100 standard cubic feet per hour (SCFH) gas flow meter. A pressure sensor was installed on top of the digester tank. Liquid level sensors were used to control and maintain the working volume of 10 L. A programmable logic controller (PLC) system was used for operation control and data collection. Reactor two was a 1 L glass bottle that was heated using a 35°C water bath and mixed using a magnetic stirrer. To waste and feed, a plastic tube went through the top plastic screw lid down below the solution level. A peristaltic pump was used for wasting and feeding. Biogas was released through two connected lines: one attached to a 60 mL syringe for gas composition analysis and a biogas collection bag.

6.2.2.4. ITHP operation
The semi-continuous anaerobic digestion system was operated for 210 days. Before the reactors were used to collect data, both reactors had to transition from a start-up period to
steady state. During the start-up period both digesters were operated at a hydraulic retention time of 12 days for three cycles (36 days) to ensure the establishment of viable methanogenic cultures and to purge the seed sludge. Afterwards each digester was subjected to a program of steady-state operation for different hydraulic retention times (see Figure 33).

Figure 6-32: Hydraulic retention time conditions for the first and second anaerobic digester reactors.

Anaerobic digester monitoring was split into two sections: (1) characteristics of feed and digested sludge and (2) digester health. Characterizing the feed and digestate sludge required manual measurements and were completed two to three times a week. Monitoring the digester health consisted of daily reporting feed and wasting volumes, produced biogas volumes, biogas composition, digester temperature, ORP and pH. In the case of the 1 L reactor biogas volumes were not measured. The use of the second reactor focused on the differences in feed and digested sludge characteristics for thermally treated sludge.

6.2.3. Analysis

6.2.3.1. Sludge Analysis
Total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were determined by standard methods (Method 2540-1997 and EPA Method 160.4). Chemical oxygen demand (COD) and total volatile fatty acids (VFA) were determined using Hach test vials (Hach, Canada). The pH and alkalinity of sludge samples was determined using the filtrate of sludge samples by Titra Lab 870 titration workstation (Hach, Canada). The filtered samples were prepared by centrifuging the sludge at 10,000 rpm
and 4°C for 15 min and then filtering the collected supernatant through a 0.45 um syringe filter. The biogas analysis was performed using an Agilent 6890 gas chromatography (GC) system (Agilent Technologies, USA) with a thermal conductivity detector (TCD). Argon was used as the carrier at an inlet temperature of 200°C. The oven temperature was held at 35°C for 7.5 min, and then increased from 35 to 206 °C at a rate of 24°C/min, and held at 206°C for 1 min. The peaks were identified and quantified by comparing with biogas mix standards including methane, carbon dioxide, nitrogen, oxygen, and hydrogen at various concentrations. The biogas was measured through syringe displacement using 10mL and 50mL glass syringes (Popper and Sons Inc.).

6.2.3.2. Biochemical Methane Potential Assay
The BMP serum bottle method described as the following was adapted from a combination of recommendations by Remigi and Buckley (2006), Hansen et al. (2004), Owen et al. (1979), Angelidaki et al. (2009), Raposo et al. (2011), Holliger et al. (2016), Strömberg et al. (2014) and Møller et al. (2004). In this study, anaerobic methane production was assessed at 35°C using 160 ml capacity serum bottles (American Scientific Products, McGraw Park III). The total volume of substrate and inoculum mixture in each BMP bottle was adjusted to achieve a desired substrate and inoculum ratio (SIR) and total bottle volume to headspace ratio. The SIR was defined as the ratio of gram of substrate COD added to the gram of inoculum VS added, which will affect the total methane volume production and the VFA/Alkalinity ratio in the BMP tests. For each condition, triplicate testing and blank bottles were used. The blank BMP bottles only contained DI water and inoculum.

Once the substrate and inoculum mixture were filled into the BMP bottles, the BMP bottle was immediately flushed using nitrogen gas for 15 seconds at a low flow rate with the tip of the nitrogen tube slowly rotated inside the BMP bottle to ensure the removal of air and the good mixing of the substrate and inoculum. The bottles were caped with rubber stoppers, sealed with an aluminum crimp, and placed upside down in the incubator (New Brunswick Scientific C25 Incubator Shaker) with constant shaking at 100 rpm at 35°C.
In the first 1 to 2 hours after the BMP tests were initialized, each bottle had their biogas production measured using 10mL or 50mL glass syringes equipped with a luer valve (Popper and Sons Inc.) to re-equilibrate the headspace, relieving the expansion of gas caused by the increased temperature. The glass syringe was fitted with a disposable needle to take a sample from the headspace. The needle was inserted into the butyl rubber septum and the syringe was held horizontally to minimize the effect of the weight of the plunger as the biogas was released. The readings were then verified by pushing the plunger down past the equilibrium point and checking if the plunger could return to the original position (Remigi and Buckley, 2006).

During the BMP test period, at least two of the triplicate testing bottles were sampled for biogas measurement. Serum bottles were taken out one at a time and the biogas volumes were quickly measurement with assumption of the biogas temperature to be 35°C at the time of measurement. In general, around 10mL of biogas is needed for an accurate GC measurement and the sample was injected into the GC using a 0.45 um filter to avoid contamination of the columns. The GC analysis determined the contents of oxygen, nitrogen, methane and carbon dioxide.

To standardize the BMP results, the as-measured volumes must be converted to standard conditions (0°C at 1 atm). This involves compensating for both volume occupied by water vapor (generates over-estimations of 2-8% in the gas volume at ambient temperature range) and thermal expansion effects (Richards et al., 1991; Strömberg et al., 2014). Normally, volumes are measured at one atmosphere, so no pressure correction is required (Richards et al., 1991). The biogas sampling intervals was calculated using the following equation:

\[ V_{CH_4,n}(mL) = \left( V_{biogas,n} + V_{headspace} \right) * \frac{\% of CH_4,n-1}{100} - V_{headspace} \]

\[ \quad * \left( \frac{T_{STP}}{T_{test}} \right) * \left( 1 - \frac{P_{vap}}{P_{gas}} \right) \]

Equation 6-24: Methane volume for biogas sampling intervals
where \( V_{CH4,n} \) is the methane generation volume (mL) of the mixed liquor; \( V_{biogas,n} \) is the biogas generation volume (mL) of mixed liquor; \( V_h \) is the headspace volume (mL) of each BMP bottle; \( %CH4,n \) is the current methane percentage of the generated biogas determined by GC; \( %CH4, n-1 \) is the methane percentage of generated biogas in last sampling time point; \( T_{STP} \) is the standard temperature (273.15 K); \( T_{gas} \) is the incubation temperature (K) for the BMP test; \( P_{vap} \) is the water vapor pressure (5.626 kPa) at the TMP temperature (35 °C); \( P_{gas} \) is the pressure of the measured gas (101.325 kPa).

The methane generated from the substrate was calculated by subtracting the methane volume from the blank. With the substrate methane isolated, the methane yield was calculated by dividing the STP methane volume by the mass of the VS solids or the TCOD of the substrate added into the serum bottle (reported as NmLCH4/g VS or NmLCH4/gCOD). The biodegradability was calculated by dividing the measured methane yield (NmLCH4/gCOD) by the theoretical methane yield at STP (0.35 NmLCH4/gCOD).

The accuracy and reliability of BMP test method used in this study were evaluated by using microcrystalline cellulose (Sigma-Aldrich) as the control of the BMP method. Cellulose which had a VS content of 97.2% (w/w) is the most common choice for a control substrate of BMP test because it is relatively easy to calculate the theoretical BMP (415 NmLCH4/g VS) (Koch et al., 2017). Holliger et al., (2016) stated that the BMP methods are acceptable if the methane yields of cellulose obtained from the BMP tests are between 85% and 100% of the theoretical BMP (352 - 414 NmLCH4/g VS).

6.2.4 Calculations

Removal rates of total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) were calculated after 55 days of a start-up period using the following equation:

\[
Removal_{VS} = \frac{VS_{feed} - VS_{outlet}}{VS_{feed}} \\
\text{Equation 6-25: Removal of TS, VS and COD}
\]

Substrate solubilisation was based on measurements of raw substrates total COD in comparison to the soluble COD after treatment. The following equation was used to describe the degree of disintegration:
where COD_s is the concentration of soluble COD after treatment, COD_so is the raw sludge soluble COD concentration, COD_max is the total COD of the sludge sample.

The degree of hydrolysis, acidogenesis, and methanogenesis achieved for each digestion stage was calculated from the COD balance. The total COD conversion ratios were defined using the method provided by Xiao et al., (2018), Wu et al., (2015) and Wandera et al., (2018). The following equations were used:

\[ DD_{COD} = \frac{COD_s - COD_{so}}{COD_{max} - COD_{so}} \]  
Equation 6-26: Degree of Disintegration

\[ Hydrolysis \ (\%) = \frac{SCOD_{eff} - SCOD_{in} + COD_{CH4}}{TCOD_{in} - SCOD_{in}} \]  
Equation 6-27: Degree of Hydrolysis

\[ Acidogenesis \ (\%) = \frac{COD_{vfa} - COD_{vfa_{in}} + COD_{CH4}}{TCOD_{in} - COD_{VFA_{in}}} \]  
Equation 6-28: Degree of Acidogenesis

\[ Methanogenesis \ ratio \ (\%) = \frac{(COD_{CH4})_{reactor}}{TCOD_{inf}} \]  
Equation 6-29: Degree of Methanogenesis

where TCOD_{in} is the total influent COD; SCOD_{eff} is the centrifugal effluent supernatant SCOD; SCOD_{in} is the influent SCOD. COD_{CH4} was calculated based on the conversion factor 0.35 L CH_4/g COD under standard conditions. COD_{VFA} is the total VFA concentration calculated by the oxygen demand of individual VFAs and COD_{VFA_{in}} is the influent of COD_{VFA}. The representative conversion efficiency was calculated after three HRTs of operation.

6.2.5 Energy balance
An energy balance was used to compare the efficiency of each configuration. The assumptions for the calculations are provided in Table 20. For this study the energy required for further dewatering, transportation and land application were not accounted for. As stated
by Yuan et al., (2019) these factors are missing in anaerobic digestion mass balances in literature, preventing a method and comparison.

Table 6-20: Assumptions for energy balance

<table>
<thead>
<tr>
<th>Description</th>
<th>Unit</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>m³/d</td>
<td>100</td>
<td>(Xiao et al., 2018)</td>
</tr>
<tr>
<td>Density of water</td>
<td>kg/m³</td>
<td>1000</td>
<td>(Xiao et al., 2018)</td>
</tr>
<tr>
<td>Specific heat of water</td>
<td>kJ/kg°C</td>
<td>4.18</td>
<td>Metcalf &amp; Eddy, 2014)</td>
</tr>
<tr>
<td>Temp of air</td>
<td>°C</td>
<td>-5</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Earth next to wall</td>
<td>°C</td>
<td>0</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Incoming sludge</td>
<td>°C</td>
<td>10</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Earth below floor</td>
<td>°C</td>
<td>5</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Temp of sludge in digester</td>
<td>°C</td>
<td>35</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Treatment temperature</td>
<td>°C</td>
<td>170</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Heat transfer of walls</td>
<td>W/m³°C</td>
<td>0.68</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Heat transfer of floor</td>
<td>W/m³°C</td>
<td>2.85</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Heat transfer of roof</td>
<td>W/m³°C</td>
<td>1.5</td>
<td>(Metcalf and Eddy, 2014)</td>
</tr>
<tr>
<td>Heat recovery by heat exchanger</td>
<td>%</td>
<td>80</td>
<td>Xiao et al., 2018)</td>
</tr>
<tr>
<td>Energy consumption for pumping</td>
<td>kJ/m³</td>
<td>1800</td>
<td>(Xiao et al., 2018)</td>
</tr>
<tr>
<td>Energy consumption rate of mixing</td>
<td>kJ/m³/d</td>
<td>300</td>
<td>(Xiao et al., 2018)</td>
</tr>
<tr>
<td>Lower heating value of methane</td>
<td>kJ/m³ CH₄</td>
<td>35800</td>
<td>(Xiao et al., 2018)</td>
</tr>
<tr>
<td>Methane production rate</td>
<td>m³ CH₄/kg VS destroyed</td>
<td>0.499</td>
<td>Metcalf and Eddy, 2014)</td>
</tr>
</tbody>
</table>

The assessment was determined by the difference between the energy input and output. The energy input was calculated using equations 1–4 (Xiao et al., 2018):

\[
E_{\text{input}} = E_{\text{op}} + E_{\text{influent}} + E_{\text{Reactor loss}}
\]

\[
E_{\text{op}} = Q \times 1800 \frac{kJ}{m^3} + V \times 300 kJ/m³d
\]

\[
E_{\text{influent}} = \rho \times Q \times \frac{4.18 kJ}{kg} \times C \times (T_d - T_i)
\]

\[
E_{\text{Reactor loss}} = (A_w \times (T_d - T_{air}) \times kw) + (A_f \times (T_d - T_{earth}) \times kf
\]

\[
+ A_c \times (T_d - T_{air}) \times kc
\]

Equation 6-30: Energy input
Equation 6-31: Energy Required from Electricity
Equation 6-32: Energy required for Influent
Equation 6-33: Energy Required for Reactor Heating
where $E_{\text{input}}$ is total input energy required; $E_{\text{op}}$ is the input electricity from pumping and mixing, $E_{\text{heat}}$ is the input heat for raising the influent temperature to either digestion temperature or thermal hydrolysis temperature, $E_{\text{reactor}}$ is the energy required for heating the digester; $Q$ is the feed flow rate; $V$ is the working volume of the digester; $C$ is the heating capacity of the feed; $\rho$ is the density of the feed; $T_d$ is the temperature of the digester; $T_i$ is the temperature of the influent; $A_w$, $A_f$, $A_c$ are the surface areas of the walls, floor and ceiling; $k_w$, $k_f$, $k_c$ are the energy transfer coefficients of the walls, floor and ceiling.

The output energy was determined using equations:

$$E_{\text{output}} = E_{\text{methane}} + E_{\text{recovery}}$$

Equation 6-34: Energy Output

$$E_{\text{meth}} = MY \left( \frac{CH_4}{kg \, VS} \right) \times 0.9 \times VS \, (kg)$$

Equation 6-35: Energy in Methane

$$E_{\text{recovery}} = 1000 \times Q \times \rho \times (T_d - T_i) \times 0.8$$

Equation 6-36: Energy Recovery

$$\Delta E = E_{\text{output}} - E_{\text{input}}$$

Equation 6-37: Energy Balance

where $E_{\text{output}}$ is the total energy recovered from digestion; $E_{\text{methane}}$ is the energy generated from produced methane; BMP is the biochemical methane potential of the substrate ($m^3 \, CH_4/ \, kg \, VS$); 0.9 is a correction efficiency; $E_{\text{recovery}}$ is the estimated heat recovery based on the heat of the effluent: 0.8 is a correction efficiency for the heat recovery system; $\Delta E$ is the energy balance.

6.2.6 Combined Heat and Power (CHP) and Steam Generation

Gas engines can provide electricity, hot exhaust gases and hot water through the combustion of biogas. To estimate whether a full scale anaerobic digester with intermediate thermal hydrolysis would be able to generate enough steam for the thermal hydrolysis unit to be self-sustaining the assumptions used by R. Cano et al., (2014), where a typical commercial biogas engine is 88% efficient with an electrical efficiency of 33% and thermal efficiency of 55% (25% exhaust gas and 30% hot water). Steam would be generated using an exhaust gas boiler with a 64% (Pérez-Elvira and Fdz-Polanco, 2012). The theoretical steam requirements were calculated using the following equation (Ringoot et al., n.d.):
\[ \frac{X}{\%DS_r} = (Th - Tr) \times \left( \frac{C_{p,w} - \%DS_r \times (C_{p,w} - C_{p,s})}{\%DS_r \times (H_{steam} - TH \times C_{p,w})} \right) \]

Equation 6-38: Steam Consumption

Where X is the specific steam consumption (ton steam/ton wet sludge); \(X/\%DS_r\) is the specific steam consumption (ton/ton dry solids); TH is the temperature of hydrolyzed sludge, Tr is the temperature of raw sludge; \(\%DS_r\) is the raw sludge dry solids as fed to the TH system; Cpw is the specific heat of water; Cps is the specific heat of solids; Hsteam is the enthalpy of steam fed to the THP system.

6.3. Results

6.3.1 Impact of anaerobic digestion with intermediate thermal treatment on TWAS stabilization

6.3.1.1 Performance of reactor one

Figure 34 shows the sludge characteristics, biogas production, biogas composition, pH and volatile solid destruction. It is known that as the HRT of an anaerobic digester is shortened from 30 days to 5 days there is an increase in overloading rate (gVS/L/d), biogas productivity (LCH\(_4\)/L/d) and decrease in reactor methane yields (LCH\(_4\)/gCOD). This was true for decreasing the HRT from 30 days to 12 and 9 day HRTs. But this was not reflected as clearly in the day 5 HRT volatile solids destruction or methane productivity.

Volatile solid concentrations for the feed and digestate sludge varied continuously during operation for HRT times 5, 9, 12 and 30. As the solid destruction is relative to the feed, each HRT was analyzed separately. The VS destruction for each HRT was 39.9±5.5, 34.3±2.98, 30.4±5.9, 42.2±7.2%. Normally as the HRT increases the solid destruction increases, but at an HRT of 5 days the solids measured lower than an HRT of 9 and 12 days. However based on other parameters such as BMP assay and smell, it was clear the digestate had the lowest degree of stabilization.

Methane production for HRT of 5, 9, 12 and 30 days averaged for each run at 0.50±0.24, 0.54±0.10, 0.34±0.12, 0.10±0.08 L CH\(_4\)/L reactor/d. All HRT conditions produced gas at a steady composition of 70% CH\(_4\) and 30% CO\(_2\). To test the accuracy of predicting methane generation using BMP data the feed was multiplied by the loading rates and by a correction
factor of 0.9. BMP were found to accurately predict the methane production rates of the system, except for day 5 HRT. At this time it was predicted that the system was washing out the methanogenic microorganisms. At a HRT of 5 days, it is estimated there was a washout of methanogenic bacteria.

Figure 6-33: (a) pilot methane measurements, (b) feed and digestate solid content, (c) percent VS reduction, (d) pH and ORP measurements.

Figure 35 shows the methane yield profiles of TWAS and anaerobic digestate after a HRT of 5, 9, 12 and 30 days. As the retention time of the digester increased the methane yields and kinetics decreased from 313 NmL CH4/g VS to 80 NmLCH4/ gVS. This shows that the degree of digestion improved by reducing the TWAS methane yield by 70% after 30 d HRT.
It was also worth noting that the kinetics of the methane yields also decreased when the first stage increased from 5 days to 30 days. This confirms that the longer retention times more biodegradable material in the substrate is converted to methane and carbon dioxide, thus lowering the amount of organic material remaining in the sludge sample. This range appears to be consistent with residual digestate methane potentials found in literature. For example, Ruile et al., (2015) investigated the residual methane potential of whole digestate from 21 full-scale digesters and reported methane yields varying from 24 to 126 NmL Ch4/g VS. Sambusiti et al., (2015) reported methane yield of untreated digestate of 70 NmL CH4/ g VS, and a untreated solid-separated digestate of 90 NmL/ g VS with 33 and 44% degradability (Sambusiti et al., 2015).

![Graph showing methane yield curves for TWAS and anaerobic digestate exiting the first anaerobic digester after 3 cycles.](image)

**Figure 6-34:** Left- Methane yield curves for TWAS and anaerobic digestate exiting the first anaerobic digester after 3 cycles, Right- Percent reduction in TWAS methane yield for different HRTs.

### 6.3.1.2. Thermal Hydrolysis Impact on Anaerobic Digestate

The sludge composition of untreated and thermal treated digestate samples are shown in Table 21. All digestate samples responded similarly to the thermal treatment. TS, VS, and TCOD concentrations remained unchanged, while sCOD, VFA and NH₃ and pH increased. The extent of both SCOD and VFA increases are related to the initial concentrations, where higher initial values lead to higher treated concentrations. Similar solubilisation rates were found for 20 g TS/ L TWAS by Bougrier et al., (2006) using thermal treatment (40-45%) (Bougrier et al., 2006).
Figure 36 shows the methane yield of treated TWAS and anaerobic digestate for retention times of 5, 9, 12 and 30 days. As the retention times increased the effectiveness of the treatment on raising the methane yield of the sludge also increased. With the 5 day HRT, the methane yield was close to TH-TWAS. This might show the sludge was not fully digested and contained a similar amount of readily biodegradable and non-biodegradable material. In comparison, the HRT of 9, 12 and 30 were closely grouped together. Perhaps once the readily biodegradable material has been converted, and there is only non-digestible material that TH has less material to make available for re-digestion. When the thermal treated methane yields are compared to the initial untreated values the most efficient use of TH is on fully stabilized sludge at increase longer retention times. These results agree with Chauzy et al., (2014) recommendation that for the general optimization of an anaerobic digestion process with intermediate thermal hydrolysis, the first digester should achieve at least 85-90% volatile solids reduction before thermal treatment. Similar observations were made by Pinnekamp, (1989), who through the treatment of primary sludge and anaerobically pre-stabilized sludge found that lower the volatile solids concentration, the higher the percentage increase in gas yield attainable by thermal pre-treatment. However, it is worth noting that the increased digestion time will increase the size of the digester or reduce the treatment capacity of the digester, which will have significant impact on the Capex cost the digesters for the treatment of a given waste sludge flow. The assessment of the effect of TH treatment should not be solely based on the enhancement of TH, but should be based on the overall methane recovery from the two stage AD and the system energy balance.

Table 6-21: First digester anaerobic digester properties before and after thermal treatment after 3 cycles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>5 d HRT R1</th>
<th>5 d HRT R1+TH</th>
<th>9 d HRT R1</th>
<th>9 d HRT R1+TH</th>
<th>12 d HRT R1</th>
<th>12 d HRT R1+TH</th>
<th>30 d HRT R1</th>
<th>30 d HRT R1+TH</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>17.0±0.4</td>
<td>16.6±0.2</td>
<td>17.0±0.4</td>
<td>16.1±0.3</td>
<td>25.7±1.9</td>
<td>24.6±0.6</td>
<td>24.0±2.4</td>
<td>22.1±0.5</td>
</tr>
<tr>
<td>VS</td>
<td>10.0±0.8</td>
<td>10.9±0.6</td>
<td>10.7±0.2</td>
<td>10.3±0.1</td>
<td>15.3±1.0</td>
<td>14.7±0.8</td>
<td>12.4±1.3</td>
<td>11.6±0.9</td>
</tr>
<tr>
<td>COD</td>
<td>18.9±0.7</td>
<td>17.4±0.3</td>
<td>16.7±0.3</td>
<td>16.4±0.1</td>
<td>25.5±2.7</td>
<td>24.1±0.6</td>
<td>23.9±0.2</td>
<td>18.2±0.6</td>
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<tr>
<td>SCOD</td>
<td>164±25</td>
<td>7530±300</td>
<td>914±200</td>
<td>8205±410</td>
<td>1299±301</td>
<td>10480±500</td>
<td>1076±300</td>
<td>9860±440</td>
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<tr>
<td>VFA</td>
<td>157±100</td>
<td>840±40</td>
<td>181±10</td>
<td>710±30</td>
<td>384±180</td>
<td>946±200</td>
<td>149±50</td>
<td>787±300</td>
</tr>
<tr>
<td>NH3-N</td>
<td>790±10</td>
<td>810±20</td>
<td>782±20</td>
<td>793±50</td>
<td>860±89</td>
<td>889±45</td>
<td>1340±88</td>
<td>1280±90</td>
</tr>
<tr>
<td>pH</td>
<td>6.9±0.1</td>
<td>9.0±0.1</td>
<td>7.1±0.1</td>
<td>9.0±0.1</td>
<td>7.3±0.1</td>
<td>9.0±0.1</td>
<td>7.2±0.1</td>
<td>9.0±0.1</td>
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<tr>
<td>Solubilisation</td>
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<td>0.45</td>
<td>---</td>
<td>0.37</td>
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<td>0.46</td>
<td>---</td>
<td>0.37</td>
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<td>0.38</td>
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<tr>
<td>Acidogensis</td>
<td>---</td>
<td>0.04</td>
<td>---</td>
<td>0.03</td>
<td>---</td>
<td>0.02</td>
<td>---</td>
<td>0.03</td>
</tr>
<tr>
<td>Methanogensis</td>
<td>---</td>
<td>0.01</td>
<td>---</td>
<td>0.01</td>
<td>---</td>
<td>0.01</td>
<td>---</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Figure 6-35: Methane yield curves for 30 day BMP test of thermal treated TWAS and anaerobic digestate after 5, 9, 12 and 30 day HRT, (b) Percent increases in methane yields after thermal hydrolysis.

6.3.1.3. **Reactor two system performance**

Reactor two was kept at a stable HRT of 12 days for the duration of the test. Table 22 synthesizes the performance obtained during anaerobic digestion of thermal treated sludge samples. The feed solids were different for each time correlating with the gradual decrease in TWAS feed solid content. In general, the second digester was able to remove an additional 2 g VS/L of the thermal treated sludge. From operational insights, the treated sludge had a pH raised to 9 due to the release of proteins and ammonia and soluble content. For all runs, the SCOD was constant around 8000 to 10000 mg/L. After 12 days retention time, the soluble content decreased on average to 2000 mg/L. This decrease was also observed for VFA content. The exception was that with the decrease in pH from 9 to 7.8 in the reactor which is still within healthy operation status, the ammonia concentration increased. This might signal a protein degradation which increase ammonia, and do not result in a statically significant increase in biogas production.
Table 6-22: Average data collected over stable regions during semi-continuous test before and after second digestion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>5 d HRT</th>
<th>9 d HRT</th>
<th>12 d HRT</th>
<th>30 d HRT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1-TH</td>
<td>R2</td>
<td>R1-TH</td>
<td>R2</td>
</tr>
<tr>
<td>TS</td>
<td>16.6±0.2</td>
<td>14.3±0.2</td>
<td>16.1±0.3</td>
<td>14.6±0.5</td>
</tr>
<tr>
<td>VS</td>
<td>10.9±0.6</td>
<td>7.7±0.3</td>
<td>10.3±0.1</td>
<td>7.0±0.2</td>
</tr>
<tr>
<td>COD</td>
<td>17.4±0.3</td>
<td>15.2±0.8</td>
<td>16.4±0.1</td>
<td>14.8±1.7</td>
</tr>
<tr>
<td>SCOD</td>
<td>7530±300</td>
<td>1788±116</td>
<td>8205±410</td>
<td>3152±120</td>
</tr>
<tr>
<td>VFA</td>
<td>840±40</td>
<td>255±50</td>
<td>710±30</td>
<td>216±64</td>
</tr>
<tr>
<td>NH₃-N</td>
<td>810±80</td>
<td>1142±110</td>
<td>793±80</td>
<td>1038±105</td>
</tr>
<tr>
<td>pH</td>
<td>9.0±0.1</td>
<td>7.8±0.1</td>
<td>9.0±0.1</td>
<td>7.8±0.1</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>---</td>
<td>0.67</td>
<td>---</td>
<td>0.05</td>
</tr>
<tr>
<td>Acidogensis</td>
<td>---</td>
<td>0.04</td>
<td>---</td>
<td>0.03</td>
</tr>
<tr>
<td>Methanogensis</td>
<td>---</td>
<td>0.07</td>
<td>---</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Figure 37 shows the methane yield curves of the anaerobic digestate leaving the second digester. In comparison to the decrease in the sludge methane yield of the feed after the first reactor, the difference between the thermal treated AD methane yield and the secondary reactor digestate is much greater. This had to do with the change in the rate limiting step for digestion. By applying thermal hydrolysis, methanogenesis, instead of hydrolysis, will be the rate-limiting step in anaerobic digestion. As a result selection of the HRT for the digestion of thermal treated sludge changes from selecting a longer optimal retention time for complete digestion, to an HRT closer to the washout of microorganism. As stated by Barber, (2016), it could be that running digestion plants at approximately 10 days rather than 20 days retention time may be preferable when coupled with thermal hydrolysis. Li and Noike, (1992) concluded optimum digestion retention times between 5 and 10 days based on various tests and observations of changes in methanogenic populations. Therefore further studies need to investigate the performance of anaerobic digestion of thermal hydrolyzed sludge at short hydraulic retention times and changes in the methanogenic communities.
6.3.1.4. Methane yield of TWAS through AD-ITHP process

Figure 38 shows the methane yield of the TWAS throughout the laboratory anaerobic digestion process integrated with intermediate thermal hydrolysis. The TWAS methane yield entering the stabilization process was around 300 NmL CH$_4$/g VS. As the first digester solid retention time increased, the methane yield of the digestate decreased from 200 to 80 NmL CH$_4$/g VS. After each was thermal treated the methane yield of the sludge increased between 250 and 350, with the increase related to the combination of readily and unreadily biodegradable material within the digestate. Finally after second digestion, the methane yield of all test conditions leveled out between 50 and 100 NmL CH$_4$/g VS. In the situation where a 30 day digestate is commonly said to be stabilized due to its low measurement in methane yield, the increase after thermal hydrolysis tells that this is not completely true, there are still possibilities for methane extraction.

Figure 6-37: Methane yield of TWAS through each anaerobic digestion stabilization stages.
Figure 39 shows the methane productivity for 1 m³ of the reactor per day for each digester and configuration. In terms of the total system’s methane productivity, AD with pre-treatment is predicted to have the highest performance. In comparison, the AD configurations with ITHP had improved methane productivity above convention digestion except for when the first digester has a 30 day HRT. In all ITHP configurations the methane productivity of the first reactor was greater than the second fed with thermal treated digestate. Theoretically the 5 d HRT first digester should have the highest methane productivity, but based on the experimental results, 60% of the theoretical methane yield was achieved. Therefore, the methane yield for this comparison was multiplied by 0.6.

![Graph showing methane productivity](image)

Figure 6-38: Methane productivity for convention, pretreatment and ITHP configurations

6.3.2. Analysis of AD- ITHP local system level input and output using mass balance

6.3.2.1. Methane Productivity and volatile solids destruction

The ITHP laboratory system performance was evaluated based on methane productivity and volatile solid destruction. Conventional AD, AD with thermal pre-treatment, and AD with ITHP and a second digester were compared. The mass balance assumed the treatment of 100 m³ of TWAS (20 g VS/L) for discussion on the differences of digester sizing in relationship to the selected minimum hydraulic retention time. BMP data of the sludge entering into the digesters and volatile solid reduction of TWAS after varying anaerobic digestion conditions were used for the simulation. Methane yield values for the first digester operating in series were selected based on the methane yield curve of TWAS and the values at each incubation
time. In the situation where the first digester was operated at 5, 9, 12, and 30 days, the
selected methane yields on the TWAS BMP curve were 225, 261, 265, and 291 NmL CH₄/g VS. When the thermal treated digestate entered the second digester at varying volatile solid
concentrations, the methane yields were selected off different curves corresponding to each
digestate methane yield curve using a HRT of 12 days. The methane yield for the second
digester when the first digester was operating at 5, 9, 12, and 30 were 375, 253, 214 and 217
NmL CH₄/g VS. For the conventional digester, the HRT was selected for 15 days and the
methane yield after that time of 290 NmL CH₄/g VS. For the pre-treatment comparison, the
TH-TWAS curves were used and a methane yield of 380 NmL CH₄/g VS after 12 days.
Volatile solid destruction percentages were based on the performance of the semi-continuous
digesters.

Figure 40 shows the estimated methane production per m³ of feed per day. All ITHP total
system performance were higher than both conventional and pre-treatment configurations.
But the individual anaerobic digestion stages were lower than the conventional and
pretreament. The total methane production in comparison to the sub ITHP configurations
were relatively equal. As the first digester HRT increased methane production, it was
balanced by the lowering of available methane produced in the second anaerobic digester.
The performance of the volatile solids destruction followed a similar pattern. The ITHP
system were able to raise the ultimate VS reduction to 50% respectively in comparison to
33% and 43% for the conventional and pretreament. Based on methane productivity and
volatile reduction, no first digester HRT configuration could be chosen for optimization.
Studies were also carried out by other researchers to compare the AD1+ITHP+AD2 process with other AD configurations. Shana et al. (2013, 2012) showed that the ITHP configuration produced 20% more biogas compared to the pre-treatment configuration. Similarly, Takashima and Tanaka (2014) showed that on average, the VSS reduction that was achieved in the ITHP coupled AD was 67.6% in comparison to 48.7% for the one-stage AD, 65.8% for the one stage AD with the pre-treatment, and 52% for the two-stage AD without the intermediate TH treatment (Campo et al., 2017). In addition, Shana (2015) reported the average VS destructions achieved by the ITHP ADs, AD with the TH pretreatment (THP), the two-stage mesophilic AD, and the conventional mesophilic ADs were 62%, 47%, 52% and 44%, respectively, and the methane yield of the sludge with the ITHP coupled AD was 38% higher than that with the THP AD configuration.

Although many studies showed that the ITHP coupled AD could achieve higher methane production and VS reduction, the overall benefits of the ITHP coupled AD could be subsided by the energy consumption of the additional TH treatment and the investment required for the second digester and the TH equipment. Bjerg-Nielsen et al., (2018) suggested that it is necessary to thicken the sludge prior to the intermediate TH treatment to improve the energy balance of the ITHP processes. Ortega-Martinez et al., (2016) suggested that the performance
of the ITHP AD can be improved by optimizing the retention time of AD1 and thickening the
digestated sludge prior to the TH treatment. The energy balance of ITHP could also be
improved by using the chemical-aided low temperature (70 °C to 90 °C) TH treatment
(Campo et al., 2018). Therefore, although it has been proven that the ITHP treatment is an
effective process to enhance methane yields of wastewater sludge, the optimal process design
of the ITHP coupled AD still needs to be explored to improve the overall process energy and
cost efficiencies.

6.3.2.2. Energy Balance
An energy balance was used to assess the employment of convention, pre-treatment and
ITHP anaerobic digestion configurations. As stated by Li et al., (2019), there are a large
amounts of lab-sale studies showing high potential for attractive anaerobic digestion schemes
to enhance methane production, but whether they are economically feasible for consuming
more energy than that of increased methane production is often unaddressed.

Table 23 shows the energy balance of these three strategies assuming treatment of 100m³/day
of 3% TS TWAS. Out of the required energy inputs, the heat required to raise the
temperature of the influent to either digestion or thermal hydrolysis temperature accounted
for the greatest weight. For conventional digestion, energy was needed to raise the 10°C
influent to digester temperature (35°C), while pretreatment required energy to raise 10°C
sludge to 170°C. The ITHP, required both to raise the temperature to 35°C for the first
digester and then from 35 to 170°C for the second. ITHP also required greater compensation
for digester heat losses due to the extra surface area for an additional digester, however a
lesser impact on the total energy input required than heating the influent.

The energy from produced methane was higher for ITHP configurations than conventional
and pre-treatment but the additional generated energy was not enough to counter than extra
energy requirements for heating the influent. This was similar for pretreatment. The energy
required for TH, even with the use of a heat exchanger was not enough for a positive energy
balance. Therefore, from this specific system, conventional anaerobic digestion is the
advisable option based on the energy balance, while both pretreatment and ITHP
configurations are not due to the extra energy required for thermal hydrolysis.
Yuan et al., (2019) also completed an energy assessment of pre-treatment and iTHP assuming a TS input concentration of primary sludge of 1.82%. The study assumed energy production from AD as the output energy and the energy consumption by heating the thermal reactor and supplying the heat loss from the walls of AD reactors. This the evaluation TH temperatures of 130, 150, 170, 190 and 210°C were tested. In all cases both pre-treatment and ITHP configurations had negative energy balances, with ITHP slightly higher (-57 MJ/t) than pre-treatment (-54.74 MJ/t). The authors recommended that construction and maintenance costs of the additional AD tanks and the pipelines for returning treated digestate and advanced thickening tank also be considered.

Table 6-23: Energy balance assuming treatment of 100 m³ TWAS/day (kJ/kg feed)

<table>
<thead>
<tr>
<th></th>
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<td>Conventional</td>
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<td>0</td>
<td>6</td>
<td>36</td>
<td>147</td>
<td>187</td>
<td>68</td>
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<tr>
<td>Pretreatment</td>
<td>0</td>
<td>672</td>
<td>5</td>
<td>32</td>
<td>709</td>
<td>245</td>
<td>259</td>
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<td>5 day- ITHP</td>
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<td>630</td>
<td>7</td>
<td>50</td>
<td>792</td>
<td>292</td>
<td>259</td>
<td>483</td>
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<td>9 day- ITHP</td>
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<td>802</td>
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</table>

6.3.2.3. CHP and Steam Generation

Although pretreatment and ITHP were found not feasible based off the energy balance, the combined use of a gas engine and exhaust gas boiler have been found to generate steam that can power a thermal hydrolysis reactor. If enough steam is generated, than the TH energy requirements would be reduced making pretreatment (+467 kJ/kg fed) have the highest energy balance, followed by ITHP (+389, 370, 347, 331 kJ/kg fed). The following section investigated whether the steam produced from a CHP unit anaerobic digestion system using intermediate thermal hydrolysis would be enough to be self-sustaining (see Figure 41).
Figure 6-40: Anaerobic digestion with intermediate thermal hydrolysis with CHP and steam generation.

To estimate both steam generation and steam consumption, the methodologies of papers carrying out energy balances for thermal pretreatment were followed: Barber, 2016; R Cano et al., 2014; Pérez-Elvira and Fdz-Polanco, 2012; Ringoot et al., n.d.). However the data collected form this experiment used electric thermal treatment instead of steam. Based off Mottet et al., (2009) comparison of 165°C sludge treatment comparison of electric and steam mode thermal treatment, perhaps it is possible to use electric results for a prediction. As stated by Mottet et al., (2009), in the comparison between electric and steam for WAS thermal pretreatment, there was no significant difference on the impact of heating mode on sludge anaerobic biodegradability or solubilisation.

Table 24 shows the energy produced from a gas engine for electrical output, hot water and exhaust gases. The pretreatment and ITHP configurations were able to generate 1.3 and roughly 1.5 more electric energy than conventional digestion. This electricity could be distributed to the national grid and could take advantage of public subsidies provided for the production of electricity from renewable sources (Ruffino et al., 2016). But, the steam produced would not be enough to account for all the steam required. Following the calculations for the amount of steam required per dry solids by Barber, (2016) and Ringoot et al., (n.d.) for sludge with 3%DS would require approximately 5533 kg steam/ ton DS. In this scenario, 3 tons of DS are being fed into each system, requiring 16600 kg of steam. However,
the steam produced from the exhaust gas, assuming a conversion of 0.77 kWh/kg steam (2785 kJ/kg steam) and the assumption that the boiler is 64% efficient for generating steam from exhaust gases, than each system requires energy supplementation from auxiliary fuel (natural gas) (Ruffino et al., 2016)

Table 6-24: CHP and Steam Generation assuming 3%DS feed treating 100m³ feed/day

<table>
<thead>
<tr>
<th>Configuration</th>
<th>Total Methane (m³)</th>
<th>Total Energy in Biogas (kWh)</th>
<th>Electrical Output (EE)</th>
<th>Thermal Output Hot Water (HW)</th>
<th>Thermal Output Exhaust Gas (EG) (kWh)</th>
<th>Steam produced (kg steam)</th>
<th>Steam required (kg steam)</th>
<th>Steam needed using auxiliary fuel (kg steam)</th>
<th>Steam required if sludge at 17% DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>522</td>
<td>5052</td>
<td>1667</td>
<td>1263</td>
<td>1515</td>
<td>1273</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>684</td>
<td>6621</td>
<td>2184</td>
<td>1655</td>
<td>1986</td>
<td>1668</td>
<td>16600</td>
<td>14932</td>
<td>3242</td>
</tr>
<tr>
<td>5 day- iTHP</td>
<td>813</td>
<td>7873</td>
<td>2598</td>
<td>1968</td>
<td>2362</td>
<td>1984</td>
<td>16600</td>
<td>14616</td>
<td>3242</td>
</tr>
<tr>
<td>9 day- iTHP</td>
<td>788</td>
<td>7635</td>
<td>2519</td>
<td>1908</td>
<td>2290</td>
<td>1924</td>
<td>16600</td>
<td>14676</td>
<td>3242</td>
</tr>
<tr>
<td>12 day- iTHP</td>
<td>745</td>
<td>7211</td>
<td>2379</td>
<td>1802</td>
<td>2163</td>
<td>1817</td>
<td>16600</td>
<td>14783</td>
<td>3242</td>
</tr>
<tr>
<td>30 day- iTHP</td>
<td>783</td>
<td>7588</td>
<td>2504</td>
<td>1897</td>
<td>2276</td>
<td>1912</td>
<td>16600</td>
<td>14688</td>
<td>3242</td>
</tr>
</tbody>
</table>

These results show that a full scale anaerobic digestion system would not be energy feasible in comparison to the lower complexity of conventional digestion. This highlights the need for sludge thickening before thermal treatment in order to decrease the steam required per kg of sludge. As stated by Pérez-Elvira and Fdz-Polanco, (2012), a secondary sludge concentration of 17%DS going into a TH pretreatment unit would generate all the biogas necessary to get the steam needed in the TH unit from the exhaust gases. In this case, the steam requirements would decrease from requiring 16600 kg steam for 100m³/day of sludge to 3242 kg steam.

Future laboratory studies investigating anaerobic digestion with intermediate thermal treatment should therefore, adjust the following parameters to minimize steam consumption: (1) sludge inlet temperature, as lower steam consumptions will be realized at higher inlet temperatures, (2) sludge outlet temperature, since the pressure in the flash vessel can be reduced to even slight vacuum to reduce the outlet temperature and the steam usage at the cost of increased hydrolyzed sludge pumping needs, (3) losses of process vapors increase the steam usage (Barber, 2016; Pérez-Elvira and Fdz-Polanco, 2012).
6.4. Conclusion
This study inspected the stabilization of TWAS from a municipal wastewater treatment plant using a semi-continuous laboratory scale anaerobic digester followed by intermediate thermal treatment (165°C/30 min) for enhanced methane productivity. The AD+TH in this experiment was viewed as a two stage pre-treatment to anaerobic digestion. The first anaerobic HRT was changed from 5, 9, 12 and 30 days to observe the influence on the substrates methane yield through the whole stabilization process. As the first reactor retention time increased, the methane yield of the sludge decreased. The most efficient application of thermal treatment on raising the digestate methane yield was found for longer retention times, with the thermal treatment of 30 day HRT digestate increasing methane yield by 200%, in comparison to TWAS (+29%) and 5 d HRT digestate (+90%). A mass balance was carried out using the BMP data and input and output parameters of the semi-continuous system. Methane productivity of the total three stage anaerobic digestion system was found to be lower in all cases than if thermal pre-treatment was applied. When the methane production is compared based on the total size of reactors required for 1m³ of TWAS per day, all ITHP systems produced more methane than conventional or pre-treatment configuration. Although ITHP may produce more methane per volume of feed, it is yet to be determined whether it is economically feasible for consuming more energy than that of the increased methane production.
CHAPTER 7- CONCLUSION AND RECOMMENDATIONS

7.1 Conclusions
This research explored the potential experimental methane production for an anaerobic digestion system using a laboratory scale intermediate thermal hydrolysis. This investigation resulted in three papers: (1) a review and development of a biochemical methane potential (BMP) method, (2) an investigation into the methane potential of thermal treated anaerobic digestion, and (3) an assessment of a lab-scale anaerobic digestion with intermediate thermal treatment performance. This thesis made the following conclusions for each section:

1. BMP tests are the most common tool to evaluate a substrate’s experimental biodegradability and methane potential. As of 2018, BMP testing lacks standardization of procedures, resulting in a lack of comparable values. Studies continue to further the elimination of systematic errors. To determine the ultimate methane potential requires the proper design of a BMP test to ensure conditions are not limiting or inhibitory. The central variable appears to be control over the substrate to inoculum ratio (SIR). Too low a SIR may prevent induction of enzymes necessary for biodegradation, and too high a SIR may produce low methane production due to overloading of the seed by excessive volatile fatty acids. Therefore, for each substrate an operator may have to complete initial testing of various SIR to find the substrate’s optimal conditions. Additional factors that could impact the accuracy of BMP tests include the selection of blank and control bottles, headspace flushing, mixing, pH control, methane production monitoring method, and methane correction calculations.

2. The methane potential of thermal treated anaerobic digestate was investigated to evaluate the potential of secondary anaerobic digestion. A series of batch experiments were carried out analyzing different aspects of thermal treated digestate. Thermal treating digestate was found to have a greater impact on raising the methane yield than treating primary or thickened waste activated sludge. The excess control over thermal treatment temperature and time for anaerobic digestate between 160 to 190°C and 30 to 60 minutes appears to be unnecessary. The selection of optimal conditions within this range should be based instead on economic factors such as total solid concentration, net energy, and sludge dewaterability. Thermal treatment of 2% DS anaerobic digestate was found to increase the solid content released into the liquid
fraction, resulting in an increase of methane yield from 0 mL CH₄/g VS to 650 mL CH₄/g VS, suggesting redigestion of liquid fraction as a possibility for further investigation.

3. A lab-scale anaerobic digester with intermediate thermal treatment, fed TWAS, was operated for 210 days. During this time the first digester hydraulic retention time was altered from 5, 9, 12 and 30 days, while the second digester was maintained at 12 days. As the retention time increased the methane potential of the sludge after thermal treatment also increased, showing that a more stabilized sludge has a greater ability to improve the percent increase of a digestate’s methane yield. In terms of total system performance, anaerobic digestion with intermediate thermal hydrolysis may produce more methane per m³ per day than conventional anaerobic digestion or anaerobic digestion with thermal pre-treatment. However, an energy balance simulating the treatment of 100m³/day of 3%DS TWAS showed that methane pre-treatment or ITHP have negative energy balances due to the heating required to raise the temperature of sludge from 10°C to 170°C, making them undesirable options. In comparison, conventional anaerobic digestion is a less complex option and has a positive energy balance. Therefore a pre-treatment or ITHP system without thickening before thermal treatment would not be desirable from an energy balance standpoint. Further investigations are required at a pilot scale, and computer simulations evaluating.

7.2 Outlook and recommendations
This study focused on the substrate-level impacts of anaerobic digestion with intermediate thermal hydrolysis. But for a treatment to be considered feasible it must be assessed on different levels of scale in comparison to conventional digestion such as the local AD system with inputs and output, and the expanded AD system including its placement within a WWTP. Therefore the results of this study were dependent on both the specific process conditions and the measurement limitations. BMP test results for this work could be limited by the specific test conditions used and the semi-continuous reactor assessment is also tied to the specific process conditions tested. Further use of these results for process design and framework conditions would only be valuable to the treatment of the specific TWAS used.
Improvements to the accuracy of the experiments could be made in the following ways:

1. Run the digester with continuous daily feeding and treatment, instead of semi-continuous. This would minimize surges in acid and hydrogen production causing potentially detrimental decreases in pH if not sufficient alkalinity is not present.

2. Up scale the reactor sizes. Larger reactors could have higher concentrations of microorganisms, and would provide more realistic predictions of gas generation (Pearse et al., 2018).

3. Increase sampling times for TWAS feed to avoid putrication. This would maintain consistant substrate conditions and measurements in biodegradability.

4. Provide more in-depth characterization of sludge through the digestion process. These additional parameters may include alkalinity, VFA composition, sludge dewaterability, EPS, soluble proteins, soluble carbohydrates, humic acid, and pathogen kill.

5. Increase the frequency of BMP measurements during operation. Instead of taking one measurement after the reactors reached stable operation, multiple measurements would provide more statistically significant results.

6. Increase the frequency of SIR tests for different substrates being tested. Instead of testing one wastewater sample and carrying that SIR for treated digestate samples, regular SIR would provide more accurate and reliable results.

7. Operate BMP tests longer than 30 days. In situations where a comparison between untreated digestate and thermal treated digestate is made, BMPs operated for more than 30 days would allow the operator to observe whether the difference in methane yield values were due to increased hydrolysis rate or actual increases methane yield.

Finally, future studies could focus on the individual stages of the ITHP process. These might include the following at a laboratory scale:

1. Investigate into thickening before thermal hydrolysis at different solid concentrations. This could result in lower methane production, but also lower steam requirements for thermal hydrolysis.
2. Investigate the dewaterability of the digestate after second digestion. Once the dewaterability of digestate is known full computer simulations of the biosolids train could be completed, along with disposal cost analysis.

3. Observe the effects of altering the HRT of the second digester for performance optimization. Similar to the HRT for the first digester, perhaps due to the second digester treating thermal treated anaerobic digestate with methanogensis as the rate limiting step, a HRT for the second digester could decrease from the industry norm of 15 days to under 10 days.

4. Microbial analysis of the second digester for treating thermal treated digestate, in comparison to thermal treated TWAS.

5. Investigate the digestion of thermal treated liquid fraction. Digestate from a WWTP could be thermal treated and the liquid fraction fed into a semi-continuous reactor to observe the methane productivity and effluent quality. A performance evaluation would then be used to assess the feasibility of this option.

6. Complete a computer simulation using mass and energy balance for cost-benefit analysis. This would determine whether the cost of extra methane is decreased through the added complexity of this AD+ITH process.
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