1-11-2022

Improving Biomethane Recovery from Municipal and Industrial Wastes

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IMPROVING BIOMETHANE RECOVERY FROM MUNICIPAL AND INDUSTRIAL WASTES

By

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A Thesis
Submitted to the Faculty of Graduate Studies
through the Department of Civil and Environmental Engineering
in Partial Fulfillment of the Requirements for
the Degree of Master of Applied Science
at the University of Windsor

Windsor, Ontario, Canada

2022

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ABSTRACT

In Ontario, Canada, landfill capacity is rapidly decreasing, and inadequate waste management has resulted in increased greenhouse gas emissions and leachate volumes. Municipalities are left to design and implement their own organics waste management solution. Energy recovery through anaerobic digestion (AD) is attractive. However, AD can be costly for small and medium-sized communities. Two methods of improving economics of AD are studied in this thesis: co-digestion and pre-treatment of wastes.

Making use of industrial wastes can be an excellent method of supplementing AD of municipal wastes. The effect of mixing ratios on methane yield, substrate compatibility, and kinetics were studied for AD of distillery wet cake, source-separated organics (SSOs), and wastewater sludges. Mesophilic AD (37ºC) at an F/M ratio of 0.5 in a batch setup was performed using the AMPTS II unit. The addition of SSOs at higher ratios (50% and 75% VS) in the substrate mix resulted in a 14-15% higher yield per gram COD added, as compared to mono-digestion of wet cake. Mesophilic AD of the stillage and SSO mixtures resulted in a considerable lag phase, implying that degradation kinetics could be improved by acclimation of inoculum. This could help reduce operational costs and overall digestion time. Co-digestion studies revealed compatibility between the substrates, thus making AD a feasible alternative.

Microwave (MW) pre-treatment on distillery wet cake was investigated at temperatures of 50ºC,70ºC, and 90ºC at 480W and 1080W, respectively. MW pre-treatment of distillery wet cake did not have a significant effect on the solubilization of COD and biomethane yield. At 480W, 20-35% decreases in methane production rate were observed. At 1080W, 22-30% decreases were observed. This suggests the production of phenolic compounds that slowed the degradation of stillage.

ACKNOWLEDGEMENTS

I would like to thank my advisors, Dr. Rajesh Seth, Dr. Nihar Biswas, and Dr. Hisham Hafez, for their support and guidance. Their knowledge and patience have been inspiring to me. I am very proud and grateful to have begun my career in the field of environmental engineering with them.

Thank you to my committee members, Dr. Rajeev Ruparathna and Dr. Edwin Tam for providing their insightful comments and giving me a unique perspective on my research.

I would also like to thank the past and present members of my research group: David Lee, Ahmed ElGhanam, and Hyeongu Yeo. Their contributions and suggestions are deeply appreciated and were crucial to the success of my work.

My special thanks to Bill Middleton for his kind help and encouragement, especially with difficulties encountered throughout the pandemic. I wish him a happy and peaceful retirement.

Finally, I would like to express my sincere gratitude towards Giselle St. Louis and Winnie the Therapy Dog. They have been a safe supportive space for me during a difficult period in my life. My appreciation towards them is indescribable.

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LIST OF ABBREVIATIONS/SYMBOLS

AD	Anaerobic Digestion
ADS	Anaerobic Digested Sludge, seed, inoculum
AMPTS	Automatic Methane Potential Test System
BMP	Biochemical Methane Potential
BOD	Biochemical Oxygen Demand
CH_4	Methane
COD	Chemical Oxygen Demand
COD:N:P	Chemical Oxygen Demand to Nitrogen to Phosphorus ratio
C/N	Carbon to Nitrogen ratio
DDGS	Dried Distillers' Grains with Solubles
FA	Free ammonia, unionized ammonia
FOG	Fats, Oils, and Greases
FOW	Food and Organic Waste
IC&I	Industrial, Commercial, and Institutional
MW	Microwave
OFMSW	Organic Fraction of Municipal Solid Waste
Organic N	Organic Nitrogen
RRCEA	Resource Recovery and Circular Economy Act
SCOD	Soluble Chemical Oxygen Demand
SRB	Sulfate-Reducing Bacteria

SSO	Source-Separated Organics
TAN	Total Ammoniacal Nitrogen
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
TS	Total Solids
TSS	Total Suspended Solids
TVFA	Total Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids
WCK	Wet Cake
WDTA	Waste Diversion Transition Act
$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NH_3	unionized ammonia, free ammonia
NH_3-N	ammonia-nitrogen
NH_4^+	Ammonium ion, ionized ammonia
$\Delta G^{0\prime}$	Gibbs free energy

CHAPTER 1
INTRODUCTION TO THE STUDY

Over 80% of the world's energy supply is extracted from fossil fuels, which results in harmful greenhouse gas emissions [1]. Worldwide reliance on fossil fuels has resulted in resource depletion. Increases in population has resulted in higher levels of waste produced – translating to nearly 5% of global greenhouse gas emissions [2]. The Canadian province of Ontario has responded by introducing a ban on the landfilling of food and organic wastes (FOW), moving towards a closed-loop approach to organic waste management [3] [4]. Now, the responsibility of creating an organic waste management solution has been left to individual municipalities.

Anaerobic digestion (AD) is a waste-to-energy technology that is capable of confronting both the energy crisis and growing waste management concerns. In AD, organic waste is converted into biogas in the absence of oxygen by microbes. Biogas is mainly composed of methane and carbon dioxide, with trace amounts of hydrogen sulfide and water [5]. Depending on end usage, biogas must be scrubbed of its impurities. For heat and electricity generation via combined heat and power units, only hydrogen sulfide and water vapor need to be removed [6]. Alternatively, biogas can be upgraded to natural gas purity for injection into the existing grid infrastructure or the pipeline. However, biogas projects are almost always hampered by high capital and operating costs – which makes implementation difficult in small and medium-sized communities.

The centralized model approach to tackle FOW could certainly be an option for small and medium-scale municipalities that have economic concerns about implementing a biogas facility. Denmark has implemented centralized biogas plants since the 1980s [7]. Typically, centralized biogas plants involve digesting various farm manure with food waste and the organic fraction of municipal solid waste, agricultural residues, and in some cases, industrial residues [7] [8]. These plants provide many advantages, such as larger digester capacity, higher energy recovery and sales, income from tipping fees, centralized storage, production of nutrient-rich fertilizer, and reduction of transport distance [8] [9].

The success of the centralized approach relies on the following:

- Availability of feedstock
- Long-term financing

- Ownership, social context, and organization
- Nurturing of relationships between stakeholders, researchers, and other groups
- Supporting policy [7] [10]

The benefits from economies of scale are realized through the volume organics being diverted into anaerobic digestion, which ultimately lowers the cost per unit volume of waste.

1.1 Opportunities for Biogas Recovery

Pairing industrial wastes with municipal wastes creates a more integrated approach to waste management. This section will introduce sectors that represent valuable opportunities for anaerobic digestion.

1.1.1 Distillery Stillage

Global distilled spirits revenue reached $142.9 billion in January 2022 and is expected to grow at an annual rate of 2.2% until 2027 [11]. Distilleries generate 8-20 L of stillage for every liter of alcohol produced [12] [13]. Stillage is a high-strength liquid effluent that remains after the distillation process. It is often identified by its acidic nature, high nutrient content, dark brown colour, and high values for chemical oxygen demand (COD) and biochemical oxygen demand (BOD) [14]. If discharged into water bodies, the coloured characteristic of stillage can obstruct sunlight availability - putting the aquatic life and their habitat at risk due to decreased photosynthetic activity and reduced oxygenation of the water [14] [15]. Further, the high organic content (COD) of stillage can deplete the dissolved oxygen content, causing eutrophication and death of the ecosystem [13] [14] [15]. Land-usage application of stillage is also restricted due to its high nutrient content and high solids content, which can be fatal to crops in large volumes and can block oxygen uptake through the soil, respectively [14].

Thus, environmental discharge of stillage is considered hazardous and must be treated before disposal or re-use. There are several available methods outlined in the literature to appropriately manage and treat stillage waste [13] [14] [15]. These include physical, chemical, physiochemical, and biological treatment. All methods are subject to restrictions such as treatment efficiency, cost, climate, land use, regulatory constraints, and public perception [14]. Many distilleries employ a physical or mechanical separation method to treat stillage. The stillage can be separated into various constituents and undergo an evaporation and drying process

to generate a product that can add value back to the distillery. There is an added benefit of treating the different fractions separately – which could result in added profit with flexibility.

In a typical distillery, the whole stillage from the distillation process is centrifuged into a solid by-product called ‘wet cake’ (or Wet Distillers Grains) and a liquid by-product called ‘thin stillage’. Thin stillage is evaporated into syrup and re-combined with wet cake. The bulk product is sent to the dryer to produce ‘Dried Distillers Grain with Solubles’ (DDGS). Ethanol by-products, such as DDGS are usually sold as animal feed, due to its high protein and digestible fiber content [16][17].

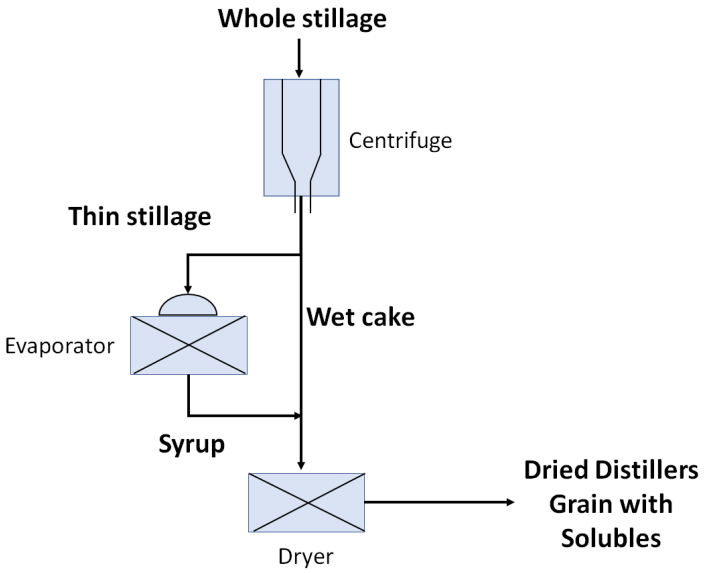

Figure 1.1 Processing of stillage in a typical distillery. Adapted from [18].

Although DDGS production generates a significant revenue for distilleries, there are many reasons to pursue alternatives for stillage processing. For instance, DDGS production is costly and energy intensive. One-third of the thermal energy demand of a bioethanol facility can be attributed to processing stillage via separation and drying [18]. With increasing natural gas prices, it is in the interest of distilleries to reduce their carbon footprint to cut costs [19]. Many distilleries have attempted to combat this, by shipping Wet Distillers Grains instead. However, this may further complicate matters, as the increased moisture content makes the feed more susceptible to spoilage and decreased revenue [17].

The rise of biofuels has led to an increase in ethanol production and consequentially an increase in ethanol co-products. The excess supply of DDGS influences profitability. Higher production volumes of ethanol co-products can drive down the market value of the product [17]. Hidden costs may arise to address infrastructure issues. For instance, it may be necessary to have increased transportation or longer storage periods of products to compensate for excess supply.

Anaerobic digestion of stillage fractions is a viable alternative. As mentioned earlier, stillage is rich in organic content (COD). Through biological conversion, anaerobic digestion of stillage can achieve COD removal rates of nearly 90% and generate biogas as a valuable product [13] [14] [20]. The resulting biogas can be upgraded to natural gas purity and injected into the grid or can be used to offset carbon dioxide emissions onsite in a combined heat and power unit [15]. Additionally, the high nutrient content present in stillage fractions is retained throughout the anaerobic process as semi-digested solids or 'digestate'. Additional profit could be sourced from the application of digestate as fertilizer and promotes a more circular economy [13] [15]. Moreover, it is estimated that at least 5-15% of the global ethanol energy consumption could be supplied by bioenergy recovery from stillage – regardless of feedstock origin [20].

1.1.2 Source Separated Organics

Source Separated Organics (SSO) refers to the organic fraction of municipal solid waste and consists of food waste, leaf and yard waste, soiled paper products, and wood waste [21] [22]. SSOs constitute nearly 40% of the residential waste stream in Canada [21]. Recently, there has been an interest in diverting organics from the landfill as their disposal results in the release of harmful greenhouse gas emissions, increased leachate contamination, and strain on landfill space [4] [22].

To move towards a circular economy, the Government of Ontario introduced Bill 151, the Waste-Free Ontario Act in 2016 [23]. This legislation is comprised of the Resource Recovery and Circular Economy Act (RRCEA) and the Waste Diversion Transition Act (WDTA). The RRCEA outlines provincial motivation to invest in waste reduction strategies and value-added activities and requires municipalities to follow suit [4].

SSO diversion programs are a natural stride in the progression to a more circular economy. Ontarians generate a significant amount of food and other organic waste – and much of it is

diverted to the landfill [3]. So, there is potential to re-integrate this waste back into the economy through value-added activities to recover energy and nutrients. As a result, Ontario released the Food and Organic Waste Policy Statement outlining SSO diversion targets for municipalities in 2018 [3].

1.1.3 Raw Sludge

Raw sludge is an amalgamation of humic and mineral matters from wastewater treatment operations [24]. In the form of a slurry, it contains organic carbon compounds, and nutrients, such as nitrogen and phosphorus, heavy metals, inorganic compounds, and pathogens [25]. Sludge processing is a cumbersome and costly initiative that cannot be avoided, as improper disposal can lead to groundwater and crop contamination [26]. Drinking water and wastewater treatment are integral to public and environmental health; therefore, sludge management is a necessary part of treatment.

There are several sludge management and disposal methods available, including incineration, landfill disposal and stabilization [26]. Incineration of sludge was used in the past; however due to concerns with greenhouse gas emissions, this strategy is heavily regulated. Sludge may be disposed of in landfills however, it is restricted by available capacity, regulatory compliance, and public perception [26]. Stabilization of sludge can be achieved through composting or anaerobic digestion. Composting is an attractive method as it provides a pathway to land application of wastewater biosolids by taking advantage of the organic matter and nutrient content in sludge [27]. For wastewater sludge to qualify as fertilizer, it must meet regulatory restrictions. Issues plaguing composting initiatives include cost, odour production, presence of pathogens, and inconsistency in fertilizer quality [26] [27]. Public perception of the usage of the biosolids as fertilizer is also a factor [26].

The most common sludge processing method is anaerobic digestion [26]. The benefit of anaerobic digestion is two-fold – energy recovery from the production of biogas and nutrient recovery in the form of a semi-solid effluent known as digestate. The biogas produced from the process can be recycled back into the treatment process to offset greenhouse gas emissions. More recently, raw sludge has been utilized as an effective co-substrate in the process of ‘co-digestion’ (the digestion of more than one substrate). When digested, raw sludge can supply the necessary

amount of nutrients for digestion, maintain a stable pH level, and supply a diverse microbial community [28] [29].

1.2 Enhancing Energy Recovery

Recent literature surrounding AD is focused on enhancing methane yield. Two methods have been popularized amongst researchers: (1) Co-digestion and (2) Pre-treatment of waste.

1.2.1 Co-digestion

Co-digestion refers to the anaerobic digestion of multiple feedstocks to exploit their unique characteristics to improve digestion. Esposito et al. [30] suggests that co-digestion can provide the following benefits:

- Dilution of toxic constituents
- Balance out excess or supply additional nutrient content
- Provide natural buffering capacity
- Increase organic loading to feedstock mixture
- Diversify microbial community in feedstock mixture to improve organics degradation

1.2.2 Pre-treatment

Pretreating the feedstock can increase the rate of solubilization (or hydrolysis) of large compounds [30]. This can help improve the kinetics of the AD process and potentially result in higher biogas volume produced. Pre-treatment can help make biogas production from feedstock that are recalcitrant to hydrolysis (lignocelluloses) more economically feasible [31]. For pre-treatment strategies to be effective, they should have low initial and operating costs [32]. Preparation and handling steps should also be minimized. There are many different pre-treatment methods, including physical, thermal, biological, chemical, or combinations of multiple pre-treatments.

1.3 Motivation for Research

As mentioned earlier, due to issues with the landfilling of SSOs, the Government of Ontario has mandated diversion targets for municipalities. From the municipality's perspective, AD of SSOs can give value back to the wasted organics in the form of added income from bioenergy recovery. However, due to high capital and operating costs, it may not be feasible to pursue

alone. This may mean that municipalities will need to find other organic waste providers to make AD more practical.

Distilleries demonstrate significant potential for waste-to-energy opportunities. As mentioned earlier, distilleries divert stillage waste to animal feed production. However, rising natural gas prices, fluctuations in the co-product market, and infrastructure costs are reasons to consider alternatives.

Cooperation between the two parties can provide mutual benefits and result in a more comprehensive waste management solution. Partnerships can be manifested as a municipally owned central digester, where distilleries can offer stillage through a waste collection service.

To be able to pursue such a partnership, a preliminary assessment of the biomethane potential of the substrates is required. Moreover, techniques used to enhance the biomethane potential can be evaluated at the lab scale to generate projections of how commercial scale AD may behave.

1.4 Thesis Objectives

This thesis will focus on enhancing the biomethane recovery from distillery stillage. There will be an emphasis on improving the feasibility of anaerobic digestion by bridging the gap between the municipal and industrial sectors. The specific objectives are to:

1. Study the effect of mixing ratios on batch co-digestion of wet cake, source-separated organics, and municipal sludges.
2. Study the effect of microwave pre-treatment on batch anaerobic digestion of wet cake.

Chapter 2 is a broad literature review of the anaerobic process and microbiology. Factors affecting AD are examined. Co-digestion and feedstock pre-treatment as techniques to enhance biomethane recovery are considered. The importance of substrate compatibility for co-digestion is discussed. Various pre-treatments and methods to determine their effectiveness are reviewed.

Chapter 3 explores the effect of mixing ratios on the anaerobic digestion of wet cake stillage from a local distillery with source-separated organics. The anaerobic biodegradability was measured in a batch biomethane potential study. One assay included raw sludge to extend the discussion of centralized AD. The results were scanned for potential synergism exhibited by

mixing substrates. Improvements in the degradation kinetics using the Modified-Gompertz model were explored.

Chapter 4 examines the effect of microwave pre-treatment of wet cake stillage from a local distillery on solubilization and anaerobic biodegradability. Solubilization of wet cake was determined by tracking the organic content before and after treatment (as chemical oxygen demand and solids concentration). The anaerobic biodegradability was measured in a batch biomethane potential study. Improvements in the degradation kinetics using the Modified-Gompertz model were explored.

Finally, Chapter 5 determines the engineering significance of this research and summarizes future recommendations based on the findings of this thesis.

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CHAPTER 2
LITERATURE REVIEW

Energy recovery from the anaerobic digestion (AD) of novel feedstock has been of interest since the late 1800s, when biogas produced from the treatment of sewage sludge was used to light streetlamps in England [1]. Since then, there have been considerable developments in the field of AD, such as multiple reactor configurations, co-digestion studies, pre-treatment of feedstock, bio-augmentation of microbial community [1] [2] [3].

Now, the depletion of fossil fuels, resource recovery, and waste management have become the forefront of environmental issues. There is a large portion of research in AD dedicated to biomethane enhancement strategies [4] [5] [6] [7] [8] [9]. Optimizing biomethane production from a substrate can help reduce overall costs and improve the economics of implementing commercial AD facilities. Making sufficient use of a feedstock is critical for enhanced bioenergy recovery.

2.1 Anaerobic Process and Microbiology

Anaerobic digestion is a complex, biochemical process, carried out by microbial consortia to produce an energy-rich product known as biogas. The anaerobic degradation pathway consists of four distinct processes: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis.

Complex organics are broken down into simple monomers, which are degraded to form short-chain (or volatile) fatty acids, carbon dioxide, and hydrogen. Some acids are further broken down to form acetate, carbon dioxide, and hydrogen. These end-products are used in the final stage, methanogenesis, to form methane [10]. Thus, AD is often described as a series of syntrophic interactions – meaning that the products generated in earlier stages are consumed in subsequent stages.

Microorganisms that carry out the hydrolysis, acidogenesis, and acetogenesis stages may consist of facultative or obligate anaerobes [10]. Facultative anaerobes are capable of metabolizing in aerobic or anaerobic environments, whereas obligate anaerobes will be killed in the presence of oxygen. The archaea that carry out methanogenesis are strict obligate anaerobes [10].

Methanogens found in anaerobic digesters have also been found in the stomachs of ruminant animals and humans and in organic sediment from water bodies [10].

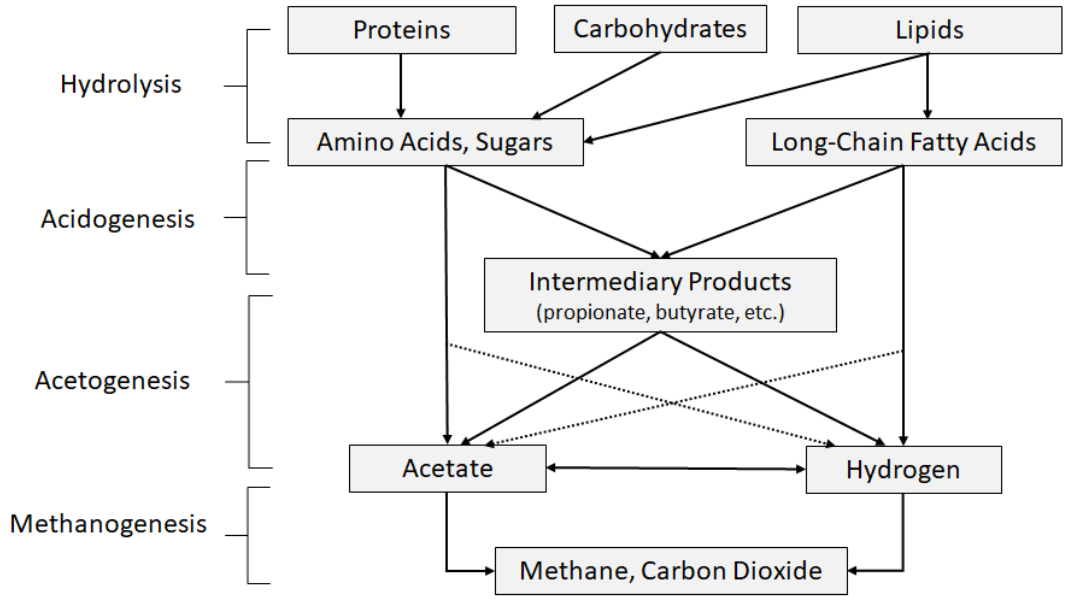

Figure 2.1 Four-step anaerobic digestion process. Adapted from [11]

2.1.1 Hydrolysis

In hydrolysis, complex organic compounds (such as lipids, polysaccharides, proteins, nucleic acids, etc.) are converted into soluble organic polymers, which are subsequently degraded into simple monomers or dimers [10] [11] [12] [13] [14]. Hydrolytic bacteria are unable to use complex organic compounds directly, due to the size and form of the compounds [11] [13] [14] [15]. Thus, they must secrete extracellular enzymes, which can penetrate and break down the larger molecules, making them accessible for microbial consumption and energy utilization [15] [13] [14] [16]. Under anaerobic conditions, hydrolysis is considered the rate-limiting step of bioconversion [14]. The rate of hydrolysis relies on the free surface area of the particles – as the soluble organic compounds produced from hydrolysis are further broken down into precursors for biogas production [17] [18].

2.1.2 Acidogenesis

In acidogenesis, the simple monomers produced from the hydrolysis step are either fermented or oxidized to form a collection of volatile fatty acids, (e.g., acetic acid, formic acid, propionic acid, butyric acid, valeric acid, isovaleric acid, caproic acid, etc.) alcohols, carbon dioxide, hydrogen,

and ammonia [10] [11] [13] [15] [17] [19]. Some amount of biomass is also produced in this stage [11] [17]. It is important to point out that the degradation of fatty acids into hydrogen is in dependent on the partial pressures of hydrogen [11] [13]. Thus, high levels of hydrogen may result in the inhibition of this process [11].

2.1.3 Acetogenesis

In acetogenesis, volatile fatty acids (except for acetate) are converted to acetate and hydrogen, and carbon dioxide [13][15] [17]. Intermediate acids such as propionate acid and butyric acid play a key role in the conversion to acetate.

The function of this reaction can be explained using the concept of Gibbs free energy ($\Delta G^{o\prime}$) exchange. The successful oxidation of substrates such as propionate, butyrate, and ethanol requires thermodynamically favourable conditions (i.e., a negative $\Delta G^{o\prime}$ value) to generate a positive bacterial energy yield [15] [17]. The value of $\Delta G^{o\prime}$ associated with the oxidation of alcohols and fatty acids is influenced by the partial pressure of the hydrogen [11] [13] [15]. If AD is stable, low concentrations of hydrogen (between 10^{-4} atm and 10^{-6} atm) are maintained due to the rapid consumption of hydrogen by 'hydrogen-consuming' bacteria [10] [14] [17]. Thus, the $\Delta G^{o\prime}$ associated with this reaction will be negative and the reaction will be exergonic [15] [17].

If the microorganisms are unable to consume hydrogen, then the degradation of fatty acids is slowed down, resulting in accumulation of intermediary products such as propionate and butyrate, and in some cases, lactate, and alcohols [10] [11] [17] [19]. Formation and accumulation of acids results in a pH drop which can result in AD process instability [10]. In this case, the oxidation will not occur as the conditions are thermodynamically unfavourable – resulting in a positive $\Delta G^{o\prime}$ and negative energetic bacterial yield [15] [17]. For this reason, it is crucial that the concentrations of reaction products, such as acetate and hydrogen, be consumed by methanogens [15]. Reactor failure is commonly caused by an imbalance between the acid-formers and methane-producing bacterial groups [20].

The syntrophic relationship between the production and rapid usage of hydrogen is known as inter-species hydrogen transfer [10] [15] [17] [21].

2.1.4 Methanogenesis

In methanogenesis, there are two main pathways to producing methane. Around 65-72% of methane formation is driven by the decarboxylation of acetate into methane [10] [11] [14] [19]. Methane is also formed via hydrogenotrophic (hydrogen-utilizing) methanogens. This process occurs via the reduction of carbon dioxide with hydrogen as the electron doner [17].

2.2 Environmental and Operational Parameters

Microbial population dynamics and the anaerobic process are impacted by a whole host of environmental conditions and operational parameters, such as temperature, pH, alkalinity, nutrient requirements, substrate composition, and mixing [10] [19] [20] [22] [23].

2.2.1 Temperature

Microbial kinetics and process stability are highly influenced by temperature-controlled environments [10] [23] [24] [25]. Optimal bacterial growth may occur in specific temperature regimes, such as the mesophilic range (25-40ºC), thermophilic range (55-65ºC), or psychrophilic range (12-18ºC) [10]. Anaerobic digestion studies within the psychrophilic temperature range are seldom pursued as microbial activity is low [26]. Mesophilic bacteria can tolerate temperature fluctuations of around 2-3ºC, whereas thermophilic bacteria are more sensitive and can only withstand a change of less than 1ºC [20].

Most anaerobic digesters operate in the mesophilic temperature range as it is generally more stable [22] [24] [27]. However, research has shown that thermophilic digestion offers advantages such as enhanced biochemical reaction rates, increased organic destruction efficiency, and increased biomethane yield [22] [25] [28] [29]. A major challenge in pursuing thermophilic AD is process instability [22] [25] [28] [29]. The enhanced hydrolysis of complex organics results increased concentrations of VFAs, which depress pH and hence cause inhibition [30]. The added heating cost is also considered as a negative aspect of thermophilic AD [22] [25] [28] [29]. For thermophilic AD to be more economical, the benefit of increased organics destruction and increased methane yields must offset the energy cost [22].

2.2.2 pH and Alkalinity

The anaerobic process can operate in a pH range of 6.5 – 7.6 [31]. Deviation from this range may result in decreased organic conversion efficiency and could result in inhibition [10] [31]. The

methanogenic bacteria are highly sensitive to changes in environment and function best at a narrower range of 7.0-7.2 [31]. Since methanogenic growth is slow, it is important that anaerobic treatment be modeled around the optimum environmental conditions for methanogens, so that more rapid and effective treatment can be achieved [13] [31].

Also termed ‘buffering capacity’ - alkalinity is described as the ability to control the pH of a microbial environment after the addition of acids [10] [31]. Since optimal AD occurs at pH ranges near neutral, sufficient buffering capacity must be present to stabilize the pH, particularly during the production of volatile fatty acids [13] [22] [31]. In AD, buffering capacity exists as bicarbonate alkalinity system [22] [31]. Alkalinity can also be supplied by an external buffer or can be naturally produced by the degradation of proteins and amino acids [10]. The addition of alkalinity is significant to process stability. However it should be approached cautiously, as excess supplementation may lead to ammonia-nitrogen inhibition due to the elevated pH level [22].

2.2.3 Volatile Fatty Acids

As mentioned earlier, the anaerobic process relies on a series of syntrophic relationships - particularly the relationship between acid-formers and methane-producing bacteria. In a healthy digester, the acids are immediately consumed by the methanogens. As a result of unbalanced digestion, the acids cannot be degraded, which leads to an accumulation of intermediary products. The acids consume the alkalinity and consequentially, the pH level drops, thus leading to a higher VFA concentration [17] [19]. This is known as ‘digester souring’. Some researchers have proposed that it is the unionized form of VFAs that are the perpetrators of inhibition [17] [32]. Their ability to easily pass through the cell membrane and dissociate results in the depression of pH and thus disturbs the homeostasis condition [32].

Severe inhibition of the anaerobic process is difficult to reverse. pH, alkalinity, and VFA concentration can be good indicators of process instability [33]. Typically, acids accumulation is common in overloaded digesters. There have been reports of various inhibitory concentrations of VFAs in literature, however concentration can vary on substrate composition, organic loading rate, and acclimatization. Analysis of individual VFA concentrations can provide valuable information on the anaerobic process and what may be causing accumulation or inhibition [19] [33].

2.2.4 Nutrients

Nutrients play an important role in the growth and function of organisms [10]. However, their concentrations should be monitored as they can have limiting effects on the degradation of organic wastes, if available in excess. The required ratio between organic content and nutrients for anaerobic processes is 250:5:1 as COD:N:P, where COD is the chemical oxygen demand, N is the total nitrogen content, and P represents phosphorus [34].

2.2.4.1 Nitrogen

Nitrogen exists in various charge states and can be converted into different forms via bacterial activity. As mentioned earlier, nutrients are necessary for microbial growth and function. Ammonia concentrations up to 200 mg/L are considered essential [35]. However, the presence of certain forms of nitrogen can lead to inhibition, therefore nitrogen tracking throughout AD is important [10] [35]. Metcalf & Eddy et al. [10] report the total nitrogen (TN) as the summation of organic nitrogen (Organic N), ammoniacal nitrogen, nitrite (NO_2^-), and nitrate (NO_3^-).

The degradation of proteins and urea can lead to the production of ammoniacal nitrogen, which can exist as ammonium ion (or ionized ammonia $NH4^+$) or as free ammonia (FA) (unionized ammonia, NH_3) [35]. The species of ammonia present in a solution is dependent on the pH of the solution. At pH levels above neutral, ammonia exists mainly as FA, whereas at pH levels below neutral, $NH4^+$ is dominant [10]. Higher operating temperatures can also lead to increased FA levels due to increased hydrolysis of organic compounds [35].

Elevated FA levels are the main cause of ammonia-nitrogen inhibition. The unionized species of ammonia can freely pass through a membrane and cause proton imbalance and potassium deficiency [35] [36]. It has been said that higher total ammoniacal nitrogen levels (TAN) may slow hydrogen consumption and result in VFA accumulation [35] [36]. When the pH level drops, the ionization equilibrium shifts towards ammonium. This interaction has been seen in literature and is referred to as an ‘inhibited steady state’ – the process is stable but produces less methane gas [36].

Organic nitrogen may be stored in feedstock as amino acids, proteins, amino sugar, or urea and can also be converted to ammoniacal nitrogen through microbial activity [10] [37]. This further

increases the TAN concentration during anaerobic degradation. At alkaline pH levels, TAN levels could shift towards higher FA concentrations and potentially inhibit the anaerobic process.

There have been many inhibitory concentrations of ammonia reported in literature. The inhibitory FA level that causes 50% reduction in methane production ranges from 1.7 to 14 g/L [35]. It is important to note that limiting concentrations be reported in the context of substrates and inoculum used, digester conditions, pH level and acclimation periods [35].

2.2.4.2 Phosphorus

Phosphorus is important for the conversion of organic matter to biogas. Orthophosphates (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4) are already bioavailable without requiring further reduction [10]. High concentrations of orthophosphates, ionized ammonium, and magnesium ions can lead to struvite formation – although this more common in larger-scale reactors [24]. Excess phosphorus can be removed via chemical precipitation.

2.2.4.3 Sulfur

Sulfur is produced when proteinaceous matter is degraded. Under anaerobic conditions, sulfate is converted to sulfide via sulfate-reducing bacteria (SRB) [10] [35]. SRBs compete with acetogens and methanogens for hydrogen and volatile fatty acids [35]. The result is the formation of hydrogen sulfide - which can corrode concrete sewer piping and compromise their structural integrity [10] [35]. Although toxic levels are rare (> 200 mg/L), sulfate levels should be monitored carefully [10]. Iron can be added to digesters to precipitate the sulfide concentrations [10].

2.2.5 Substrate Composition

Substrate composition can provide valuable insight to the theoretical methane yield produced when anaerobically degraded. It can also supply information on potential inhibitory effects that may be observed. Biomass consists of many organic compounds, such as carbohydrates, lipids, and proteins. The reported maximum gas yields and methane content for common organic compounds are shown in Table 2.1.

Table 2.1 Maximum theoretical biogas yield and corresponding methane content for common organic compounds. Adapted from [38]**.**

Substrate	Biogas (Nm^3/t TS)	CH_4 (%)
Carbohydrates	790-800	50
Raw protein	700	70-71
Raw fat	1200-1250	67-68
Lignin	0	0

Carbohydrates are the preferred substrate for methanogens because they are highly biodegradable. When digested, they convert to sugars, which are broken down to form VFAs [6]. Issues may arise during the AD of carbohydrate-rich waste if the acidification rate is faster than the methanogenic process rate, as it may lead to VFA accumulation and possible inhibition [5] [6]. Examples of such wastes include source-separated organics and food wastes [5].

Lipids can produce high methane yields due to the presence of a high number of carbon and hydrogen atoms in their molecule [5]. However, lipids usually require long retention times, and may result in clogging or washout due to adsorption onto the surface of biomass [5] [38] [39]. Examples of lipid-rich wastes may include fat, oil, and grease (FOG) waste, and slaughterhouse or dairy wastewaters [5] [39].

Proteins are generally characterized by high BOD levels and higher levels of nitrogen [5]. This may pose an issue during AD since conversion of nitrogenous matter may lead to elevated levels of ammonia content [5]. Examples of nitrogen-rich wastes include animal manures and distillery stillage [5] [40] [41].

Lignocelluloses are made up of cellulose, hemicellulose, lignin, and other inorganic compounds [9]. The structure and content of lignocelluloses make them unresponsive to biological or chemical treatment [9] [42]. Lignocellulosic biomass includes wood, grass, and agricultural and forest residues [42]. Pre-treatments are usually done on lignocellulosic biomass to improve degradation and biogas yield [43].

2.2.6 Mixing

Mixing intensity is another factor that impact digester efficiency. Common mixing patterns in AD are as follows: continuous mixing, intermittent mixing, or no mixing [23] [44]. Effective mixing ensures a homogenous distribution of substrates, nutrients, and alkalinity [22]. Good mixing will have contact between substrates and bacteria for degradation and releases gas

bubbles entrapped in biomass or sludge [22] [23]. Since the reactor environment must be temperature-controlled, mixing provides an even temperature distribution and prevents stratification [22] [23].

Contrarily, vigorous mixing may also cause negative effects in AD, such as significant shear stress which can destroy flock formations and can disrupt adhesion between cells and bacteria – thus resulting in reduced gas production [44]. Ineffective mixing may also lead to lower organics destruction efficiency [23]. In a batch study, the mixing patterns should emulate those of a full-scale reactor [23].

2.3 Measuring Biodegradability

The biodegradability or Biochemical Methane Potential (BMP) of a substrate is the ultimate methane volume produced if the substrate is completely degraded. The BMP of a substrate can be expressed as mL CH_4/g VS_{added} or mL CH_4/g COD_{added} at standard temperature and pressure conditions [23]. As explained by Filer et al. [23], the BMP of a substrate can be used to predict the bioenergy recovery from commercial anaerobic digesters, determine size of AD reactors, and potential avenues for energy enhancement. Furthermore, kinetic data can be extracted from BMP data to predict process performance [23].

Batch experimental assays are commonly used to determine the BMP of a substrate due to their simplicity and low cost [45]. Theoretical methods to determine the biodegradability of a substrate have been used in the past but rely on access to accurate and comprehensive data on substrate composition and fractions of soluble matter [45].

2.3.1 Bio Methane Potential Test

The BMP test was originally developed by Owen et al. [46]. The BMP study is a batch experimental procedure used to measure the biodegradability of a substrate under anaerobic conditions [23] [46]. In replicates, bottle reactors containing inoculum and substrate are flushed with nitrogen gas to achieve anaerobic conditions. Then, the bottles are incubated in a bio-shaker to ensure continuous mixing and stable incubation temperature. The degradation of the substrate via microbial activity produces gas in the headspace of the bottle. Biogas volume and percentage methane content are measured daily over the incubation period. Controls and blanks are used to

ensure accurate results and account for the endogenous methane production produced by the inoculum.

There is no single protocol for the BMP assay. Several standards exist such as DIN 38414 TL8 (1985), ASTM D 5210 (1992), ASTM D 5511 (1994), ISO 11734 (1995), ISO 14853 (1998), and ISO 15985 (2004) [47]. However, these standards still leave room for interpretation which results in different BMP setups .There also exists several methods for BMP studies. As summarized by Pham et al. [48], the German standard procedure, Verein Deutscher Ingenieure (VDI) 4630, the Møller method, and the Hansen method are popular [48] [49] [50] [51]. These methods differ in selected operational conditions (i.e., incubation temperature, inoculum temperature, etc.) [45]. Pham et al. (2013), showed the differences in the BMP of pig manure, cow manure, and cellulose using the aforementioned methods were not statistically significant [48].

Not only can BMP studies determine the anaerobic biodegradability of a substrate, but they can also reveal degradation kinetics and potential inhibition [52]. The main drawbacks to the conventional BMP test are laborious and resource and time-consuming. A typical BMP test lasts around 30 – 100 days [45].

2.3.2 Automatic Methane Potential Test System

To address the shortcomings of the conventional BMP test, Bioprocess Control Sweden (BPC) Company developed the Automatic Methane Potential Test System (AMPTS). The AMPTS unit follows the same principle as the BMP test – the bottle reactors are submerged in a water bath to maintain incubation temperature. However, when biogas is produced, the unit strips the carbon dioxide and other contaminants from the gas to directly measure the methane volume. The methane production is measured using liquid displacement and buoyancy method [45]. Using an embedded data acquisition system, the AMPTS unit logs the biomethane volume and flow rate in real-time whilst normalizing the data for pressure and temperature differences [53]. It also corrects for overestimation of gas volume due to flush gas [53]. The capacity for a single AMPTS unit is 15 bottle reactors.

2.4 Kinetics

Methane production in a batch reactor follow an exponential growth pattern. A typical biomethane production profile is shown in Figure 2.2. Deviation from this profile may indicate potential instability or inhibition.

Figure 2.2 Typical net cumulative methane curve plotted against time.

Methane production profiles can be modeled via empirical kinetic models. There are many models available – enzymatic (Monod, Michaelis-Menten, etc.), chemical (i.e., first-order model, variable time dependency, etc.), statistical distribution (Weilbull, Cauchy, Gaussian), and microbial growth model (i.e., Gompertz, Logistic, etc.) [54]. Currently, there is no standard model that can describe bacterial growth patterns [23] [54]. Despite this, mathematical models can still provide valuable insight into the growth and degradation kinetics of feedstock, inoculum adaptation periods, estimation of ultimate biomethane yield, and reactor scale-up potential [23] [54].

The most popular model used is the exponential (or first-order) model, due to its simplicity. The first-order model is founded on the principle that substrate utilization is directly proportional to biomass growth . However, the first-order model is limiting – as cell growth rate is not constant over time. The Monod model attempts to remedy this and introduces a ‘maximum cell growth

rate' which is present during the beginning of organics degradation. The growth rate then decreases over time when the substrate is consumed.

The Monod equation has been a well-received modification to the first-order model. In fact, Monod models are used on the simulation platform: Anaerobic Digestion Model No.1, developed by the International Water Association [55]. However, it still has some limitations. Model accuracy is dependent on knowing the substrate concentration and biomass concentration and may require iterative methods [55]. According to Jeyakumar et al. [56], the variation in biomass concentration as the substrate is consumed is not defined very well using the Monod equation. This can be critical if higher retention times are to be used in real-world applications [56].

The Gompertz model has been widely used to illustrate the growth of animals and plants, and the volume of bacteria and tumor cells [57]. The Gompertz model also employs Monod's adjustment for a variable cell growth rate, but also theorizes that there is no maximum cell growth rate. Zwietering et al. provide a modification (known as the Modified-Gompertz model) to express the Gompertz equation with more biologically relevant parameters (lag phase, growth rate, maximum biogas production rate) to make the model easier to use [55] [58]. In any case, the Modified-Gompertz model is attractive for anaerobic digestion researchers as it only requires accumulated biogas data to determine kinetic constants and goodness of fit. For this reason, the Modified-Gompertz model will be used in this thesis [55].

2.5 Co-digestion

Co-digestion is when two or more substrates are anaerobically digested together with the goal of increasing methane production [4]. Co-digestion is used to dilute toxic compounds, balance nutrients, and increase the organic load [4] [5] [59]. Other advantages include the stabilization of pH, supplementation of natural alkalinity, and diversification of the microbial community [4] [5]. Co-digestion also generates economic savings due to shared equipment between wastes, easier handling, and economies of scale [4] [59] [60].

The ratio of carbon to nitrogen (C/N ratio) present in the feedstock is an important metric when considering co-digestion. The optimum C/N ratio for stable anaerobic digestion reported in literature is 20/1 – 30/1 [5]. Although, successful AD has been achieved outside this range. Elbeshbishy and Nakhla reported successful batch studies on powdered starch and lyophilized

powdered bovine serum albumin at a C/N ratio of 12.8 [6]. Mshandete et al. concluded that a C/N ratio of 16/1 was suitable for batch co-digestion of sisal pulp and fish waste [61]. Itodo and Awulu reported success at C/N ratios of 6/1 and 9/1 for the co-digestion of poultry, cattle, and piggery wastes [62]. If poorly degradable compounds such as lignin are considered, lower C/N ratios can be tolerated [61].

An unbalanced C/N ratio can lead to digestion issues. For instance, a high C/N ratio may have a nutrient deficiency. Feedstock with low C/N ratios are prone to free ammonia inhibition due to the presence of nitrogenous compounds. Wastes rich in nitrogen can also supply buffering capacity. Ammonium ion combined with carbon dioxide and water forms ammonium bicarbonate (a natural buffer) [63]. Pairing carbohydrate-rich wastes and proteinaceous wastes for anaerobic digestion is a common approach. Due to their biodegradability, carbohydrates can quickly be converted into VFAs [5]. Mono-digestion of carbohydrates may be problematic due to possible VFA accumulation and subsequent pH drop. Many researchers have turned toward proteinaceous wastes to provide alkalinity to ensure stable AD.

Successful co-digestion may result in synergistic observations – meaning that the methane yield achieved from a mixture is greater than the methane yield achieved from the mixture as calculated from the mono-digestion of the feedstock. Alternatively, co-digestion could result in antagonistic effects, shown by decreased biomethane yield and presence of inhibitory compounds.

Enhancing bioenergy recovery from co-digestion requires proper selection of a co-substrate. Exploiting complementary characteristics of substrates is an effective way to make use of available waste and enhance the yield.

2.6 Pre-treatment

Lignocellulosic materials account for nearly 50% of the available biomass globally – translating to an estimated 350 million tonnes per year [64]. Abundance and low cost establish reasoning to consider lignocelluloses as viable feedstock for energy recovery [8] [64] [65].

Unfortunately, conversion of lignocelluloses to bioenergy is difficult, as the structure of these materials render them recalcitrant to microbial and enzymatic degradation [66]. To access the untapped potential presented in lignocelluloses, pre-treatment is necessary to be able to

hydrolyze these compounds. Pre-treatments can change the physiochemical, structural, and compositional properties of lignocelluloses to make them more amenable to hydrolysis, and therefore anaerobic digestion [66].

Lignocellulosic biomass generally consists of three polymers: (1) cellulose, (2) hemicellulose, and (3) lignin [8] [65]. The goal of pre-treatment is to disrupt the cellulose structure and destroy the hemicellulose and lignin components in the lignocellulosic feedstock [65]. The pre-treatment should allow for microbes to convert the polymers into easily digestible substrate, whilst avoiding the production of inhibitory compounds [8] [65]. Although, there is some research that suggests some inhibitory compounds may be converted to methane after adaption [67]. For the pre-treatment process to be feasible, it should be able to succeed the processing and operational costs [8].

2.6.1 Cellulose

Cellulose forms the foundation of plant cell walls by way of a linear polymer that consists of β-1,4 glycosidic bonds and repeated cellulose subunits, known as cellobiose [8] [65] [67] [68]. Cellobiose units form long chains which are organized in fibrous ligaments called microfibrils. The microfibrils are bundled together in parallel and stabilized through hydrogen and covalent bonds, and van der Waals forces [65] [66] [67] [68]. The hydrogen bonding determines the structure of cellulose with respect to the ‘straightness’ of the cellulose chains [8]. Cellulose usually forms a rigid, crystalline structure that has a half-life of 100 million years at a pH level of 7 [8] [65] [68]. Sometimes, cellulose can be found in an amorphous structure, where the cellulose chains are disorganized. This form of cellulose is more receptive to enzymatic degradation [65]. Forms of cellulose can be found in cotton, wood, and the cell walls of primitive microbes [66].

2.6.2 Hemicellulose

Hemicellulose contributes around 20-50% of lignocellulosic compounds [8] [68]. Hemicellulose is structured as branches of short polymers such as pentoses (i.e., xylose, arabinose), hexoses (i.e., mannose, glucose, and galactose), and sugar acids [8] [65] [67] [68]. Hemicellulose serves as the link between cellulose and lignin and adds rigidity to the entire lignocellulosic compounds [67].

A key property of hemicelluloses is that they are easily hydrolyzed due its configuration and presence of sugars [65] [67] [69]. Polymers in hemicellulose are agreeable to thermo-chemical treatment. Hendricks et al. reports the solubility of different polymers in descending order: mannose, xylose, glucose, arabinose, and galactose [67]. Hemicellulose compounds begin to solubilize at 150ºC – 180ºC under neutral conditions [67]. Other factors, such as moisture content or pH level may impact solubility as well. Hemicellulose structure can differ amongst biomass, so when considering pre-treatment options, the composition should be identified [8]. For instance, xylan is receptive to acidic or alkaline treatment, whereas glucomannan requires a stronger alkaline environment for destruction [8] [67]. So, care should be exhibited when selecting pre-treatments.

2.6.3 Lignin

Compared to the other polymers, lignin is the least responsive to biological, chemical, or enzymatic treatment [68] [69]. Lignin has a complex heterogenous structure, consisting of three phenylpropane units: p-coumaryl, coniferyl, and sinapyl alcohol [65] [66] [67]. Lignin supplies structural support through linkages between hemicellulose and cellulose – which also makes it hydrophobic and even more challenging to degrade [8] [67] [68]. Normally, softwoods contain a substantial amount of lignin [65] [69]. Like hemicellulose, lignin can solubilize in water at around 180ºC under neutral conditions [67]. Their receptivity to acid and alkaline pretreatments are dependent on their precursor [67].

2.7 Pre-treatment Methods

There are several different methods to enhance the hydrolysis of feedstock. Some methods include physical, chemical, thermal, biological, or other unconventional pre-treatments.

2.7.1 Physical

Physical pre-treatments refer to size reduction techniques, such as blending, chipping, shredding, grinding, or milling of feedstock [8]. The intent behind physical pre-treatments is to reduce the particle size of the feedstock to make the substrate more accessible to microorganisms [68]. In turn, the degree of polymerization is also reduced, thus making the feedstock easier to degrade [8] [67] [68]. In most cases, the rate of hydrolysis can increase by 5-25% depending on the biomass, technique, and duration of pre-treatment [67] [68]. In addition to this, the retention time may also decrease, thus resulting in more cost-savings due to the increased hydrolysis [67].

However, reduction below a 40-mesh particle size does not produce significant changes in biomethane yield or rate of hydrolysis [8] [67]. Physical pre-treatments do not produce phenolic inhibitors – thus making it attractive [67]. The cost of physical pre-treatments varies on waste characteristics and desired particle size [8] [67] [68].

Other physical pre-treatments such as irradiation and ultrasound have also been used, however these methods may be difficult to employ in industrial settings [8] [69].

2.7.2 Thermal

Thermal pre-treatments involve heating up lignocellulosic biomass to increase the rate of hydrolysis. Hemicellulose and lignin begin to dissolve in water at temperatures between 150ºC-180ºC [67]. Hemicelluloses are broken down into acids which further aid the hydrolysis of hemicellulose [67]. It is important to note that solubilization of lignin will typically produce phenolic compounds, which are toxic to methanogens at certain concentrations [67] [68]. Additionally, soluble lignin compounds can re-condense and precipitate on biomass, if not removed immediately. In acidic environments, the production of inhibitory compounds is even more pronounced [67].

2.7.3 Chemical

Adding of chemicals such as acids, alkali, organic solvents, and ionic liquids can also degrade lignocellulosic compounds [8].

Sulfuric acid, hydrochloric acid, nitric acid, and phosphoric acid have been applied to hydrolyze lignocellulosic biomass [8] [68] [69]. For methane production, sulfuric acid and nitric acid may not be preferable, since they can produce hydrogen sulfide and nitrogen gas, thereby reducing the overall energy recovery [67]. Generally, acidic pre-treatment can be performed at high or low temperatures with different acid concentrations [68]. However, pretreatment with acids at ambient temperatures can enhance anaerobic digestibility [67]. In acid pre-treatment, hemicelluloses, particularly xylan, solubilizes (glucomannan remains stable) [67]. The dissolved hemicelluloses can undergo further hydrolysis to produce monomers, furfural, hydrolymethalfurfural, and other volatile products, which could be converted to methane with adaptation [67]. At strong acid concentrations, lignin and hemicellulose both get hydrolyzed;

however diluted acids are normally preferred over concentrated ones. This is due to the corrosiveness and costly acid recovery treatment [8] [68] [69].

Alkaline pre-treatments are effective in destroying lignin and some parts of hemicellulose [67] [68]. Alkaline treatment causes swelling of the fibers, which results in a larger surface area and decreases crystallinity [68]. It can also reduce the degree of polymerization between lignin and carbohydrates [68]. At strong alkali concentrations, a peeling reaction may occur, which can result in the breakdown of polysaccharides and loss of carbon due to the formation of carbon dioxide [67]. Solutions typically used for alkaline pre-treatment can include sodium hydroxide, lime, potassium hydroxide, or ammonia [8] [69].

2.7.4 Biological

Biological pretreatments typically involve the addition of an enzyme-producing fungi or bacteria to aid in the breakdown of lignocelluloses [8]. The benefits of using biological pretreatments lies in its environmental impact and low financial input – as they do not require retrieval of any chemicals nor special instrumentation [70]. However, biological pretreatment requires a careful selection of parameters such as microbial consortium, pH, moisture content, incubation period, temperature, particle size and aeration [70]. Thus, it is important to fully characterize the biomass to apply biological pretreatment. Moreover, the rate of hydrolysis obtained from biological pretreatment can be very low [71].

2.7.5 Other Pretreatments

Some selected pretreatments were reviewed below. These pretreatments are those that have been popularized in literature for biomethane recovery.

2.7.5.1 Steam Explosion

Steam explosion is derived from physical pretreatment – it uses high-pressure saturated steam to disrupt the rigid structure of lignocellulosic biomass. The biomass is exposed to temperatures between 160-260ºC for a few seconds and then immediately brought to atmospheric pressure via explosive decompression [65] [66]. The rapid change in pressure causes fragmentation of the lignocellulosic structure and increases the available surface area for enzymes [65]. During the short incubation period, hemicelluloses are solubilized, and produce acetyl acids which further the hydrolysis [68]. The lignin is broken down due to the high temperature - in turn, this makes

the cellulose portion more digestible [65] [66]. Acids can be added to improve hydrolysis yield from steam explosion.

2.7.5.2 Liquid Hot Water

Liquid hot water (LHW) treatment is a more aggressive form of thermal treatment. It involves the application of pressurized high-temperature water. In the same manner as steam explosion, LHW treatment solubilizes hemicelluloses and removes lignin [65] [66]. The main drawback of LHW is its higher water consumption, however the production of inhibitors also decreases, due to dilution [67].

2.7.5.3 Ammonia Fibre Explosion

During ammonia fibre explosion (AFEX), the feedstock is submerged in a liquid ammonia solution at a moderate temperature and high pressure [65] [66] [67]. After the incubation period, the pressure is relieved and causes swelling of fibers in lignocelluloses, as with steam explosion and LHW [65] [66] [68]. The main drawback to AFEX is the cost of recovering the ammonia [68].

2.7.5.4 Microwave Irradiation

Microwaves (MW) are electromagnetic waves in the 300 MHz to 300 GHz frequency range [72] [73]. MW irradiation can break down cell walls, reduce the degree of polymerization and increase the accessible surface area for degradation [68]. MW pre-treatment operates via two mechanisms: (1) a thermal effect and, (2) an athermal effect to break down lignocellulosic compounds [74]. The heating mechanism occurs via molecular friction from dipole rotation and ionic conductance [75] [76] [77]. The athermal effect is caused by the alignment of the poles of the electromagnetic field with the polarized side chains of macromolecules. It is thought that this would lead to the breakage of weak hydrogen bonds [76] [77]. However, existence of an athermal effect has been difficult to prove [78]. Benefits to microwave pre-treatment as compared to conventional heating includes more rapid heat penetration, quicker heating and cool down time, and less energy consumption due to selective heating of sample [76]. However, microwave pre-treatment is still considered novel, and its applications are limited.

2.8 Measuring Pre-treatment Effectiveness

The effect of pre-treatment can be measured as particle size reduction, increased solubilization, production of inhibitory compounds, loss of organic material, and biogas yield [79].

Reducing particle size implies the increased free surface area of substrate, which apparently would lead to increased hydrolysis and enhanced biogas production [18] [27] [79]. However, pre-treatments centered on size reduction may be hindered by difficulties in quantifying particle shape, effect on inner surface area, and accounting for dissolved particles that have already been degraded [79].

Increased feedstock solubilization can be quantified by the measurement of soluble chemical oxygen demand (SCOD). The SCOD of a substrate represents the fraction of COD that is easily biodegradable and be quickly assimilated by biomass [10]. Comparisons between the initial COD fractions of the substrate prior to pre-treatment and the COD fractions of the substrate after pre-treatment (particularly the soluble portion) can give insight into the effectiveness of pre-treatment [79].

Inhibitory compounds may be produced after pre-treatment. This may lead to the formation of weak acids, furans, phenolic compounds, or production of melanoidins due to Maillard reaction between carbohydrate and protein-rich feedstock [79] [80]. These compounds may hinder the growth of microorganisms or decrease the biodegradability of the feedstock [79] [80]. Inadvertent destruction or loss of organic matter may also impact the bioenergy recovery available from a substrate [79].

The biogas yield is another parameter that can be used to indicate the effectiveness of pre-treatment in conjunction with substrate characterization [79]. After pre-treatment, the biodegradability of a feedstock may be enhanced or may have decreased, compared to the unpretreated feedstock. Substrate solubilization or production of refractory compounds can be checked along with the biogas yield to understand the effect of pre-treatment. The biodegradability of a substrate can be measured using a BMP test.

2.9 Conclusions

The anaerobic process and common methane enhancement techniques were reviewed in this chapter. After examining the literature, it is clear that:

1. The microbial community responsible for carrying out AD is highly sensitive to changes related to temperature, nutrient concentration, acids concentration, and pH and alkalinity. Substrates should be fully characterized to determine if inhibition could occur or if the anaerobic process stability could be compromised. Special focus should be placed on excess nutrient content, low alkalinity, and potential acids accumulation. Detailed understanding of substrate characteristics can result in potential synergies between wastes and balance the costs of anaerobic digestion.
2. Co-digestion can reduce the costs of AD by virtue of economies of scale. However, substrates should be compatible with one another to obtain benefits.
3. Selection of pre-treatment is based on feedstock and knowledge of the chemical composition can be helpful. Pre-treating lignocellulosic feedstock can unlock the greater methane potential of a substrate. However, the benefits of pre-treatments (increased solubilization of substrate and higher biomethane potential) should supersede the cost of itself.

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CHAPTER 3
CO-DIGESTION OF DISTILLERY STILLAGE AND MUNICIPAL WASTES

Source-separated organics (SSO) make up nearly 40% of the residential waste stream in Canada. Recent interest in diverting SSO from landfills arose from concerns of reduced landfill capacity, CO_2 emissions, and increased leachate volumes. Motivated by concepts of circular economy, Ontario released the Food and Organic Waste (FOW) Policy Statement, outlining SSO diversion targets for municipalities. Thus, a need to integrate SSO back into the economy through value-added activities has been established.

SSO are an attractive feedstock for anaerobic digestion (AD), due to their abundance in volume and their high carbon content – which is ideal for balancing nitrogen-rich feedstock. However, AD of SSO alone can still be a costly endeavour for small and medium-sized municipalities. Additional FOW sources within a municipality include the Industrial, Commercial, and Institutional (IC&I) sector and wastewater biosolids. Anaerobic co-digestion of multiple organic wastes has often been found to increase digester performance, dilute inhibitory compounds, and improve bioenergy recovery. Further, increased waste diversion can reduce AD costs and improve the feasibility of biomethane recovery due to economy of scale.

Amongst the IC&I sector, distilleries generate 8-20 L of organic-rich stillage for every liter of alcohol produced. Stillage is a high-strength liquid effluent that remains after the distillation process. Traditionally, stillage is centrifuged and dried to produce animal feed, generating a profit. However, processing this waste is costly and energy intensive. Diversion to AD can be an alternative to stillage processing, whilst simultaneously adding value back to the waste. However, stillage is known to have unfavourable characteristics, such as high nutrient concentration and low alkalinity, which can lead to reactor failure. Thus, it must be paired with a compatible co-substrate to achieve stable digestion.

The objective of this chapter was to determine the impact of co-digestion between distillery stillage and municipal wastes. Improvement in biomethane potential of stillage and SSO at varying ratios was evaluated in a batch study. An assay including co-digestion with wastewater biosolids was added to provide additional information about co-digestion of industrial and municipal wastes.

The scope of the study is outlined below:

1. Study the effect of mixing ratios on substrate composition
2. Investigate the effect of mixing ratios on anaerobic process stabilization
3. Investigate potential synergistic or antagonistic effects
4. Use a simple microbial growth model to fit experimental data to predict kinetic parameters

3.1 Literature Review

Mono-digestion studies on various stillage fractions have been covered extensively by many researchers. The methane yields for the anaerobic digestion of stillage fractions in batch reactors is consolidated in Table 3.1. Observing the data, the BMP from stillage can vary based on stillage fraction, source of stillage, and temperature of AD.

Previous literature has shown that the high nitrogen content in stillage may subject them to free ammonia nitrogen inhibition [5]. A study completed by Drosg et al. suggested the dilution of stillage fractions in water or nitrogen removal processes [1]. However, the addition of water would increase reactor volume, and nitrogen removal as a pre-treatment may be costly. Co-digestion as an alternative was not considered. Other studies have illustrated that mono-digestion of stillage showed varying gas production and process failure. Eskicioglu et al. treated corn whole stillage in a semi-continuous flow reactor [3]. The authors reported instability after increased levels of volatile fatty acids, free ammonia, and fluctuating alkalinity were observed. Westerholm et al. claimed that cereals whole stillage showed inhibition after alkalinity depletion and an increase VFA concentration [5].

Due to issues with mono-digestion of stillage, some studies have looked at co-digesting stillage with other substrates. A popular substrate is livestock manure, due to its ability to provide an array of nutrients and microorganisms, which can enhance process stability, and provide buffering capacity to stabilize the pH level during AD [4] [7]. The idea of co-locating an ethanol plant and anaerobic digester near a beef feedlot is an added economic benefit, as transportation can be a hindrance for waste initiatives.

Table 3.1 Compiled biomethane yields of various stillage fractions from selected literature.

Source	Stillage Fraction	Temperature	Methane Yields		References
Mixed Grain - mostly corn & wheat, with trace amounts of triticale & molasses	Whole stillage	Mesophilic/35ºC	290 ± 1.5 NmL/g COD_{added}	469 ± 2.4 NmL/g VS_{added}	[1]
	Thin stillage	Mesophilic/35ºC	303 ± 3.0 NmL/g COD_{added}	500 ± 17.4 NmL/g VS_{added}	
	Wet cake	Mesophilic/35ºC	267 ± 9.0 NmL/g COD_{added}	425 ± 14.3 NmL/g VS_{added}	
	Syrup	Mesophilic/35ºC	298 ± 9.9 NmL/g COD_{added}	470 ± 15.6 NmL/g VS_{added}	
Corn	Whole stillage	Mesophilic/35ºC	-	0.43 ± 0.03 L/g VS_{added}	[2]
Corn	Whole stillage	Mesophilic/35ºC	-	401 ± 17 mL/g VS_{added}	[3]
			-	406 ± 14 mL/g VS_{added}	
			-	441 ± 2 mL/g VS_{added}	
			-	458 ± 0 mL/g VS_{added}	
		Thermophilic/55ºC	-	693 ± 17 mL/g VS_{added}	
			-	560 ± 24 mL/g VS_{added}	
			-	529 ± 27 mL/g VS_{added}	
			-	429 ± 8 mL/g VS_{added}	
Wheat	Whole stillage	Thermophilic/55ºC	-	533 ± 18 mL/g VS_{added}	[4]
			-	578 ± 14 mL/g VS_{added}	
	Thin stillage		-	483 ± 59 mL/g VS_{added}	
			-	592 ± 37 mL/g VS_{added}	
	Wet cake		-	485 ± 19 mL/g VS_{added}	
			-	493 ± 32 mL/g VS_{added}	
Cereals	Whole stillage	Mesophilic/37ºC	-	0.41 NL/g VS_{added}	[5]
Corn	Thin stillage	Mesophilic/37ºC	0.26 L/g COD_{added}	-	[6]

Town et al. pursued batch-AD of stillage fractions with cattle manure and found that the mixture increased the methane production rate and resulted in more consistent methane volume for all fractions [4]. Whole stillage and wet cake demonstrated a 20% and 12% rate increase, respectively when mixed with manure [4]. Co-digestion of manure and thin stillage showed signs of synergism – demonstrated by a 25% increase in methane volume compared to expected methane yield. Westerholm et al. reported stable performance after the addition of manure in the semi-continuous AD of cereals whole stillage [5] . Mono-digestion of whole stillage initial resulted in elevated VFA levels, which in turn, resulted in a drop in pH and a decline in gas production. After the addition of manure, the acids were quickly degraded, and the pH and methane content of the gas increased [5].

Blending stillage with other feedstock can alleviate some issues with anaerobic digestion. Although co-digestion with manure has been successful, it can also be troublesome. Manure typically results in low methane yield due to its high water and fiber content. Manure as a co-substrate also brings about higher sanitation costs due to the potential of pathogen spread [5].

Co-digestion of stillage with source-separated organics (SSOs) is a viable option. Source Separated Organics (SSO) refer to the organic fraction of municipal solid waste and consist of food waste, leaf and yard waste, soiled paper products, and wood waste [8] [9]. Landfilling of SSOs results in greenhouse gas emissions, increased leachate volumes, and strain on landfill space [8] [10]. Recently, the Government of Ontario introduced new legislation to develop a waste diversion system for SSOs [10] [11]. Waste reduction targets for municipalities have been outlined in the 2018 Food and Organic Waste Policy Statement [11]. Provincial motivation to move towards a more circular economy makes industrial-municipal partnerships more attractive to both parties.

SSOs are carbohydrate-rich substrates, easily biodegradable, and have high C/N ratios – which makes them ideal for balancing out the low C/N ratio of protein-rich stillage, whilst substantially increasing the methane yield [12]. Degradation of proteins can increase the risk for ammonia-inhibition. Co-digestion with SSOs can make digestion of stillage more feasible due to enhanced stability. Co-digestion with SSOs can also make it easier to achieve Class A Biosolids requirements for digestate. For some substrates, including animal slurries, sludges, and industrial

residues, there may be strict requirements to limit environmental pollution due to presence of pathogens, heavy metals, or other hazardous materials [13].

Wastewater biosolids (raw sludge) may also make an interesting co-substrate. Raw sludge is characterized by a low C/N ratio, which may make its selection as a co-substrate counterintuitive. However, raw sludge has a high buffering capacity [14]. As mentioned earlier, mono-digestion of stillage has resulted in rapid depletion of alkalinity and poor performance. The natural source of alkalinity supplied by raw sludge can help stabilize the pH level. Like manure, raw sludge can also provide a diverse source of microorganisms to enhance process performance.

Sharing a single digester can improve the feasibility of anaerobic digestion in a small or medium-sized community. Co-digestion of multiple wastes can make anaerobic digestion for small and medium-sized communities more feasible. Sharing of a digester can reduce the cost per unit volume of waste, resulting in economies of scale.

3.2 Materials and Methods

3.2.1 Preparation of Inoculum

Anaerobic digested sludge (ADS, or seed sludge) served as the inoculum and was collected from Ontario Clean Water Agency (Stratford, Ontario, Canada). The raw sludge is pumped to the primary digesters, which operate at an average temperature of 37ºC and a residence time between 16-20 days. Most of the sludge settles in the primary clarifiers, where return activated sludge is wasted to primary clarifiers and settled with raw sludge. The primary ADS was stored in a cold storage room at 4 ºC at the University of Windsor (Windsor, Ontario, Canada). It is reported that seed sludge can be stored at 4 ºC for 14 days and maintain methanogenic activity like that of fresh inoculum [15]. The seed sludge was sieved (2000 μm) after visual inspection for large particles and hairs.

3.2.2 Preparation of Substrates

A 20 L sample of wet cake with syrup addition was collected from Hiram Walker & Sons Limited (Windsor, Ontario, Canada). The wet cake was blended with distilled water at a 1:2 ratio to achieve a homogenized mixture using an electric blender (Ninja model, model number NJ 600WMW). The blending power used, and duration of blend were 900 W and 5 minutes,

respectively. Afterwards, the wet cake blend was sieved (2000 µm) and homogenized in the blender for an additional 10 minutes.

The sample was stored in a cold storage room at 4 ºC at the University of Windsor (Windsor, Ontario, Canada). Previous studies have shown that storage of stillage fractions at 4 ºC are effective in reducing volatile solids loss over the course of one week (less than 2%) [2] [3] [4] [16] Long term storage studies have not yet been completed on stillage fractions, so the waste was used as soon as possible.

A 20 L sample of raw sludge was sourced from Little River Pollution Control Plant (Windsor, Ontario, Canada). The waste-activated sludge is co-thickened with primary sludge and collected before centrifugation. The raw sludge was sieved (2000 µm) after visual inspection. Then, the raw sludge was homogenized in a blender for 5 minutes at 900 W. The sample was stored in a cold storage room at 4 ºC at the University of Windsor (Windsor, Ontario, Canada) to prevent degradation, as recommended by [17] [18].

Figure 3.1 Substrate and inoculum samples used for characterization.

A 20 L sample of SSO slurry was received from StormFisher (London, Ontario, Canada). The SSOs were sieved (2000 µm) after visual inspection. Then, the sample was homogenized in a blender for 5 minutes at 900 W. The sample was stored in a cold storage room at 4 ºC at the

University of Windsor (Windsor, Ontario, Canada). Previous BMP studies reported storage of SSOs in slurry form at 4 ºC for short periods (less than 2 weeks) [19] [20] Therefore, when the SSO slurry was received, characterization was carried out immediately.

A 500 mL sample of each feedstock and the inoculum were collected in plastic bottles and stored in the cold room (See Figure 3.1). Before characterization, the bottles were placed in a 25ºC - 30ºC water bath for 20 minutes. The remaining feedstock samples were stored in a 20 L container in the cold room and later used to create the mixing ratios needed for the BMP.

3.2.3 Analytical Methods

Substrate and inoculum characterization was carried out prior to BMP test setup. Total and soluble chemical oxygen demand (TCOD and SCOD), total volatile fatty acids (TVFAs), sulfate (SO_4), total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH_3-N), and total phosphorus were measured using HACH methods and test kits (DR6000 Benchtop Spectrophotometer). Solids (TS, VS, TSS, and VSS) were analyzed in accordance with Standard Methods [21]. The pH level was measured using the Easy pH EasyPlus Titrator from Mettler Toledo (Mettler Toledo, USA). The pH meter was calibrated using buffers at pH 4.0 ± 0.01 and 7.0 ± 0.01. Soluble parameters were determined after 0.45 µm filtration.

The results of the characterization are outlined in Table 3.2.

Table 3.2 Average substrate and inoculum characteristics with standard deviations.

Parameter	Units	Wet Cake	Raw Sludge	Source-Separated Organics	Inoculum
TCOD	g/L	148.0 ± 1.3	50.1 ± 2.3	158.4 ± 5.8	29.8 ± 0.5
SCOD	g/L	50.0 ± 1.6	4.4 ± 0.04	69.8 ± 0.2	***n.d.***
TVFA	mg/L	5267 ± 49.0	3791 ± 13.6	12980 ± 171	***n.d.***
TN	mg/L	876 ± 4.9	365 ± 23.3	1578 ± 7.5	794 ± 27.6
TKN	mg/L	864 ± 4.4	353 ± 23.8	1596 ± 7.7	829 ± 29.1
NH_3-N	mg/L	174 ± 3.8	287 ± 4.8	233 ± 1.8	679 ± 11.9
SO_4	mg/L	96.9 ± 0.3	97.6 ± 0.2	316 ± 10.2	96.9 ± 0.1
TP	mg/L	1570 ± 153	683 ± 11.3	641 ± 33.7	832 ± 2.0
COD:N:P	-	250:2:3	250:2:3	250:3:1	250:7:7
TS	g/L	112.9 ± 0.9	34.4 ± 0.3	86.1 ± 0.6	34.3 ± 0.5
VS	g/L	106.0 ± 0.9	28.6 ± 0.3	78.0 ± 0.6	19.9 ± 0.5
TSS	g/L	99.9 ± 1.3	33.3 ± 0.3	60.3 ± 1.0	32.7 ± 0.6
VSS	g/L	95.8 ± 1.1	27.9 ± 0.2	57.1 ± 0.9	19.0 ± 0.3
VS/TS (%)	%	94.0	83.1	90.6	57.9
pH	-	4.2 ± 0.03	5.9 ± 0.01	3.8 ± 0.02	7.6 ± 0.02
Alkalinity	mg$CaCO_3$/L	***n.d.***	2077 ± 45.0	***n.d.***	7484 ± 311

n.d. – no data for parameter.

A total solids analysis was completed on the solid wet cake sample to determine if the dilution and homogenization with distilled water (DIW) was accurate. A comparison between the solid wet cake and homogenized wet cake (as g TS/g WCK) showed an error of less than 1%.

3.2.4 Preparation of Mixing Ratios

Table A.1 describes the preparation of seven mixing ratios of wet cake (WCK), source-separated organics (SSOs), and raw sludge (RS) based on the VS ratio (mass basis). The VS content in each mixture was set to 25 g/L. Figure 3.2 describes the ratios between WCK, RS, and SSOs. All bottles, including control and blank, were set up in triplicates. Preparation of mixing ratios were verified via TCOD and Solids analysis. The error between expected TCOD and expected VS content were less than 10%, thus ensuring the mixing were made properly.

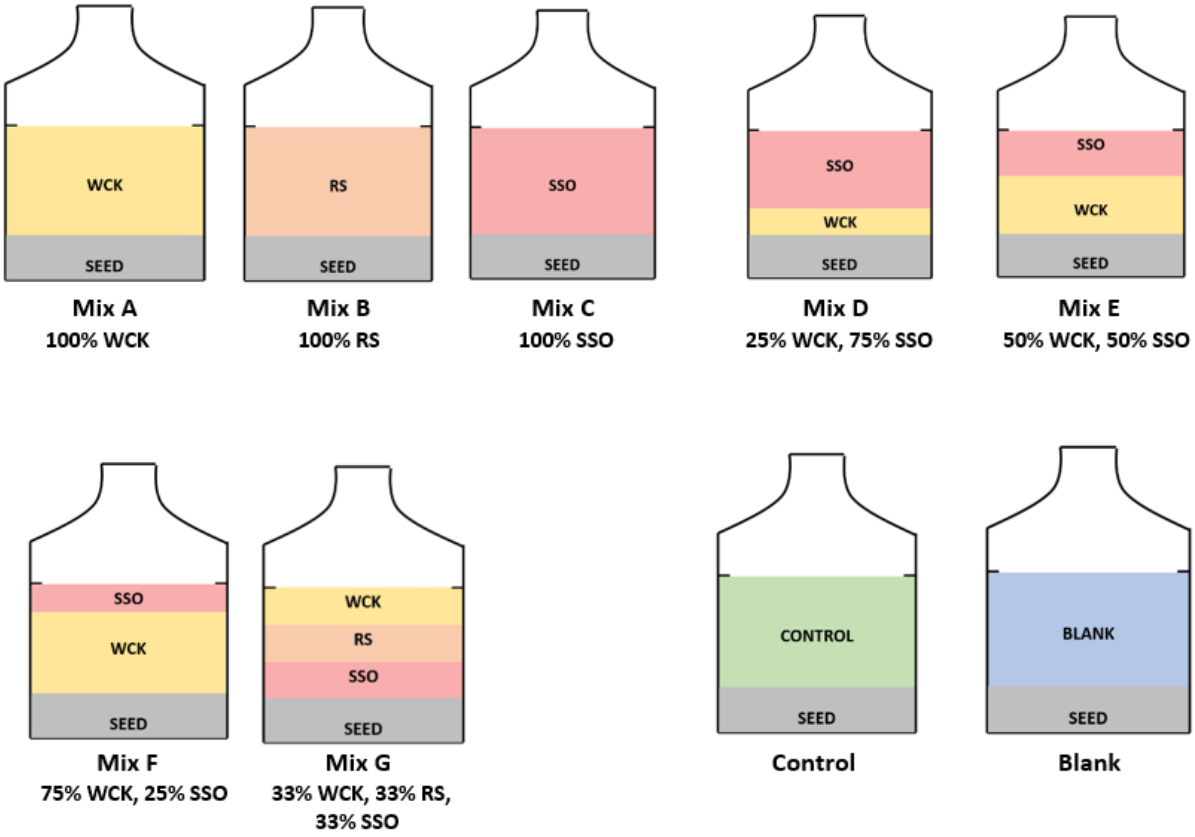

Figure 3.2 Mixture design for co-digestion study. Mixtures include ratios of wet cake (WCK), source-separated organics (SSO), and raw sludge (RS).

3.2.5 BMP Setup

The co-digestion study was carried out using 500 mL glass bottle reactors. The seed sludge in each bottle was set to 300 mL in all bottles. The food-to-microorganism ratio was set 0.5 to ensure sufficient time for the microbial activity and avoid overloading the reactor. Based on the F/M ratio, and VSS content of the seed sludge, the volumes of substrate were calculated using (1). Distilled water (DIW) was added to each bottle to equalize the volumes across all reactors. The equation is shown below:

$$\frac{F}{M} = \frac{TCOD_{substrate} \times V_{substrate}}{VSS_{seed} \times V_{seed}} \quad (1)$$

Where $\frac{F}{M}$ is the food-to-microorganism ratio as g $TCOD_{substrate}$/g VSS_{seed}, $TCOD_{substrate}$ is the TCOD of the substrate in grams per liter (g/L), $V_{substrate}$ is the volume of substrate in liters (L), VSS_{seed} is the VSS concentration of the seed sludge in g/L, and V_{seed} is the volume seed sludge in L. Details of the bottle setup can be seem in Table A.2.

Initial pH was set to 7.1 ± 0.1 at the beginning of the experiment with the addition of either 4.5 N sodium hydroxide or concentrated hydrochloric acid. External buffer was added in the form of sodium bicarbonate ($NaHCO_3$). A 50 mL sample was removed from each bottle and stored in a refrigerator for analysis, leaving a working volume of 450 mL. Bottles were flushed with compressed nitrogen gas (Linde Canada, Canada) at 8 psi for 2 minutes. Incubation temperature was set at 38 ± 1ºC, which falls into the mesophilic temperature range.

The bottle setup was verified via TCOD and Solids analysis. Since the error between expected and measured values were less than 10%, the setup is considered acceptable.

The bio methane potential study was carried out using the Automatic Methane Potential Test System (AMPTS II) supplied by Bioprocess Control [22]. The AMPTS II consists of three units: (1) a sample incubation unit, (2) a CO_2 capture unit, and (3) a gas volume measuring device. The sample incubation unit (or water bath) has a capacity of up to 15 glass bottle reactors (500 mL each). The media in each bottle is mixed using a slow-rotating stirrer. Biogas produced accumulates in the headspace of the vessel. Then, the biogas passes through vials containing an alkaline solution (NaOH). Carbon dioxide, and hydrogen sulfide are captured by the solution, whilst allowing CH_4 to flow through to the gas measuring unit. The methane gas from the CO_2-fixing unit is measured using a wet gas flow measuring device with a multi-flow cell arrangement (15 cells). Using principles of liquid displacement and buoyance, the device monitors gas flows and generates a digital pulse for a specified volume measurement. The embedded data acquisition system records and normalizes the data in real-time. Figure 3.3 illustrates the AMPTS setup.

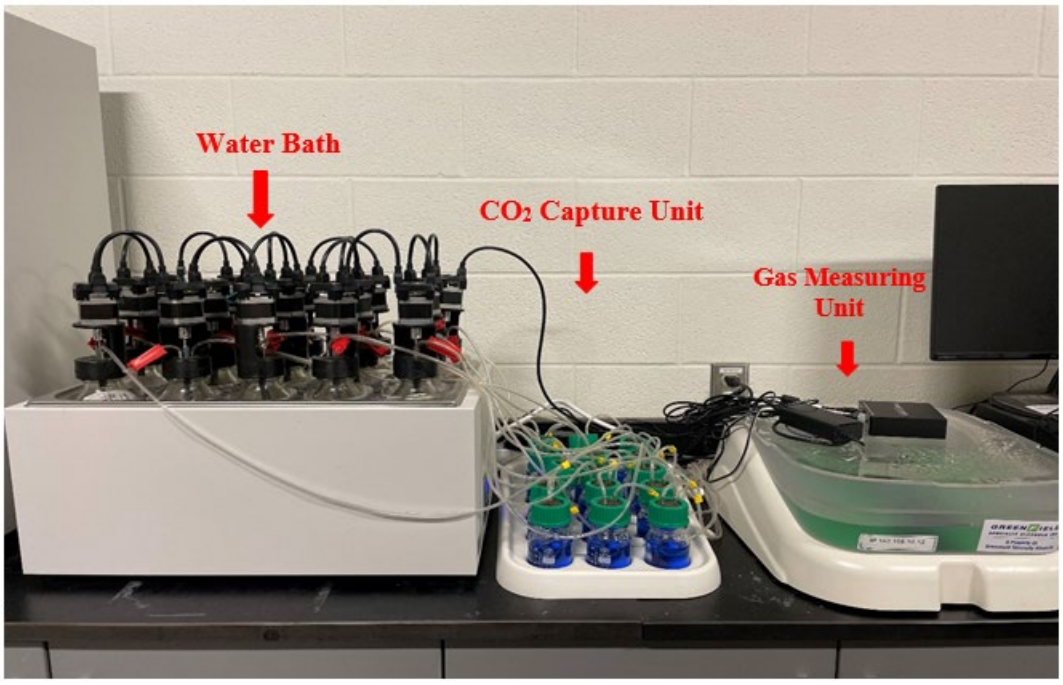

Figure 3.3 BMP setup in Automatic Methane Potential Test System (AMPTS II).

3.3 Data Analysis

3.3.1 Statistical Analysis

Comparisons of biomethane yields between mixture groups were evaluated using single factor ANOVA testing and Tukey's post-hoc test. Synergistic and antagonistic effects were determined using the student's t-test. Statistical significance was established at $P < .05$ level.

3.3.2 Kinetic Modeling

The Gompertz model has been widely used to illustrate the growth of animals and plants, and the volume of bacteria and tumor cells [23]. The Modified-Gompertz model re-expresses the Gompertz equation with more relevant parameters for anaerobic digestion (lag phase, growth rate, maximum biogas production rate) to make the model easier to use [24] [25]. The Modified-Gompertz model is attractive for anaerobic digestion researchers as it only requires cumulative methane production to determine kinetic constants and goodness of fit [25]. The equation is shown below:

$$y = Aexp\left\{-exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\} \qquad (2)$$

Where y is the cumulative methane production in mL at time t. A is the maximum methane production in mL, μ_m is the methane production rate in mL/day, t is the time in days, and λ is the lag phases in days.

Model parameters were obtained using Excel non-linear regression solver, where the residual sum of squares between the experimental and predicted data was minimized.

As explained by Koppar and Pullammanappallil [26], cumulative methane production curves only asymptotically approach the maximum methane yield. Thus, the time taken to achieve 95% of the methane potential was selected to use as an estimate for the hydraulic retention time of an anaerobic digester [27]. The equation is shown below:

$$t_{95} = \frac{A}{\mu_m}(1 - ln(-0.95)) + \lambda \qquad (3)$$

Where t_{95} is the time required to achieve 95% of the maximum methane yield in days. A is the maximum methane production in mL, μ_m is the methane production rate in mL/day, and λ is the lag phases in days.

The effective digestion time, t_{eff}, can be found from subtracting the lag phase from t_{95}.

$$t_{eff} = t_{95} - \lambda \qquad (4)$$

3.4 Results and Discussion

Based on the bottle setup in Table A.2, the characteristics of the initial triplicate bottles are calculated and summarized in Table 3.3. Looking at the Table, it appears that no inhibition is expected from any of the substrates. There are enough nutrients to ensure successful AD of all mixtures.

Table 3.3 Initial BMP bottle characteristics calculated based on initial waste characterization.

Parameter	Units	Mix A	Mix B	Mix C	Mix D	Mix E	Mix F	Mix G
TCOD	g/L	23.3	23.3	23.3	23.3	23.3	23.3	23.3
TKN	mg/L	529	536	557	548	543	535	540
NH_3-N	mg/L	414	439	416	415	415	414	423
SO_4	mg/L	62	69	70	68	66	64	67
TP	mg/L	557	575	523	528	536	543	548
COD:N:P	-	250:5:6	250:6:6	250:6:6	250:6:6	250:6:6	250:6:6	250:6:6
TS	g/L	24.5	24.4	23.6	23.5	23.9	24.0	24.1
VS	g/L	15.6	15.1	14.6	14.6	15.0	15.2	15.1
VS/TS	%	64	62	62	62	63	63	63

The total COD balance for all bottles ranged from 92% to 104%, which is within the range reported in literature (see Table A.3 [28]. The control bottles achieved an average of 88% of the expected volume.

3.4.1 Mono-digestion Yields

The average methane yields for each mixture and their biodegradability are consolidated in Table 3.4. The net cumulative methane curves are plotted on Figure 3.5.

Table 3.4 Average biochemical methane potential (BMP) of mixtures based on COD and VS added with standard deviations.

Mix	Biomethane Yield		Biodegradability
	NmL CH_4/g COD_{added}	NmL CH_4/g VS_{added}	
A	251 ± 6^{b}	359 ± 5	72%
B	184 ± 5^{c}	267 ± 7	53%
C	307 ± 11^{a}	458 ± 16	88%
D	288 ± 7^{a}	435 ± 4	82%
E	286 ± 2^{a}	424 ± 8	82%
F	263 ± 18^{a}	402 ± 22	75%
G	245 ± 10^{a}	358 ± 18	70%

Different letters represent statistical difference ($P < .05$) between mixtures.

The average methane yield for AD of wet cake was observed to be 251 ± 6 NmL/g COD_{added}, which falls just short of the yields reported in literature (see Table 3.1). A study conducted by Drosg et al. tested stillage fractions from a mixed dry-grind facility, mostly consisting of corn and wheat with trace amounts of triticale and molasses [1].. The obtained yield for wet cake was 267 ± 9 NmL/g COD_{added} [1]. Moreover, mono-digestion of wet cake resulted in 72% biodegradability, which suggests that there may be some compounds in the waste that are not as easily broken down.

One of the reasons for a lower methane yield may be due to the source of the grain used. As mentioned earlier, stillage from Hiram Walker & Sons Ltd. originates from a mixed blend of corn, wheat, rye, and barley. Distilleries or ethanol plants may use different ratios of grains depending on crop price and marketability of the resulting animal feed to decrease the cost of production [29] [30] [31] [32]. In turn, this can have an impact on the composition of the feedstock and potentially the methane yield.

Several studies have investigated the differences in chemical composition and nutritive value of various cereal grains [29] [30] [31] [33]. According to Mustafa et al. barley grains contain higher percentages of fermentation residue relative to other grains due to its high hull content [29]. The hull content of barley is normally indigestible and requires pretreatment to break down its complex structure [34]. Buenavista et al. summarized nutrient variation amongst cereal DDGS and reported higher crude protein content amongst corn, wheat, and sorghum as compared to barley [33]. Generally, protein-rich wastes are known to be good producers of biogas [35].

Much of the literature surrounding AD of stillage focuses on whole stillage and thin stillage (see Table 3.1). However, the degree of processing can have an impact on feedstock composition and methane yield [29] [31] [32]. Crude protein and sugars concentration can vary with stillage processing [31] [32]. According to Kim et al., the xylan and arabinan contents in wet distillers' grains were twice that of DDGS, even though the dried grains also contained the condensed solubles from thin stillage [32]. Thus, the availability of more sugars indicates that the wet distillers' grains are more easily digested. Some distilleries or ethanol plants also extract fat content from stillage to produce corn oil, which can impact nutrient content [33]. Fats, oils, and greases are highly digestible and energy-dense feedstock [33] [35]. Thus, oil removal from stillage can reduce the overall digestibility of the waste, and subsequently the methane yield.

Sludge stabilization is widely practiced, although results may vary depending on locality. The methane yield of raw sludge in this study was 184 ± 5 NmL CH_4/g COD_{added}. Bahreini et al. conducted a BMP study on primary sludge from the Greenway Wastewater Treatment Plant in London, Ontario [36]. Using the same inocula that was obtained in this study, the authors achieved a methane yield of 218 NmL CH_4/g COD_{added}. Thus, the results from this study are consistent with literature from similar municipalities.

Stormfisher processes wastes that contain high amounts of fats and proteins which, as mentioned earlier, have high biomethane potential [35] This is clearly exhibited through the high methane yield obtained from this study (307 ± 11 NmL/g COD_{added} or 458 ± 16 NmL/g VS_{added}). The food waste from Stormfisher also contained a higher VFA content comparative to wet cake and raw sludge (see Table 3.2). This can also explain the high methane yield and high biodegradability (88%), as the VFAs are already easily accessible to the bacteria without further degradation required. A study by Li et al. investigated the effect of different proportions of organics on mesophilic-AD of food waste [37]. By varying the ratios of carbohydrates, proteins, and lipids in the feedstock, they achieved methane yields from 385 mL/g VS to 685 mL/g VS. Thus, the methane yield obtained from this study is within the range found in literature.

3.4.2 Co-digestion Yields

The addition of source-separated organics in the substrate mix increased the biomethane yield based on gram COD added (see Figure 3.4). Mix D and Mix E, which contained 75% VS and 50% VS of SSO, respectively, resulted in a 14-15% biomethane yield as compared to mono-digestion of wet cake ($P < .05$). Mix F, which contained the lowest concentration of SSOs (25%), only resulted in 5% improvement. Post-hoc testing confirmed that this was not statistically significant.

The increase in BMP is likely due to the highly biodegradable nature of the SSOs. As mentioned earlier, the SSOs obtained from Stormfisher contained high amounts of SCOD and VFA content, which are more accessible to the microbial community. This can be confirmed by the increase in biodegradability of Mix D and Mix E, which both achieved 82% of the expected volume, as opposed to Mix F, which only achieved 75% of the expected volume (see Table 3.4). The biodegradability of Mix F was much closer to that of Mix A (72%) – which further confirms that the higher concentration of wet cake resulted in decreased biodegradability, likely due to the

lignocellulosic content present in the substrate. Similar results were seen by Moestedt et al., who compared single-stage AD of municipal solid waste (OFMSW) and thin stillage with two-stage AD [38]. Single-stage AD of OFMSW and thin stillage at a 50:50 ratio (VS basis) decreased gas yield as compared to mono-digestion of OFMSW.

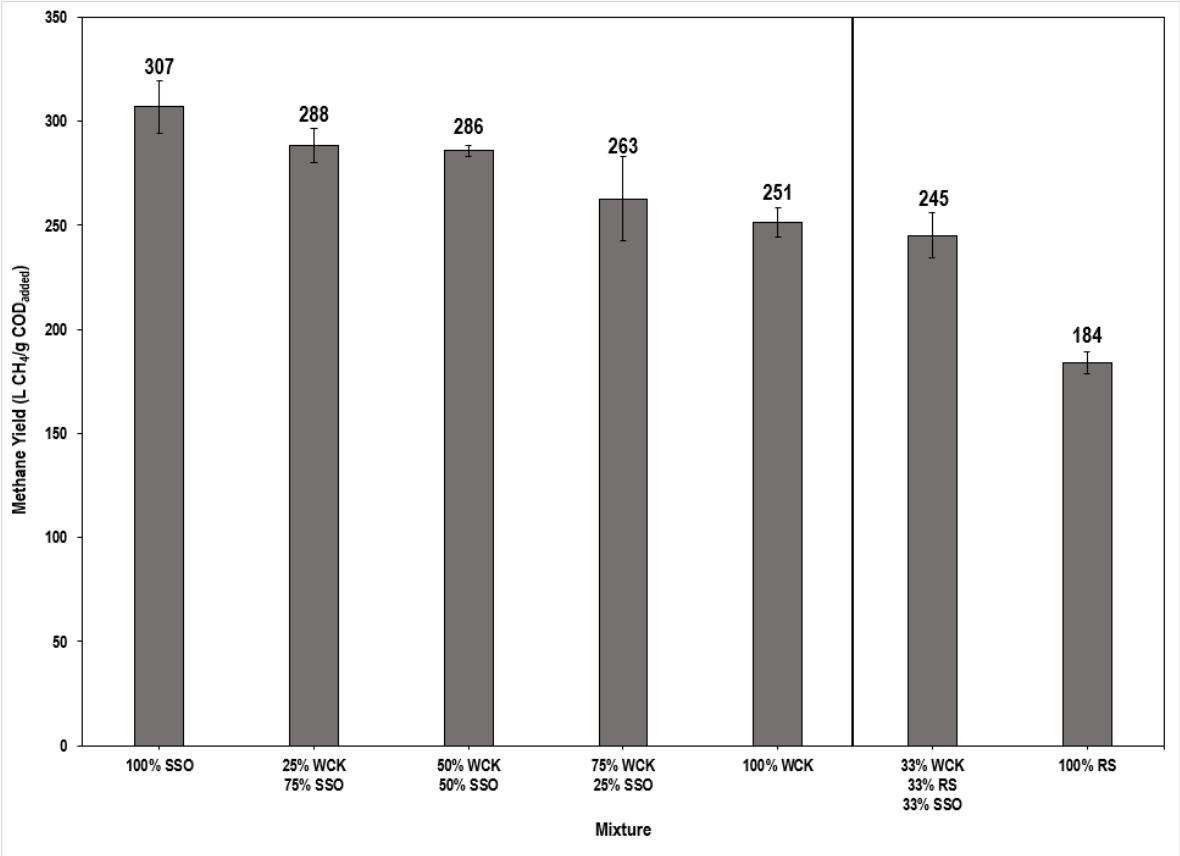

Figure 3.4 Average BMP yields based on COD added of mixtures, plotted with 95% confidence intervals ($P < .05$).

Mix G (which contained equal parts of all substrates) resulted in a statistically similar BMP as mono-digestion of wet cake (see Figure 3.4). Co-digestion of municipal sludge and SSOs is a common practice, as the inclusion of sludge prevents rapid VFA accumulation and supplies nutrients and additional buffering capacity. Also, the addition of sludge can help balance the high suspended solids concentration of stillage and SSOs, as high-solids feedstock can result in issues like increased contact times between substrate and bacteria, clogging, scum formation in mixed liquor, and difficulties with adequate mixing [39] [40] [41]

Combining municipal and industrial wastes is a more integrated approach to anaerobic digestion and aids in the shift towards a more circular economy. Additionally, many researchers have reported the benefits of sharing a common digester to co-digest wastes as opposed to building separate digesters [42].

Interestingly, Tukey's test illustrated that co-digestion of wet cake and SSOs at varying ratios (Mix D, Mix E, and Mix F) resulted in biomethane yields that were statistically similar to one another (See Table 3.4). The broader implication of this result is that the availability of either wet cake or source-separated organics is not a hindrance in co-digestion and can produce a consistent biomethane yield. This can be seen as advantageous to distilleries, as the market for crop pricing varies [29] [30]. Distilleries can have greater flexibility in obtaining profits – a portion of stillage waste could be used for DDGS production whilst the remaining amount can be diverted to anaerobic digestion. Moreover, this can be seen as an advantage for micro-distilleries or breweries who may produce a smaller amount of stillage waste fractions, but still want to divert their waste towards green energy. Communities could implement an organics pick-up service and send the waste off to an AD service provider.

3.4.3 Synergism and Antagonism

Synergism can be a result of additional nutrients, alkalinity, or organic content that could lead to a positive differential between the experimental BMP yield and the weighted BMP (calculated using the experimental BMPs from the mono-digestion mixtures). Antagonism can be the result of nutrients in excess, high acids concentration, or presence of inhibitory compounds. This would lead to a negative differential between the experimental BMP and the weighted BMP [43]. Table 3.5 displays the ratio between the weighted BMP yield and experimental methane yield of each co-digestion mixture, which were tested for significance using the t-test.

Table 3.5 Average observed BMP with standard deviations from co-digestion study as compared to weighted BMP yield.

Mixtures	Experimental BMP Yield	Weighted BMP Yield	Differential
Mix D (25% WCK, 75% SSO)	288 ± 7	293	0.98
Mix E (50% WCK, 50% SSO)	286 ± 2	279	1.02*
Mix F (75% WCK, 25% SSO)	263 ± 18	265	0.99
Mix G (33% WCK, 33% RS, 33% SSO)	245 ± 10	247	0.99

Statistical significance denoted by *.

Statistical testing of the BMP obtained from co-digestion mixtures D, F, and G did not result in any significant difference from the weighted BMP. This can be confirmed by the fact the difference in yield is within the standard deviation obtained from the experiment. The only mixture that indicated significance was Mix E ($P < .05$), with a synergistic impact of 2.0%. Since the increase in BMP yield is not substantial - it is unlikely that this result is true representation of synergism.

One sign that the data illustrated a false impression of synergism is the lack of pattern in the data. There is no indication that increasing the amount of wet cake in the mixtures leads to synergistic impacts, as Mix F does not exhibit any significant results. Low synergistic effects were also exhibited in a study conducted by Kim et al., who co-digested spent coffee grounds with food waste, marine microalgae, and whey [44]. The differential in their study ranged from 0.97 to 1.05. Thus, a differential close to 1 may suggest neutrality between the substrates.

Additionally, the acceptable average percentage error (APE) used in this study was <10%, as demonstrated by Akobi et al. [45] Thus the "true" COD added into each bottle lays within this range, which can impact the methane yield and may give the impression of slight synergism or antagonism.

Some researchers have discussed the difficulties in achieving accurate COD results, particularly for heterogenous and high solids wastes. Yadvika et al. devised a modified method to determine the COD of cattle dung slurry [46]. The researchers achieved a higher reproducibility and accuracy for samples with solids concentrations higher than 14.0 g/L, as compared to results

from samples tested using Standard Methods [21]. Shanmugam et al. calculated the theoretical COD of mixed solid wastes using the empirical composition of the wastes – although this method requires detailed chemical analysis [47]. When comparing the theoretical COD to the experimental COD of the mixed wastes, they were in good agreement, likely due to the processing and homogenization of the feedstock prior to analysis [47] The wet cake sample is a complex, high-solids waste and requires proper preparation prior to the feedstock to achieve accurate results. Similarly, the SSO sample contained large chunks of food waste even after homogenization by Stormfisher, which is why additional homogenization in a kitchen blender was done prior to characterization and the BMP.

3.4.4 Kinetics

The biomethane production data fit well with the predicted data outputted from the Modified-Gompertz model. This is reflected by the high R^2 values achieved for all mixes (ranging from 0.9730 to 0.9963). A summary of the kinetic constants for each mixture are shown in Table 3.6.

Table 3.6 Summary of averaged kinetic parameters and their standard deviations from Modified-Gompertz modeling.

Mix	A NmL	μ_m NmL/day	λ day	t_{95} day	t_{eff} day	R^2
A	652 ±24	44 ± 4^{b}	4.3 ± 2.2^{a}	26.1 ± 1.9^{b}	21.8 ± 1.6^{a}	0.9844
B	442±9	76 ± 13^{a}	0.1 ± 0.1^{b}	8.8 ± 1.7^{c}	8.7 ± 1.7^{b}	0.9963
C	836±36	51 ± 2^{b}	6.6 ± 0.6^{a}	30.6 ± 0.6^{b}	24.0 ± 1.0^{a}	0.9795
D	823±82	43 ± 2^{b}	6.2 ± 1.6^{a}	34.1 ± 4.9^{a}	27.9 ± 3.7^{a}	0.9780
E	799±5	43 ± 0.5^{b}	5.7 ± 0.5^{a}	32.7 ± 0.3^{b}	27.0 ± 0.2^{a}	0.9775
F	707±46	46 ± 12^{b}	6.5 ± 2.0^{a}	30.2 ± 4.4^{b}	23.8 ± 6.3^{a}	0.9844
G	680±42	42 ± 1^{b}	7.5 ± 0.1^{a}	31.4 ± 2.1^{b}	23.9 ± 2.0^{a}	0.9730

Different letters represent statistical differences ($P < .05$) between assays for each parameter (μ_m, λ,t_{95}, t_{eff}).

Curve fitting for each the average net methane volume of each mixture is illustrated through Figure A.1 to Figure A.7

The lag phases across most of the mixtures is quite long – this can clearly be illustrated in Figure 3.5.

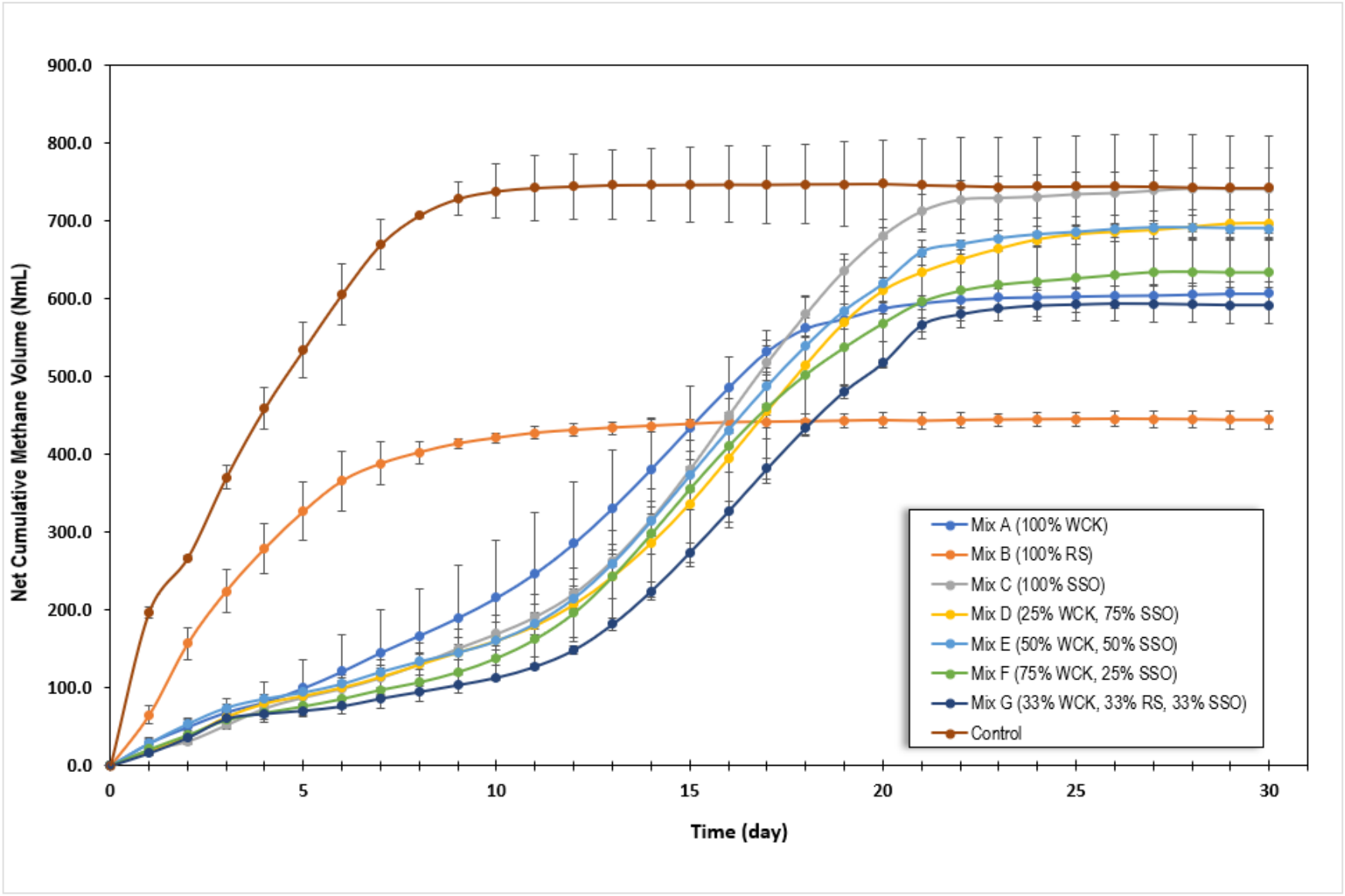

Figure 3.5 Average cumulative net methane production curve plotted against time with respective standard deviations (n=3).

Looking at Table 3.6, it appears that the lag periods for all samples except Mix B (containing 100% raw sludge), are statistically similar to one another. The long lag period of SSOs and wet cake in this study could be attributed to unacclimated inoculum [48]. For example, Nasr et al. found that the use of acclimatized anaerobic digester sludge for biohydrogen production from thin stillage reduced the lag phase from 4.4 hours to 2.3 hours due increased diversity in the microbial community [40]. The Ontario Clean Water Agency in Stratford already processes municipal biosolids and explains the short lag period exhibited by the raw sludge mixture.

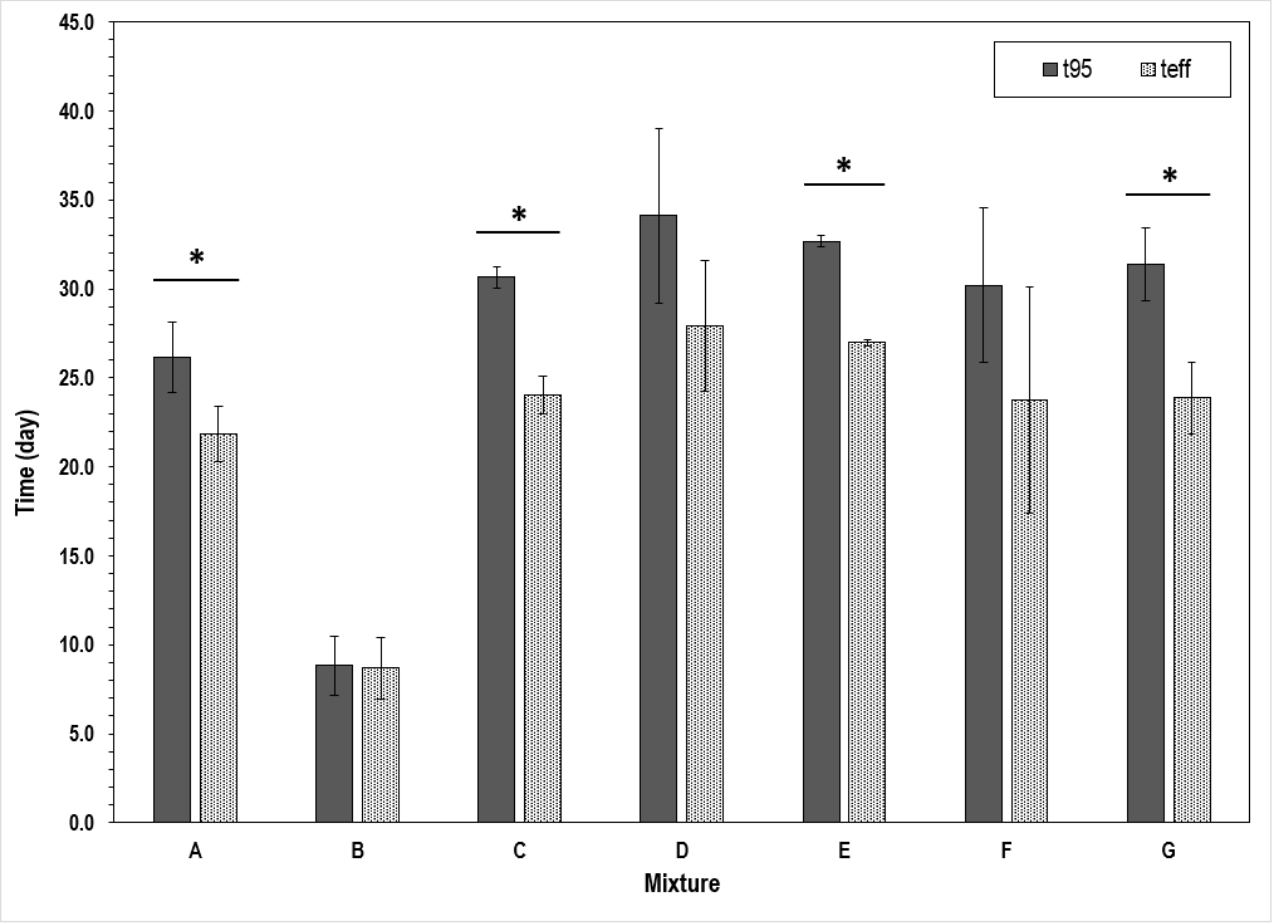

Figure 3.6 Comparison of t_{95} and t_{eff} – Averages plotted with their respective standard deviations. Statistical differences represented by asterisks ($P < .05$).

The time taken to achieve 95% of the biomethane yield for Mixes A, C, and D-G range from 26.1 to 34.1 days (see Table 3.6). As mentioned earlier, Mix B had the shortest lag phase and therefore the time taken to achieve 95% of the biomethane yield was only 8.8 days.

The effective digestion time represents the actual time taken for methane production by a substrate whilst neglecting the lag phase. Figure 3.6 illustrates that elimination of the lag phase for Mixes A, C, E and G is significant, compared to t_{95} ($P < .05$). The implication is that it may be possible to achieve similar biomethane yields at shorter retention times. This is a particularly important conclusion for the co-digestion mixtures, E and G. Anaerobic digestion projects are often plagued with high capital and operational costs. Reduction digestion time can result in improved digester efficiency and a lower operations cost over time.

3.5 Conclusions

The purpose of this study was to determine the effect of mixing ratios on the biomethane yield resulting from the batch co-digestion of distillery stillage and source-separated organics. Interactions between the substrates were evaluated for synergism or antagonism and changes in kinetics were studied using the Modified-Gompertz model. Based on the results of the study:

1. A higher VS concentration of SSO (50% VS and 75%) in the substrate mix resulted in a 14-15% higher yield per gram COD added, as compared to mono-digestion of wet cake
2. Co-digestion of wet cake and SSOs at varying ratios resulted in statistically similar biomethane ($P < .05$). This implies that anaerobic co-digestion of these substrates is not hindered by feedstock availability.
3. Co-digestion of source-separated organics and distillery stillage at different mixing ratios had neutral effects – thus the substrates are compatible with one another and did not display any initial signs of instability or inhibition during anaerobic digestion.
4. There was no sign of improvements in kinetics in relation to the methane production rate and lag phase due to co-digestion. However, the study did illustrate that acclimation of the inoculum could decrease the lag phase exhibited by the mixtures, which in turn could reduce overall digestion time and reduce operations costs over time.

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CHAPTER 4
LOW-TEMPERATURE MICROWAVE PRE-TREATMENT OF DISTILLERY STILLAGE

Increases in natural gas pricing has forced the IC&I sector to move towards 'green energy' projects to reduce energy consumption and greenhouse gas emissions. The distilled spirits industry is an excellent candidate for waste-to-energy initiatives. Distilleries are known to have high natural gas consumption, mainly due to stillage processing. Because of this, recent literature has focused on improving the net energy balance of distilleries [1] [2] [3] [4] [5] [6].

Stillage fractions are rich in organic content and have high biomethane potential, thus making them suitable substrates for anaerobic digestion (AD). Despite its benefits, stillages typically contain lignocellulose – which is difficult to degrade. Consequently, stillage requires extra processing to access the energy stored in this component [7]. Further, researchers are looking to improve the degradation kinetics of stillage [8]. In AD, the hydrolysis stage is regarded as the 'rate-limiting step' [9]. Therefore, any improvements in the breakdown of complex organic compounds could have an impact on the overall kinetics of digestion.

Pre-treatment of stillage can help unlock the greater methane potential that is stored in the lignocellulosic component and accelerate the rate of hydrolysis [10]. Effective pre-treatment should provide benefits that supersede the processing and operating costs of itself and anaerobic digestion [11].

This study focuses on testing a range of microwave parameters on wet cake prior to batch anaerobic digestion. Successful pre-treatment will be characterized as improvements in solubilization, biomethane yield, and reaction kinetics.

The scope of the study is outlined below:

1. Evaluate the effects of MW pre-treatment on various substrate characteristics including soluble COD, COD removal, solids concentration.
2. Investigate the effect of pre-treatment on methane yield.
3. Examine the effects of MW pre-treatment on reaction kinetics using the Modified-Gompertz model.

4.1 Literature Review

According to Cheng and Brewer [1], stillage fractions typically contain higher concentrations of cellulose and hemicellulose and lower levels of lignin, because it has low dissolvability in the fermentation liquor in ethanol production. Kim et al. [12] compiled a detailed compositional analysis on various corn dry-grind stillage fractions over a five-year period. They concluded that wet distillers' grain contained 20.9% xylan and arabinan contents (hemicelluloses) and 12.6% cellulose content on a dry matter basis. Wu [13] measured sugars of various corn co-product fractions and found that they consisted of pentoses and hexoses, which are the branches that make up hemicellulose structures. Therefore, pre-treatments to target cellulose and hemicelluloses should be selected to improve the biomethane production of wet cake.

Under neutral conditions, hemicelluloses can begin to break down at around 150ºC-180ºC [14]. Heating above 160ºC can also cause lignin to solubilize and induce the formation of phenolic compounds, which are toxic to methanogens [14] [15] [16]. Ethanol co-products normally contain low amounts of lignin, so thermal pre-treatment is a desirable option. However, there are other barriers to conventional heating as a pre-treatment. A significant portion of energy goes towards heating up the material [17]. Above boiling point, some of the energy is lost towards water vaporization [15]. Improved solubilization can occur at low temperatures and longer pre-treatment times, but this can result in Maillard reactions between carbohydrates and amino acids. Maillard reactions can also occur at temperatures above 150ºC [15]. Ethanol co-products are often rich in amino acids, making them ideal feed for cattle [18]. However, for AD this can be a concern.

Microwave (MW) pre-treatment is a novel technique. Eskicioglu et al. [19] compared conventional heating and microwave treatment on waste-activated sludge and found that microwave treatment illustrated superior methane production (16% increase at 96ºC). The use of a MW has many benefits, including shorter interaction times, resulting in a better energy balance than conventional heating [20] [21]. It is also much quicker to achieve the desired temperature due to rapid start-up and stopping time [21]. Other benefits include better control over heating rates, selective heating of material, reduced energy losses, and space savings compared to conventional heating units [21] [22]. Table 4.1 displays a summary of microwave pre-treatment conditions for various feedstock prior to anaerobic digestion.

There is some evidence that MW treatment can be beneficial for stillage and other similar feedstock. For instance, Bochmann et al. [23] conducted microwave pre-treatment on brewer's spent grains at temperatures between 100ºC to 200ºC. The authors observed a direct correlation between temperatures up to 200ºC and increased COD solubilization and sugars concentration. The BMP of pre-treated samples were 10% to 27% higher than the control up to 140ºC. Carbohydrate analysis revealed evidence of a Maillard reaction at temperatures above 160ºC.

Literature on microwave pre-treatment is mostly focused on treatment at high temperatures. This is because solubilization enhancement is normally better. However, low-temperature pre-treatments can still have a considerable impact on the solubilization of COD and biogas yield [21] [24]. For example, low-temperature and near-boiling point MW treatment on sludges result in similar or better COD solubilization than moderate temperatures (around 120ºC) [21]. Low-temperature pre-treatment can be more energy efficient because little of the MW energy is going towards water vaporization [15] [25]. Eskicioglu [24] studied the effects of low temperature MW pre-treatment on waste activated sludge. The treatment exhibited a three-fold increase in soluble COD at 75ºC and improvements in biogas production of up to 15% and 20% compared to the control.

Recent studies on low-temperature MW pre-treatment are normally accompanied by the use of acids and alkalis to enhance the solubility of the lignocellulosic structure. Gunes et al. [26] reported extraordinary results from low-temperature microwave pre-treatment with sodium hydroxide addition. Treatment at 240 W and 99ºC tripled the biomethane yield as compared to the control. Treatment at 400 W and 110ºC resulted in decreased lignin removal. However, this study imposed a fixed duration as opposed to a fixed temperature, so potential benefits of reduced time could not have been examined.

Although inclusion of chemicals in microwave studies has reported excellent results, it is possible that benefits may be hampered by economics. Disregarding cost, chemical type and dosage must be selected carefully so as to not inhibit the anaerobic process. For instance, sulfuric acid should be used with care, to not inadvertently stimulate hydrogen sulfide production and reduce overall energy recovery [14]. Moreover, for successful anaerobic digestion of organics, the sample needs to be brought back to neutral, which implies even more costs. In order for pre-

treatments to be effective, the other effects on the AD system as a whole should quantified, such as the economics, energy balance, and technical feasibility [27].

A compilation of literature on microwave pre-treatments prior to anaerobic digestion is shown in Table 4.1.

4.1.1 Background on Microwave Pre-treatment

Microwave irradiation can be used to break down lignocellulosic compounds and enhance hydrolysis [16] [28]. The release of thermal energy is the response from the vibration of dipolar molecules, such as water, proteins, lipids, and other organics, and from the migration of free salts [21] [24] [29]. The rapid, oscillating movement of the electromagnetic field causes polarized molecules to align themselves with the poles of the magnetic field [21] [24] [30]. In turn, the kinetic energy is released as heat [24]. The secondary mechanism associated with microwave heating is ionic conductance, in which the movement of ions in the electric field generates an electric current [24] [30]. Thermal energy is released when ions collide with molecules or atoms [21] [30]. Often, these mechanisms work simultaneously to generate heat and cannot be distinguished from one another [30] [31]. Some researchers have reported that MW pre-treatment can also provide an athermal effect, which is thought to occur due to polarized side chains of macromolecules attempting to align with the electromagnetic field, which could result in the breaking of hydrogen bonds [21] [29].

According to Vollmer [32], absorbance of MW energy is dependent on the state of matter. Physical and chemical properties may help determine the type of interaction a sample could experience when subjected to MW pre-treatment [24].

An important parameter to consider is the dielectric constant. The dielectric constant is a representation of the ability of a substance to obstruct electromagnetic waves – therefore, the higher the dielectric constant, the more energy the sample can absorb [24]. As such, materials can be categorized as (1) absorptive, (2) transparent, or (3) reflective. Absorptive materials include aqueous substrates, whereas metals and graphite are examples of reflective materials [24]. Transparent materials can be good options for vessels to heat substances, as microwave energy passes through them without heating. Examples of transparent materials are Pyrex glass and Teflon [24].

Table 4.1 Literature review for microwave pre-treatment studies prior to anaerobic digestion.

Feedstock	Conditions	Results	References
Brewer's spent grains	• Temperature: 100ºC-200ºC • Pressure: 40 bar • Duration: 15 minutes	• Up to 140ºC, improvements in biomethane yield up to 28% were observed • Beyond 160ºC, there were indicators of Maillard reaction intermediates and end products	[23]
Waste-activated sludge	• Temperature: 50ºC, 75ºC, 96ºC • Intensity: 625 W, 1250 W	• Higher solubilization at lower intensity for all temperatures due to better interaction between substrate and MW energy • Highest SCOD/TCOD ratio at 625 W and 75ºC. • 20% improvement in biogas yield at 96ºC for both intensities	[24]
Kitchen waste	• Temperature: 175ºC • Heating rate: 1.9ºC/min, 3.9ºC/min, 7.8ºC/min • Holding time: 1 minute	• Highest solubilization was achieved at a heating rate 1.9ºC/min • Anaerobic biodegradability was improved at the highest heating rate	[33]
Wheat straw	• Temperature: 100ºC, 120ºC, 150ºC, 180ºC • Power range: 400 W – 1600 W • Heating rates: 5ºC/min, 3.75ºC/min, 2.5ºC/min • Holding time: 0, 15, 30 minutes	• SCOD/TCOD ratio and VDS/TDS ratio increased with increasing temperature • Maximum improvement of 28% compared to control at 150ºC • Pre-treated samples reached t_{80} quicker than control • No improvements in anaerobic biodegradability or kinetics attributed to different heating rates or holding time	[34]
Whiskey pot ale	• Temperature: 20ºC-110ºC • Power range: 80 W, 240 W, 400 W • Duration: 11 minutes • Holding time: 4 minutes for 240 W samples • Addition of 1 M NaOH solution	• Improved biodegradability as compared to control at 80 W and 240 W (at 60ºC and 99ºC, respectively) – illustrated by enhanced hemicellulose fraction • Tripled biomethane yield at 240 W • Some delay in kinetics attributed to microbial acclimation to alkalinity	[26]

Sun et al. describe the penetration depth of a sample as the distance from the surface to the place where the strength of the electromagnetic field drops to e^{-1} (≈ 0.368) of its value at the surface [30]. The penetration depth of a sample should be proportional to the dimensions of the sample.

Too shallow of a penetration depth may reduce heating efficiency of a sample and create hot spots in the sample, even if the substance has a high dielectric constant [30]. The depth should be selected such that the entire sample can be heated uniformly with minimal losses. Eskicioglu [24] summarized a table from Decareau [8], describing the effect of moisture content of a semi-solid substrate on penetration (see Table 4.2)

Table 4.2 Suggested penetration depths for low, moderate, and high moisture samples. Adapted from [8] [24].

		Penetration Depth (cm)	
Moisture	**Dielectric constant (ε')**	**915 MHz**	**2450 MHz**
High	60	8.4	3.1
Moderate	20	11.7	4.4
Low	10	22.1	8.2

4.2 Materials and Methods

4.2.1 Preparation of Inoculum

Anaerobic digested sludge (ADS, or seed sludge) served as the inoculum and was collected from Ontario Clean Water Agency (Stratford, Ontario, Canada). The raw sludge is pumped to the primary digesters, which operate at an average temperature of 37ºC and a residence time between 16-20 days. Most of the sludge settles in the primary clarifiers, where return activated sludge is wasted to primary clarifiers and settled with raw sludge. The primary ADS was stored in a cold storage room at 4 ºC at the University of Windsor (Windsor, Ontario, Canada). It is reported that seed sludge can be stored at 4 ºC for 14 days and maintain methanogenic activity like that of fresh inoculum [35]. The seed sludge was sieved (2000 μm) after visual inspection for large particles and hairs.

4.2.2 Preparation of Substrates

A 20 L sample of wet cake with syrup addition was collected from Hiram Walker & Sons Limited (Windsor, Ontario, Canada). The wet cake was blended with distilled water at a 1:2 ratio to achieve a homogenized mixture using an electric blender (Ninja model, model number NJ

600WMW). The blending power used, and duration of blend were 900 W and 5 minutes, respectively. Afterwards, the wet cake blend was sieved (2000 μm) and homogenized in the blender for an additional 10 minutes.

The sample was stored in a cold storage room at 4 ºC at the University of Windsor (Windsor, Ontario, Canada). Previous studies have shown that storage of stillage fractions at 4 ºC are effective in reducing volatile solids loss over the course of one week (less than 2%) [36] [37] [38] [39]. Long term storage studies have not yet been completed on stillage fractions, so the waste was used as soon as possible.

A 500 mL sample of the wet cake and the inoculum were collected in plastic bottles and stored in the cold room. Before characterization, the bottles were placed in a 25ºC - 30ºC water bath for 20 minutes. The remaining feedstock samples were stored in a 20 L container in the cold room and later used to create the mixing ratios needed for the BMP.

4.2.3 Microwave Pre-treatment

Microwave pre-treatment was carried out using 1.5 ft^3 capacity household microwave (LG Electronics Inc. LMC1575 + inverter, 1200 W, 2450 MHz frequency and 12.24 cm wavelength). Temperatures were measured using a high accuracy digital thermometer (OMEGA Engineering DP97, resolution of 0.01ºC over -199.99 ºC to +849.99 ºC range). Wet cake samples were microwaved to 50ºC, 70ºC, and 90ºC at 480W and 1080W, respectively (with an average power output efficiency of 76%)

4.2.4 Analytical Methods

Substrate and inoculum characterization was carried out prior to BMP test setup. Total and soluble chemical oxygen demand (TCOD and SCOD), total volatile fatty acids (TVFAs), sulfate (SO_4), total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH_3-N), and total phosphorus (TP) were measured using HACH methods and test kits (DR6000 Benchtop Spectrophotometer). Solids (TS, VS, TSS, and VSS) were analyzed in accordance with Standard Methods [40]. The pH level was measured using the Easy pH EasyPlus Titrator from Mettler Toledo (Mettler Toledo, USA). The pH meter was calibrated using buffers at pH 4.0 ± 0.01 and 7.0 ± 0.01. Soluble parameters were determined after 0.45 μm filtration.

The results of the characterization are outlined in Table 4.3.

Table 4.3 Average substrate and inoculum characteristics with standard deviations

Parameter	Units	Wet Cake	Inoculum
TCOD	g/L	183.8 ± 8.4	23.7 ± 0.2
SCOD	g/L	55.9 ± 0.2	***n.d.***
TVFA	mg/L	9508 ± 28	***n.d.***
TN	mg/L	932 ± 19.4	506 ± 17.0
TKN	mg/L	901 ± 20.0	507 ± 18
NH_3-N	mg/L	133 ± 2.0	85 ± 0.6
SO_4	mg/L	99 ± 0.4	95 ± 0.1
TP	mg/L	2497 ± 58	779 ± 49
COD:N:P	-	250:1:3	250:5:8
TS	g/L	157.7 ± 3.1	32.8 ± 1.3
VS	g/L	149.3 ± 3.8	18.9 ± 0.7
TSS	g/L	104.3 ± 2.9	34.4 ± 2.8
VSS	g/L	100.9 ± 2.9	20.3 ± 1.4
VS/TS (%)	%	94.7	57.6
pH	-	3.7 ± 0.0	7.5 ± 0.0
Alkalinity	$mgCaCO_3/L$	***n.d.***	4906 ± 257

A total solids analysis was completed on the solid wet cake sample to determine if the dilution and homogenization with distilled water (DIW) was accurate. A comparison between the solid wet cake and homogenized wet cake (as g TS/g WCK) showed an error of less than 10%.

4.2.5 Experimental Design

The selected penetration depth of the wet cake sample was 3.1 cm, in accordance with Table 4.2, as there is a lack of microwave pre-treatment studies on distillery stillage. The desired penetration depth translated to approximately 100 mL of sample (Wheaton BOD Bottle, USP Type I borosilicate glass, 300 mL capacity).

A temperature range below boiling point (50ºC, 70ºC, and 90ºC) was selected to avoid loss of substrate and to minimize liquid evaporation, which reduces the efficiency of MW pre-treatment [25].

Intensities of 40% and 90% MW power translate to approximately 480 W and 1080 W, respectively. These power levels were selected based on the ranges given in Table 4.1. Since some lower temperature levels were selected (50ºC and 70ºC), a slightly higher initial power level was selected as the starting point. As mentioned earlier, prolonged interaction time at low

temperatures can induce Maillard reactions. The higher power level was selected to determine the effect of a faster heating rate on the sample and to provide an array of parameters. See Table 4.4 for a summary of the design parameters for the microwave treatment study.

Fresh samples were obtained for the solubilization analysis and the BMP. After treatment, the samples were allowed to cool until they reached room temperature. The samples were poured into a graduated cylinder and adjusted with distilled water to account for losses due to evaporation.

Table 4.4 Experimental design for microwave pre-treatment

	Intensity	
	480 W	**1080 W**
Temperature	50ºC	50ºC
	70ºC	70ºC
	90ºC	90ºC

4.2.5.1 Microwave Calibration

Prior to conducting pre-treatment studies, the microwave needed to be calibrated to determine average efficiency at the desired power levels. This calibration was carried out as specified by Saxena [41]. Pyrex glass beakers filled with 1 liter of distilled water were submerged in a water bath set to 25ºC for 30 minutes. Then, the average energy output of the microwave was determined by heating the distilled water for two minutes at the desired power levels. The initial and final temperatures of the water were measured using a high accuracy thermometer. Power output was reported as an average of triplicates (see Table B.1).

As mentioned by Eskicioglu [24] and Vollmer [32], absorption of MW energy is unique to sample characteristics and concentrations. A calibration curve was prepared to determine ramp time to achieve desired pre-treatment temperatures (50ºC, 70ºC, and 90ºC). Prior to MW pre-treatment, the bottles were submerged in a water bath at 25ºC for 30 minutes. Next, 100 mL of wet cake was added to 300 mL glass bottles ((Wheaton BOD Bottle, USP Type I borosilicate glass, 300 mL capacity). The samples were microwaved at 30 second intervals to build a calibration curve, shown in Figure B.1.

4.2.5.2 BMP Setup

The pre-treatment study was carried out using 500 mL glass bottle reactors. The seed sludge in each bottle was set to 300 mL in all bottles. The food-to-microorganism ratio was set 0.5 to ensure sufficient time for the microbial activity and avoid overloading the reactor. Based on the F/M ratio, and VSS content of the seed sludge, the volumes of substrate were calculated using (1). Distilled water (DIW) was added to each bottle to equalize the volumes across all reactors.

$$\frac{F}{M} = \frac{TCOD_{substrate} \times V_{substrate}}{VSS_{seed} \times V_{seed}} \quad (5)$$

Where $\frac{F}{M}$ is the food-to-microorganism ratio as g $TCOD_{substrate}$/g VSS_{seed}, $TCOD_{substrate}$ is the TCOD of the substrate in grams per liter (g/L), $V_{substrate}$ is the volume of substrate in liters (L), VSS_{seed} is the VSS concentration of the seed sludge in g/L, and V_{seed} is the volume seed sludge in L. Details of the bottle setup can be seen in Table B.6.

Initial pH was set to 7.1 ± 0.1 at the beginning of the experiment with the addition of either 4.5 N sodium hydroxide or concentrated hydrochloric acid. External buffer was added in the form of sodium bicarbonate ($NaHCO_3$). A 50 mL sample was removed from each bottle and stored in a refrigerator for analysis, leaving a working volume of 450 mL. Bottles were flushed with compressed nitrogen gas at 8 psi for two minutes (Linde Canada, Canada). Incubation temperature was set at 38 ± 1ºC, which falls into the mesophilic temperature range.

The bottle setup was verified via TCOD and Solids analysis. Since the error between expected and measured values were less than 10%, the setup is considered acceptable.

The bio methane potential study was carried out using the Automatic Methane Potential Test System (AMPTS II) supplied by Bioprocess Control [42]. The AMPTS II consists of three units: (1) a sample incubation unit, (2) a CO_2 capture unit, and (3) a gas volume measuring device. The sample incubation unit (or water bath) has a capacity of up to 15 glass bottle reactors (500 mL each). The media in each bottle is mixed using a slow-rotating stirrer. Biogas produced accumulates in the headspace of the vessel. Then, the biogas passes through vials containing an alkaline solution (NaOH). Carbon dioxide, and hydrogen sulfide are captured by the solution, whilst allowing CH_4 to flow through to the gas measuring unit. The methane gas from the CO_2-

fixing unit is measured using a wet gas flow measuring device with a multi-flow cell arrangement (15 cells). Using principles of liquid displacement and buoyance, the device monitors gas flows and generates a digital pulse for a specified volume measurement. The embedded data acquisition system records and normalizes the data in real-time. Figure 4.1 illustrates the AMPTS setup.

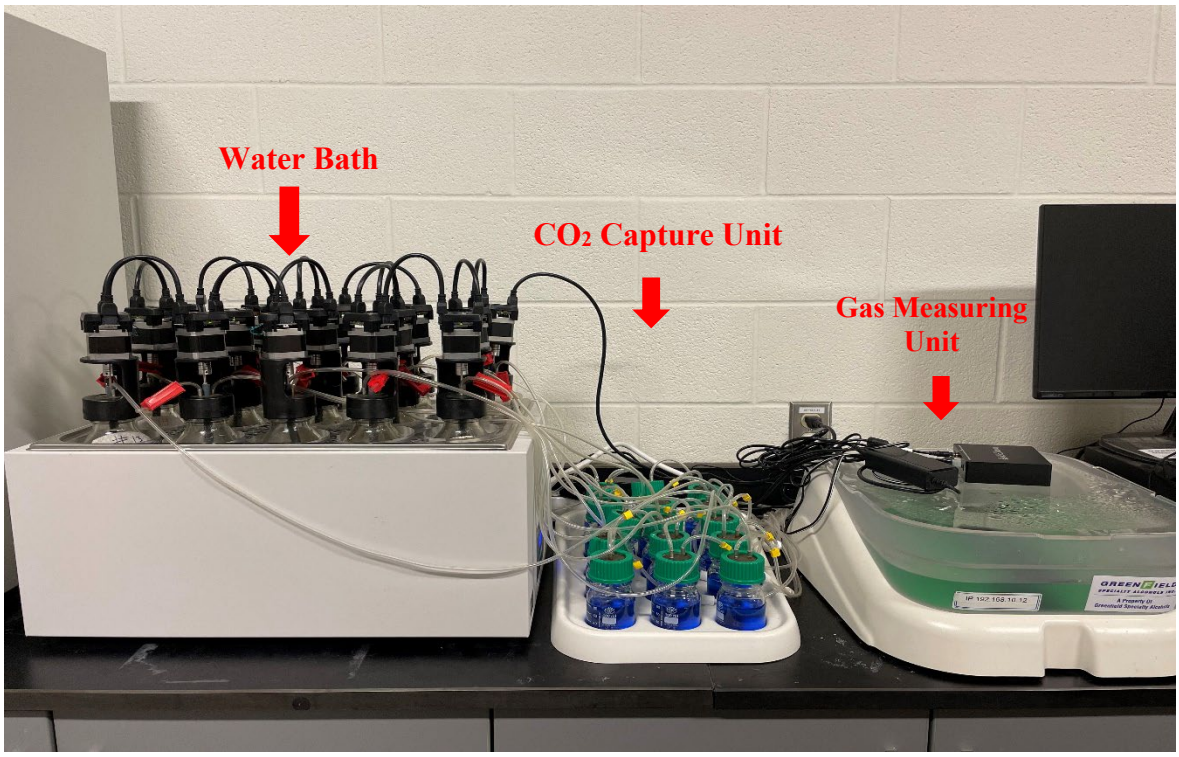

Figure 4.1 BMP setup in Automatic Methane Potential Test System (AMPTS II).

4.3 Data Analysis

4.3.1 Statistical Analysis

Comparisons of biomethane yields between mixture groups were evaluated using single factor ANOVA testing and Tukey's post-hoc test. Synergistic and antagonistic effects were determined using the student's t-test. Statistical significance was established at $P < .05$ level.

4.3.2 Kinetic Modeling

The Gompertz model has been widely used to illustrate the growth of animals and plants, and the volume of bacteria and tumor cells [43]. The Modified-Gompertz model re-expresses the Gompertz equation with more relevant parameters for anaerobic digestion (lag phase, growth rate, maximum biogas production rate) to make the model easier to use [44] [45]. The Modified-

Gompertz model is attractive for anaerobic digestion researchers as it only requires cumulative methane production to determine kinetic constants and goodness of fit [45]. The equation is shown below:

$$y = Aexp\left\{-exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\} \qquad (6)$$

Where y is the cumulative methane production in mL at time t. A is the maximum methane production in mL, μ_m is the methane production rate in mL/day, t is the time in days, and λ is the lag phases in days.

Model parameters were obtained using Excel non-linear regression solver, where the residual sum of squares between the experimental and predicted data was minimized.

As explained by Koppar and Pullammanappallil [46], cumulative methane production curves only asymptotically approach the maximum methane yield. Thus, the time taken to achieve 95% of the methane potential was selected to use as an estimate for the hydraulic retention time of an anaerobic digester [47]. The equation is shown below:

$$t_{95} = \frac{A}{\mu_m}(1 - ln(-0.95)) + \lambda \qquad (7)$$

Where t_{95} is the time required to achieve 95% of the maximum methane yield in days. A is the maximum methane production in mL, μ_m is the methane production rate in mL/day, and λ is the lag phases in days.

The effective digestion time, t_{eff}, can be found from subtracting the lag phase from t_{95}.

$$t_{eff} = t_{95} - \lambda \qquad (8)$$

4.4 Results and Discussion

Prior to the BMP study, the microwave pre-treatment was evaluated for solubilization. As mentioned in Chapter 2, typically anaerobic pre-treatment studies analyze the samples for solubilization. Solubilization can be represented as the portion of COD that is soluble (SCOD) and the amount of volatile dissolved solids that are present. Table B.2 to Table B.5 illustrate COD and Solids testing of the samples before and after MW treatment for all pre-treatment levels.

4.4.1 Solubilization

Table B.2 illustrates the results of MW-treatment on solubilization of COD. It appears as though there was no significant impact on the solubilization of COD after MW-treatment. Effective solubilization should have been indicated in a decrease in the suspended solids concentration and an increase in the soluble COD portion. Once the compounds are solubilized, they are more accessible to microorganisms. Looking at Table B.5, it appears as though there is an increase in dissolved solids concentration for the 480W, T = 50°C and 1080W, T = 70°C treatment levels. However, since there was no corresponding decrease in the total suspended solids concentration, it can be deduced that this is due to the propagation of errors from the other solids measurements.

Since there are no previous studies of low-temperature MW treatment on stillages, similarities in literature cannot be compared. However, Bochmann et al. [23] used MW-treatment on brewers' spent grain and even at the lowest temperature (100ºC), the soluble COD increased by over double. In this study, even as the temperature approaches the boiling point, there are no observable increases in SCOD. It is possible that this effect may be attributed to the use of holding time – which can be a method of improving solubilization [23].

This conclusion implies that the anaerobic biodegradability of the substrate will not be affected during the BMP study.

4.4.2 BMP Test

The total COD balance for all bottles ranged from 94% to 107%, indicating good mass closure (see Table B.7). Theoretically, it is not possible to achieve a COD mass balance over 100%, but the error found in this study falls within ranges reported in literature [50]. The control bottles achieved an average of 90% of the expected volume. The average methane yields for each mixture and their biodegradability are consolidated in Table 4.5. The net biomethane curves are displayed in Figure 4.2.

Table 4.5 Average biochemical methane potential (BMP) of treatment levels based on COD and VS added with standard deviations.

Treatment Level	Biomethane Yield		Biodegradability
	NmL CH_4/g COD_{added}	NmL CH_4/g VS_{added}	
Untreated	236 ± 25	318 ± 29	67%
480W, T=50ºC	208 ± 19	259 ± 24	60%
480W , T=70ºC	203 ± 20	258 ± 28	58%
480W , T=90ºC	184 ± 6	231 ±9	53%
1080W, T=50ºC	212 ± 8	264 ± 13	61%
1080W, T=70ºC	201 ± 16	256 ± 20	57%
1080W, T=90ºC	203 ± 14	252 ± 17	58%

The untreated sample from this study was compared to the biomethane yield achieved from the previous study (see Chapter 3). The biodegradability of the untreated mixture in this study is 67%, as opposed to the previous study where the biodegradability was 72%. A t-test showed that the methane yields are statistically similar, thus confirming reproducibility.

Although it may appear that the methane yield decreases across treatment levels, ANOVA testing of the methane yields revealed that there was no significant difference between the untreated and the treated samples ($p=0.06$). The average methane yields with the 95% confidence interval are plotted in Figure 4.3.

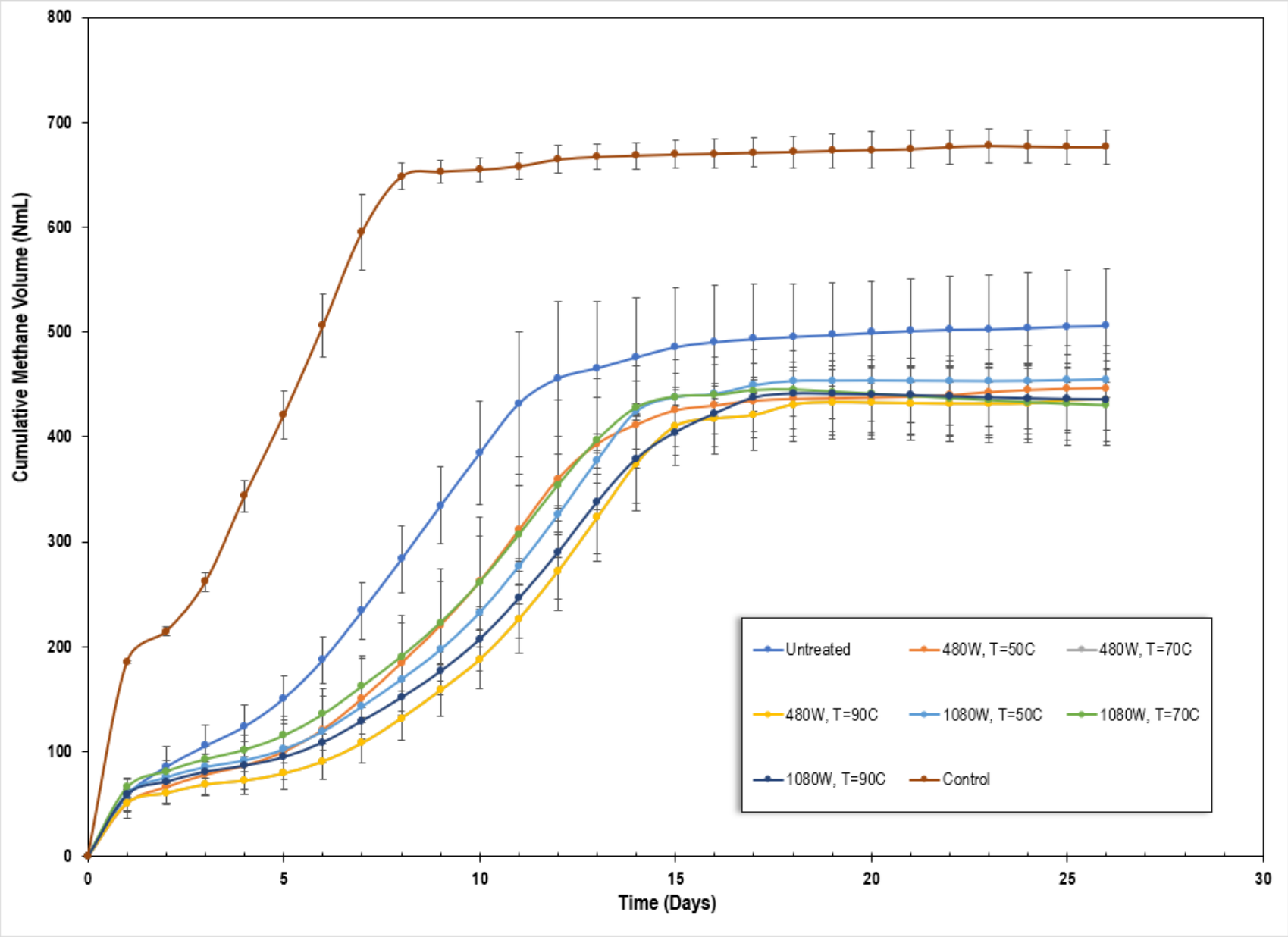

Figure 4.2 Average cumulative net methane production curve plotted against time with respective standard deviations (n=3).

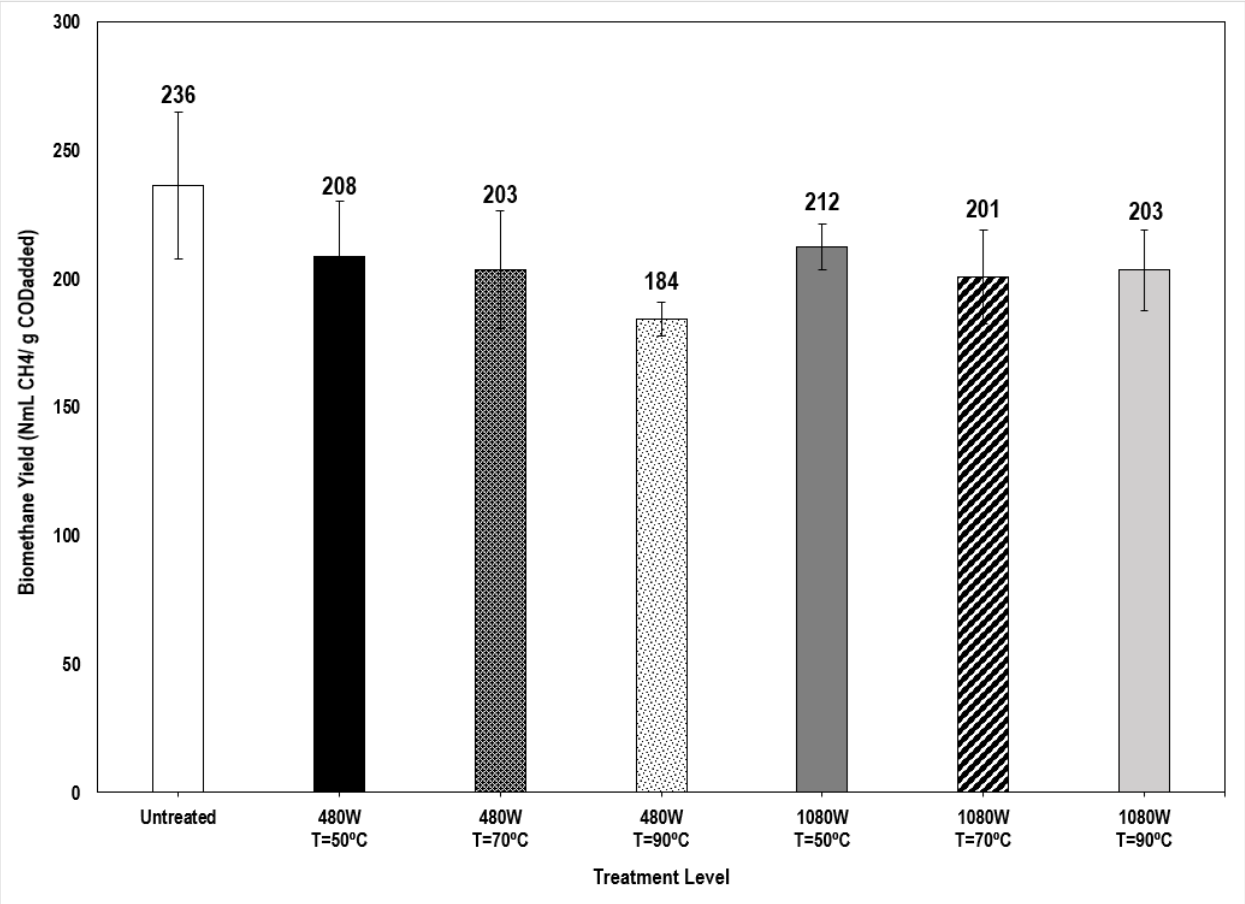

Figure 4.3 Average BMP yields plotted with 95% confidence intervals.

4.4.3 Kinetics

The biomethane production data fit well with the predicted data outputted from the Modified-Gompertz model. This is reflected by the high R^2 values achieved for all treatment levels (ranging from 0.9592 to 0.9960). A summary of the kinetic constants for each treatment level are shown in Table 4.6.

Table 4.6 Summary of averaged kinetic parameters and their standard deviations from Modified-Gompertz modeling

Treatment		A NmL	μ_m NmL/day	λ day	t_{95} day	t_{eff} day	R^2
	Untreated	520 ± 54	48 ± 6.6[a]	1.5 ± 0.1	18 ± 1.5	16 ± 1.6	0.9875
480W	T = 50ºC	467 ± 39	39 ± 3.0[b]	2.6 ± 1.0	20 ± 1.5	17 ± 1.1	0.9787
480W	T = 70ºC	472 ± 43	35 ± 1.8[b]	3.6 ± 0.7	23 ± 0.6	20 ± 1.3	0.9697
480W	T = 90ºC	420 ± 15	31 ± 5.2[b]	1.7 ± 0.5	22 ± 3.8	20 ± 4.3	0.9793
1080W	T = 50ºC	493 ± 19	36 ± 0.8[b]	2.3 ± 0.1	23 ± 1.1	20 ± 1.2	0.9702
1080W	T = 70ºC	467 ± 43	37 ± 2.4[b]	1.9 ± 0.8	20 ± 2.3	18 ± 1.6	0.9648
1080W	T = 90ºC	489 ± 58	34 ± 3.2[b]	2.6 ± 0.3	24 ± 4.8	22 ± 4.8	0.9636

Different letters represent statistical differences ($P < .05$) between assays for each parameter (μ_m).

Curve fitting for each the average net methane volume of each mixture is illustrated in Figure B.2 to Figure B.8.

Looking at Table 4.6, MW treatment at all levels resulted in a significant decrease in the production rate, as compared to the untreated ($P < .05$). At 480W, the decrease in rate was between 20% to 35%, whereas at 1080W, the decrease in rate was between 22% to 30%. This is clearly displayed in Figure 4.2, as the net methane curve for the untreated sample is steeper than that of the treated samples.

Although the maximum methane production rate decreased amongst treated samples – the time taken to achieve 95% of the total methane volume and the effective digestion time are statistically similar amongst all samples.

The slower kinetics exhibited by the microwave-treated samples can be explained by Inglett et al [51]The authors studied the effect of microwave irradiation on phenolics production from corn dried distillers' grains with solubles (DDGS). At temperatures of 23-100ºC, significant increases in phenolic compound content were detected, with major acids being *p*-coumaric, sinapic, ferulic, and caffeic. These are known to be inhibitory to anaerobic digestion. The authors noted that the presence of high levels of phenolics extracted from DDGS was surprising – since they are normally present in the bound form as lignin. So, at low temperatures, there should not be significant production of phenolics. However, the authors theorized that this was likely due to the yeast used during ethanol fermentation, which produce enzymes that free bound phenolic acids.

According to Chen et al. [52], methane production is only inhibited by phenolic acids at very high concentrations. However, there are some reports of 50% decrease in biogas production in ranges of 120 to 594 mg/L of phenolic compounds [53]. Mikucka and Zielinska [54] investigated the effect of individual phenolic acids on the anaerobic digestion of corn distillery stillage. The authors described adverse effects on the methane yield and methane production rate, even at low concentrations (0.5-1.0 g/L). The acids that had a significant effect on anaerobic digestion were vanillic, ferulic, syringic, and *p*-coumaric.

Therefore, in this study, it is likely that there was some production of phenolic acids after microwave treatment, which resulted in slower kinetics. Since there was no significant effect on the methane yield, it is likely that the total phenolic acids concentration was low, or it was converted into methane [54]

4.5 Conclusions

The purpose of this study was to perform microwave pre-treatment on distillery stillage and examine the effect on solubilization, biomethane yield, and degradation kinetics. Solubilization was determined by changes in substrate characteristics, such as COD and Solids concentration. Changes in degradation kinetics were studied using the Modified Gompertz model. Based on the results of this study:

1. Low temperature microwave pre-treatment had no significant effect on the SCOD of wet cake.
2. The biomethane yield remained the same after low-temperature microwave treatment, confirming that no significant solubilization had occurred.
3. The methane production rate decreased by 20%-35% at 480W and by 22% to 30% at 1080W. The decrease in rate could be explained by the release of phenolic compounds by the enzymes found in the yeast used during ethanol fermentation. Since t_{95} and t_{eff} statistically remained the same, this implies that the phenolics production was relatively low or was consumed by the microbes and turned in methane.

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CHAPTER 5
ENGINEERING SIGNIFICANCE AND FUTURE RECOMMENDATIONS

5.1 Engineering Significance

Chapter 3 focused on the co-digestion of municipal wastes with stillage from a local distillery. Co-digestion enhanced the biomethane yield and resulted in statistically similar yields across all ratios. This implies that these substrates can be mixed at various ratios and can provide the same energy recovery – thus providing more flexible options for diversion. A centralized anaerobic digester in a small or medium sized community can be a cost-effective waste management solution. Although degradation kinetics did not improve through co-digestion, acclimation of the inoculum can help reduce overall digestion time and operational costs.

Chapter 4 explored low-temperature microwave treatment on stillage from a local distillery. Microwave treatment resulted in the break down of larger suspended solids into colloidal solids. The treatment had no effect on the soluble COD and thus, anaerobic biodegradability was not affected. However, the methane production rate for all treated samples decreased substantially, implying that the treatment released common inhibitors. Thus, low-temperature MW treatment did not enhance AD of stillage.

5.2 Limitations of Research

- Limitations of the study are discussed below:**Full BMP bottle characterization:** In Chapter 3, the effect of mixing ratios on AD of wet cake, SSOs, and raw sludge was evaluated. Due to limited resources, full characterization of BMP bottles were not carried out. In Chapter 3, the effect of mixing ratios on co-digestion were evaluated based on biomethane yield, synergism and antagonism, and degradation kinetics. Additional characterization on the alkalinity and VFA concentration before and after AD could be used to determine how the system may have behaved during digestion.
- **Microwave equipment:** In Chapter 4, microwave pre-treatment was carried out on wet cake to enhance hydrolysis of lignocellulosic compounds. The pre-treatment was carried out using a household microwave at temperatures below the boiling point in an open vessel. The effect of higher temperatures could not be evaluated because open vessel treatment is limited to the boiling point, if losses to evaporation are to be avoided. An

industrial scale microwave can be used to achieve higher temperatures in a closed vessel without destroying it. Industrial microwaves also have pressure and temperature sensors that can determine the maximum pressure that can be achieved.

5.3 Future Recommendations

Future works to be considered are:

- **Continuous stage anaerobic digestion**: Batch test studies can only give preliminary information on the anaerobic degradability of a substrate. A continuous reactor setup can be used to experiment on different organic loading rate and determine if any instability or inhibition is observed.
- **Pre-treatment methods:** Since microwave pre-treatment did not improve digestion of stillage, other treatment options could be explored. Thermochemical pre-treatment could be revisited, despite the high costs associated with chemical dosing. Chemical pre-treatment may be more feasible when combined with conventional thermal treatment since the dosage may be smaller. A dilute acid could be used to solubilize the hemicelluloses that are present in stillage fractions.
- **Life cycle analysis (LCA):** After continuous stage AD studies have been carried out, it may be helpful to perform an LCA on the feasibility of centralized anaerobic digestion for small and medium-sized communities Capital costs, operating costs, and transportation costs should be identified. A comparative LCA can determine the potential bottlenecks, energy savings, or cost savings by digesting SSOs alone, by co-digesting with stillage, and by co-digesting with stillage and raw sludge at varying ratios. Costs for biogas upgrading and cleaning, connection lines, and potential funding should be accounted for.

Appendix A: Supplementary Information for Chapter 3

Table A.1 Mixing ratio composition calculated based on VS ratios.

Mix	Expected VS (g/L)	WCK			RS			SSO			Vol. DIW (mL)	Total Volume (mL)
		VS (g/L)	VS (g)	Volume (mL)	VS (g/L)	VS (g)	Volume (mL)	VS (g/L)	VS (g)	Volume (mL)		
A (100% WCK)	25	106.0	25.0	235.8	28.6	0.0	0.0	78.0	0.0	0.0	764.2	1000.0
B (100% RS)	25	106.0	0.0	0.0	28.6	25.0	874.1	78.0	0.0	0.0	125.9	1000.0
C (100% SSO)	25	106.0	0.0	0.0	28.6	0.0	0.0	78.0	25.0	320.5	679.5	1000.0
D (25% WCK, 75% SSO)	25	106.0	6.3	59.0	28.6	0.0	0.0	78.0	18.8	240.4	700.7	1000.0
E (50% WCK, 50% SSO)	25	106.0	12.5	117.9	28.6	0.0	0.0	78.0	12.5	160.3	721.8	1000.0
F (75% WCK, 25% SSO)	25	106.0	18.8	176.9	28.6	0.0	0.0	78.0	6.3	80.1	743.0	1000.0
G (33% WCK, 33% RS, 33% SSO)	25	106.0	8.3	78.6	28.6	8.3	291.4	78.0	8.3	106.8	523.2	1000.0

Table A.2 BMP bottle setup based on F/M ratio of 0.5.

	Mix	Bottle No.	Seed Sludge (mL)	Substrate (mL)	DIW (mL)	Total (mL)	Expected TCOD (g/L)	Expected VS (g/L)	Theoretical Yield (mL)
F/M = 0.5	**A** (100% WCK)	1	300.00	79	121	500	23.3	15.6	3662
		2	300.00	79	121	500	23.3	15.6	3662
		3	300.00	79	121	500	23.3	15.6	3662
	B (100% RS)	4	300.00	63	137	500	23.3	15.1	3662
		5	300.00	63	137	500	23.3	15.1	3662
		6	300.00	63	137	500	23.3	15.1	3662
	C (100% SSO)	7	300.00	58	142	500	23.3	14.6	3662
		8	300.00	58	142	500	23.3	14.6	3662
		9	300.00	58	142	500	23.3	14.6	3662
	D (25% WCK, 75% SSO)	10	300.00	58	142	500	23.3	14.6	3662
		11	300.00	58	142	500	23.3	14.6	3662
		12	300.00	58	142	500	23.3	14.6	3662
	E (50% WCK, 50% SSO)	13	300.00	63	137	500	23.3	15.0	3662
		14	300.00	63	137	500	23.3	15.0	3662
		15	300.00	63	137	500	23.3	15.0	3662
	F (75% WCK, 25% SSO)	16	300.00	67	133	500	23.3	15.2	3662
		17	300.00	67	133	500	23.3	15.2	3662
		18	300.00	67	133	500	23.3	15.2	3662
	G (33% WCK, 33% RS, 33% SSO)	19	300.00	63	137	500	23.3	15.1	3662
		20	300.00	63	137	500	23.3	15.1	3662
		21	300.00	63	137	500	23.3	15.1	3662
	Control	22	300.00	24	176	500	23.3	16.9	3662
		23	300.00	24	176	500	23.3	16.9	3662
		24	300.00	24	176	500	23.3	16.9	3662
	Blank	25	300.00	0	200	500	17.9	11.9	2816
		26	300.00	0	200	500	17.9	11.9	2816
		27	300.00	0	200	500	17.9	11.9	2816

Table A.3 COD balance, TCOD removal efficiency, and pH of BMP bottles.

Mix	Bottle #	TCOD of Bottles				ΔTCOD		CH_4		COD Balance	TCOD Removal Efficiency	Initial pH	Final pH
		Initial (mg/L)	Final (mg/L)	Initial (mg)	Final (mg)	mg/L	mg	NmL	mgCOD				
A	1	22083	16881	9937	7596	5202	2341	923	2637	103%	76%	7.20	7.92
	2	22237	16922	10007	7615	5315	2392	912	2607	102%	76%	7.13	8.05
	3	22555	17087	10150	7689	5469	2461	893	2552	101%	76%	7.04	7.97
B	4	22035	17284	9916	7778	4750	2138	734	2096	100%	78%	7.20	7.96
	5	22134	17424	9960	7841	4709	2119	754	2155	100%	79%	7.16	7.88
	6	21620	17605	9729	7922	4015	1807	752	2149	104%	81%	7.15	7.87
C	7	21543	15670	9694	7052	5873	2643	1024	2925	103%	73%	7.07	8.09
	8	22372	15835	10068	7126	6537	2942	1034	2954	100%	71%	7.04	8.13
	9	21572	15127	9707	6807	6445	2900	1074	3067	102%	70%	7.04	8.12
D	10	22649	16057	10192	7226	6592	2966	986	2817	99%	71%	7.10	7.99
	11	21494	15893	9672	7152	5601	2521	1019	2912	104%	74%	7.10	8.08
	12	22032	16370	9914	7367	5662	2548	993	2838	103%	74%	7.04	8.00
E	13	22450	15810	10103	7115	6640	2988	999	2855	99%	70%	7.15	8.02
	14	21853	16026	9834	7212	5826	2622	991	2831	102%	73%	7.16	8.09
	15	22261	15244	10017	6860	7017	3158	989	2826	97%	68%	7.19	8.11

Table A.3 COD balance, TCOD removal efficiency, and pH of BMP bottles (Continued).

Mix	Bottle #	TCOD of Bottles				ΔTCOD		CH_4		COD Balance	TCOD Removal Efficiency	Initial pH	Final pH
		Initial (mg/L)	Final (mg/L)	Initial (mg)	Final (mg)	mg/L	mg	NmL	mgCOD				
F	16	23635	15821	10636	7119	7815	3517	943	2693	92%	67%	7.16	7.96
	17	23436	16058	10546	7226	7378	3320	891	2547	93%	69%	7.01	8.02
	18	22600	15797	10170	7109	6803	3061	977	2791	97%	70%	7.10	8.03
G	19	21760	16271	9792	7322	5489	2470	910	2600	101%	75%	7.18	8.10
	20	21803	16240	9811	7308	5563	2503	868	2481	100%	74%	7.14	8.04
	21	22570	16145	10156	7265	6425	2891	906	2587	97%	72%	7.12	8.07
Cont.	22	21973	15054	9888	6774	6919	3114	998	2850	97%	69%	7.13	7.97
	23	22313	14627	10041	6582	7686	3459	1093	3121	97%	66%	7.16	7.97
	24	-	-	-	-	-	-	-	-	-	-	-	-
Blan.	25	17033	14801	7665	6660	2232	1004	299	855	98%	87%	7.18	7.87
	26	17117	14556	7703	6550	2561	1153	356	1018	98%	85%	7.12	7.78
	27	17590	14935	7915	6721	2655	1195	251	718	94%	85%	7.08	7.98

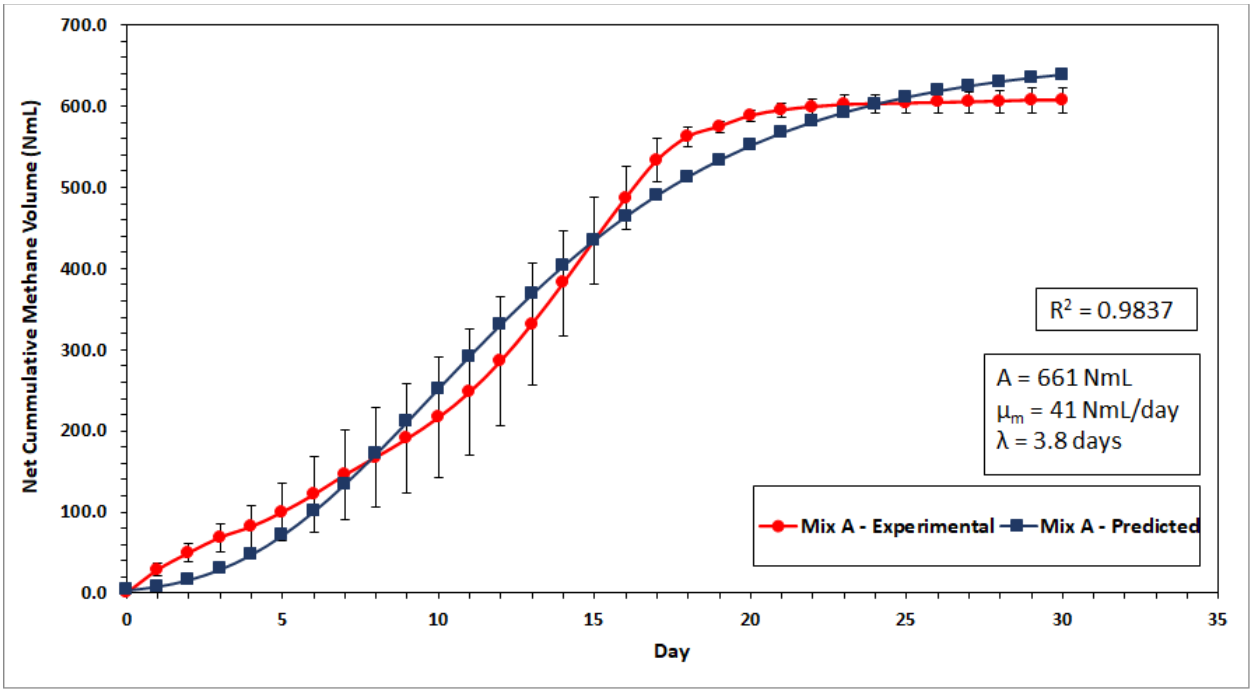

Figure A.1 Curve fitting for average net cumulative methane volume of mix A assays. Respective standard deviations of assays are plotted.

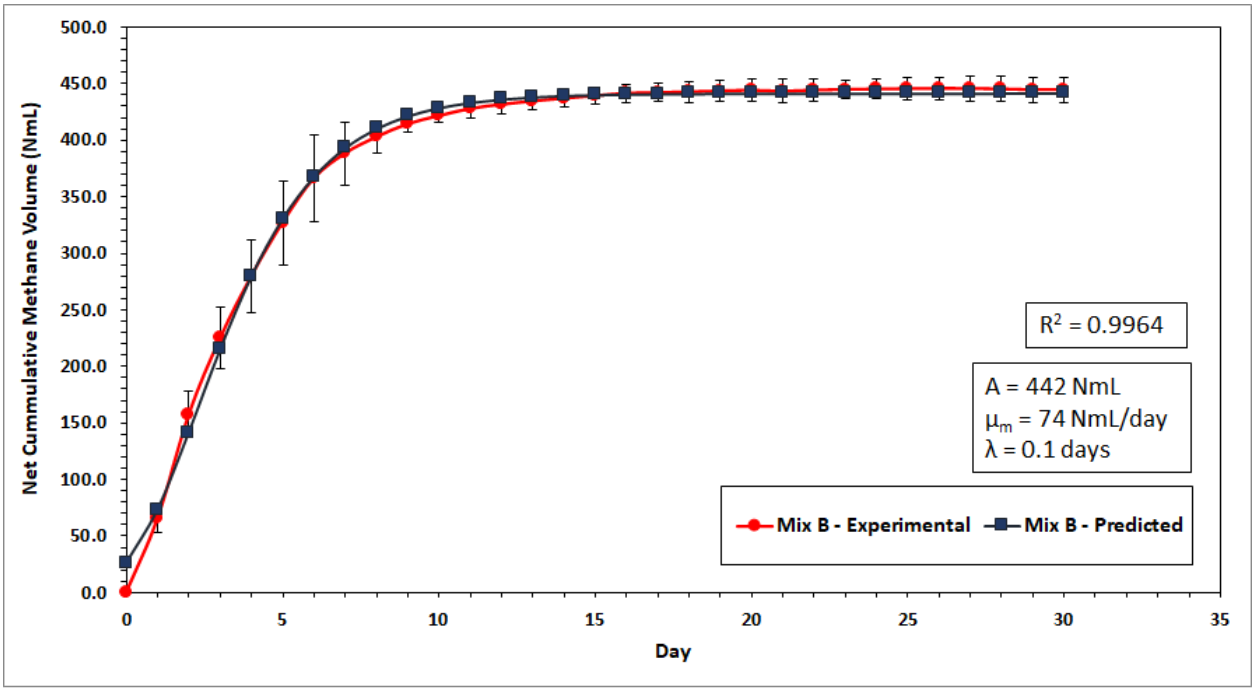

Figure A.2 Curve fitting for average net cumulative methane volume of mix B assays. Respective standard deviations of assays are plotted.

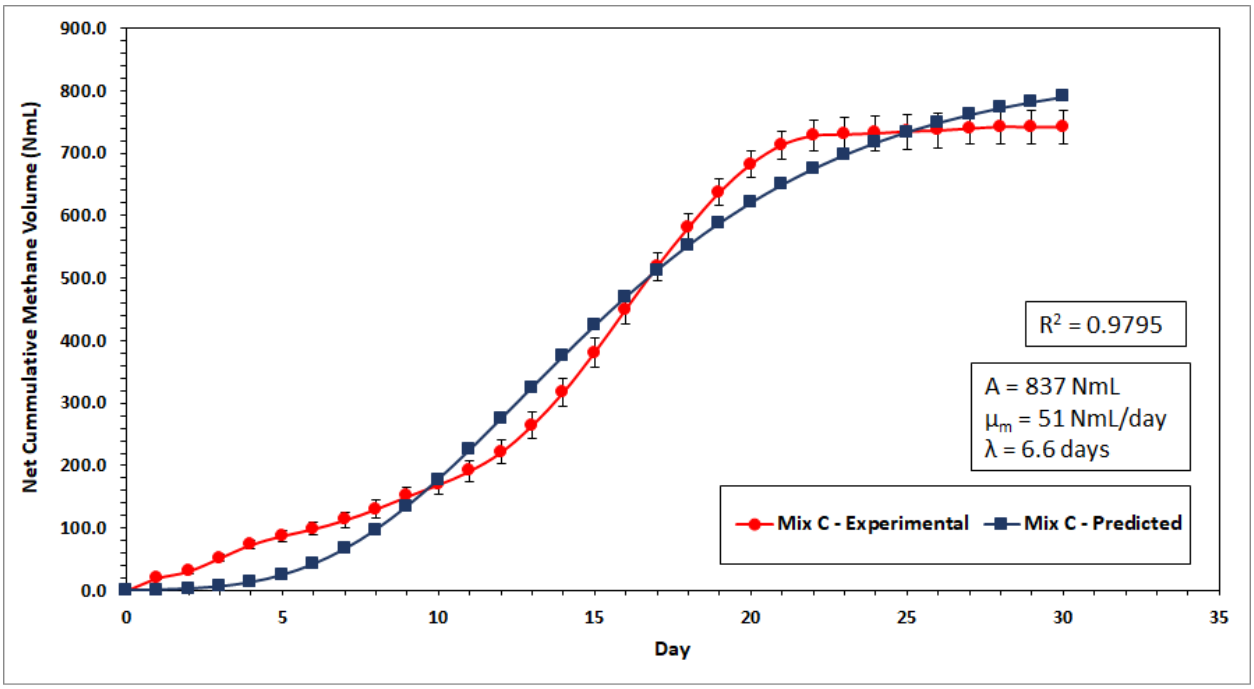

Figure A.3 Curve fitting for average net cumulative methane volume of mix C assays. Respective standard deviations of assays are plotted.

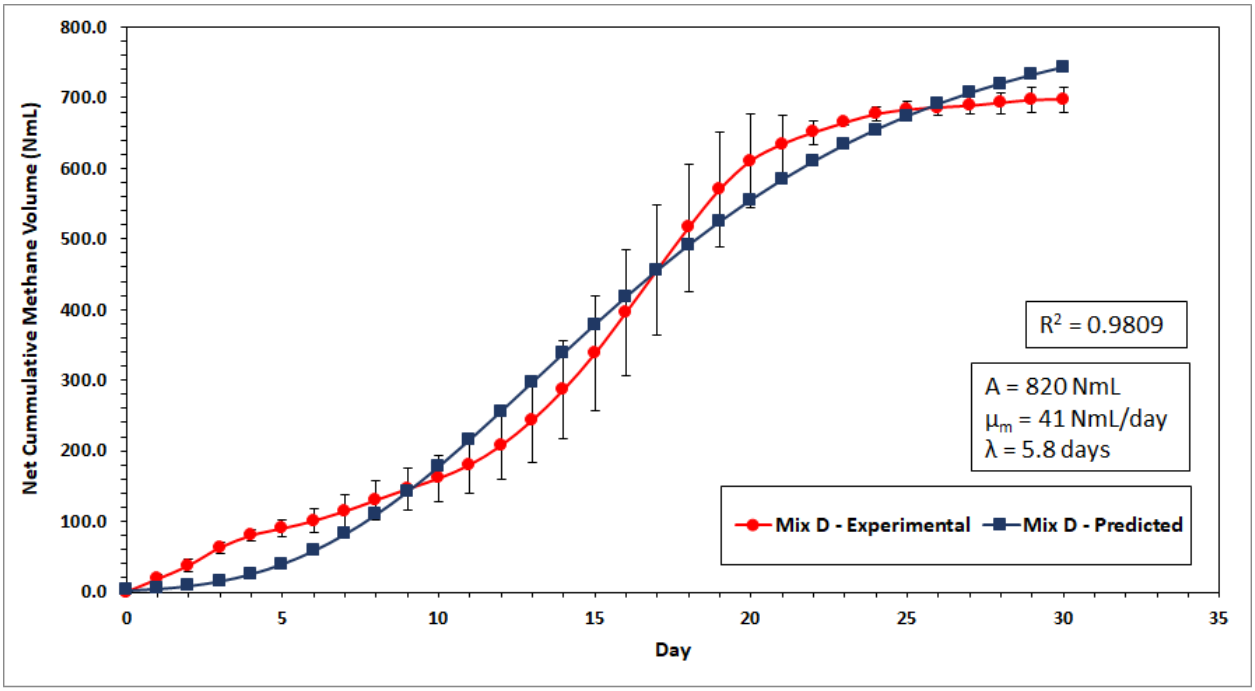

Figure A.4 Curve fitting for average net cumulative methane volume of mix D assays. Respective standard deviations of assays are plotted.

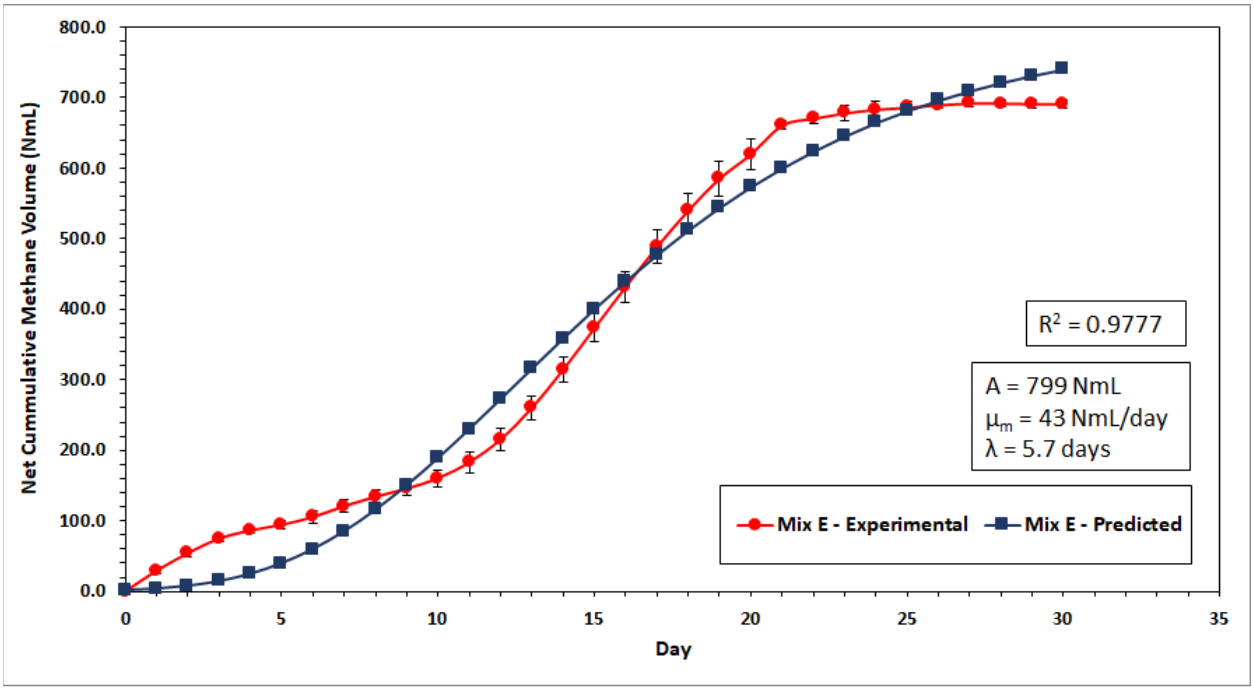

Figure A.5 Curve fitting for average net cumulative methane volume of mix E assays. Respective standard deviations of assays are plotted.

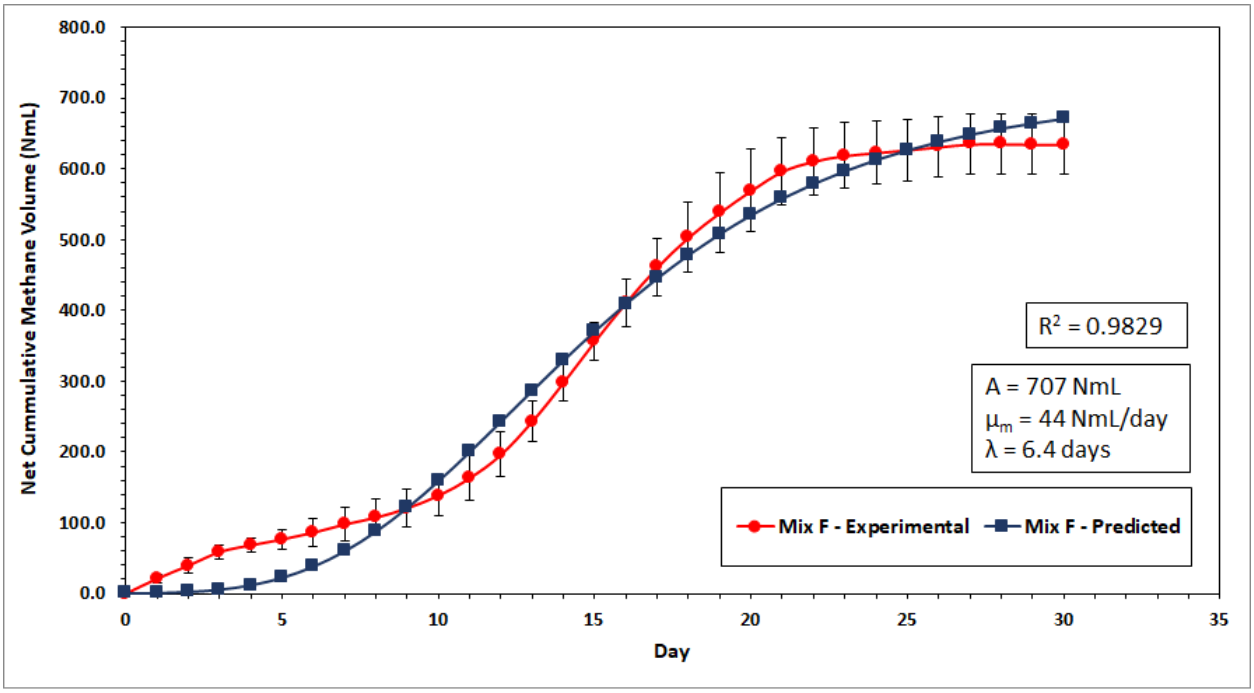

Figure A.6 Curve fitting for average net cumulative methane volume of mix F assays. Respective standard deviations of assays are plotted.

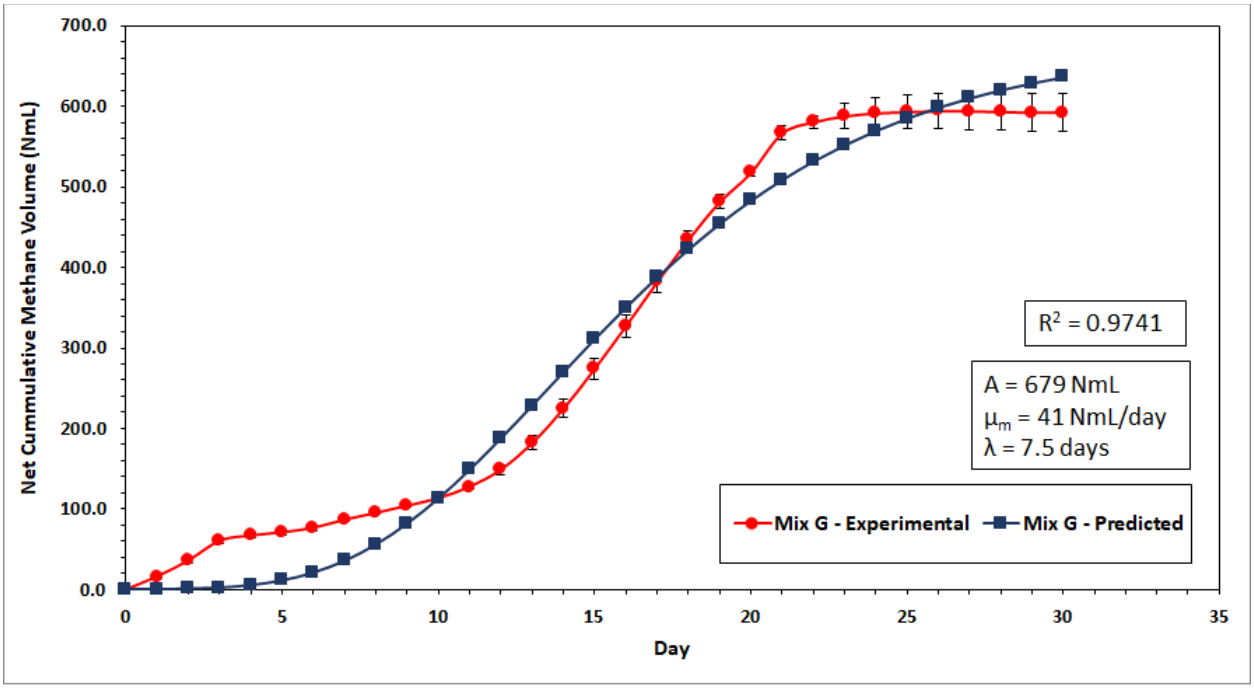

Figure A.7 Curve fitting for average net cumulative methane volume of mix G assays. Respective standard deviations of assays are plotted.

Appendix B: Supplementary Information for Chapter 4

Table B.1 Microwave calibration for power output and efficiency.

Power Level	Initial Temperature (ºC)	Final Temperature (ºC)	Power (Watts)	Average Power Output (W)	STD	Expected Power Output (W)	Efficiency
480W	23.31	33.76	363.3	367.4	4.2	480	76.5%
	23.69	34.25	367.1				
	23.04	33.73	371.7				
1080W	24.31	49.45	873.7	864.9	18.7	1080	80.1%
	22.09	46.34	843.4				
	21.94	47.17	877.6				

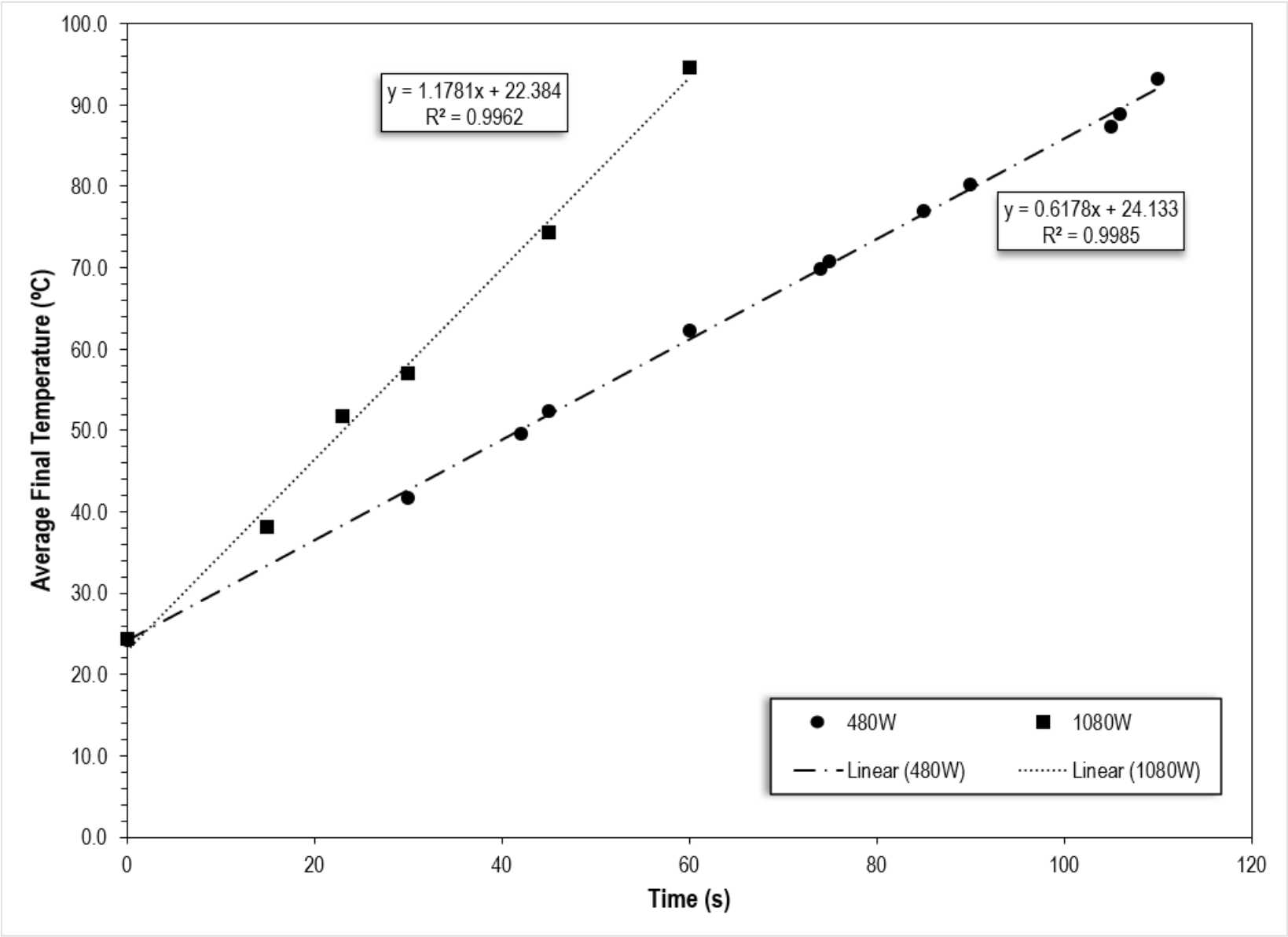

Figure B.1 Microwave calibration curve for wet cake at 480W and 1080W under the boiling point.

Table B.2 Summary of solubilization parameters and their standard deviation before and after microwave pre-treatment.

Treatment Level	TCOD			SCOD			SCOD/TCOD Ratio		
	Initial (g/L)	Final (g/L)	Change (%)	Initial (g/L)	Final (g/L)	Change (%)	Ave. Initial (%)	Ave. Final (%)	Change (%)
Untreated	190.1 ± 5.1	***n.d.***	***n.d.***	86.1 ± 0.6	***n.d.***	***n.d.***	45.3%	***n.d.***	***n.d.***
480W T=50ºC	185.8 ± 4.6	192.8 ± 2.4	+3.8%	86.8 ± 0.4	85.7 ± 1.5	-1.3%	46.7%	44.4%	-4.9%
480W T=70ºC	193.5 ± 4.9	205.6 ± 11.2	+6.2%	84.7 ± 0.3	86.8 ± 1.4	+2.5%	43.8%	42.2%	-3.6%
480W T=90ºC	202.8 ± 12.0	209.1 ± 9.8	+3.1%	89.0 ± 0.6	88.8 ± 0.4	-0.2%	43.9%	42.5%	-3.2%
1080W T=50ºC	207.3 ± 9.8	192.9 ± 2.9	-7.0%	86.1 ± 0.4	89.2 ± 1.2	+3.5%	41.6%	46.2%	+11.3%
1080W T=70ºC	185.6 ± 5.0	198.7 ± 5.9	+7.1%	85.6 ± 0.8	88.2 ± 1.4	+3.1%	46.1%	44.4%	-3.7%
1080W T=90ºC	193.9 ± 2.1	196.8 ± 7.8	+1.5%	86.1 ± 1.2	89.2 ± 2.4	+3.7%	44.4%	45.3%	+2.1%

Table B.3 Average solids (TS, VS) solubilization and their standard deviations before and after microwave pre-treatment.

Treatment Level	TS			VS		
	Initial (g/L)	Final (g/L)	Change (%)	Initial (g/L)	Final (g/L)	Change (%)
Untreated	136.3 ± 2.5	***n.d.***	***n.d.***	127.3 ± 2.6	***n.d.***	***n.d.***
480W T=50ºC	148.2 ± 7.8	156.6 ± 6.3	+5.7%	139.2 ± 6.9	147.8 ± 6.3	+6.2%
480W T=70ºC	144.8 ± 5.1	149.1 ± 3.4	+2.9%	137.0 ± 5.1	141.1 ± 3.4	+3.0%
480W T=90ºC	152.7 ± 4.5	155.5 ± 7.6	+1.9%	143.5 ± 5.2	147.0 ± 7.3	+2.4%
1080W T=50ºC	161.0 ± 6.9	156.6 ± 8.3	-2.7%	152.5 ± 6.5	151.3 ± 7.8	-0.8%
1080W T=70ºC	153.7 ± 8.0	159.1 ± 4.9	+3.5%	145.0 ± 6.8	150.8 ± 4.8	+4.0%
1080W T=90ºC	161.2 ± 3.5	162.3 ± 5.2	+0.7%	152.5 ± 3.3	153.8 ± 4.8	+0.9%

Table B.4 **Average solids (TSS, VSS) solubilization parameters and their standard deviations before and after microwave pre-treatment.**

Treatment Level	TSS			VSS		
	Initial (g/L)	Final (g/L)	Change (%)	Initial (g/L)	Final (g/L)	Change (%)
Untreated	89.7 ± 1.8	***n.d.***	***n.d.***	88.0 ± 1.8	***n.d.***	***n.d.***
480W T=50ºC	94.3 ± 3.2	89.0 ± 1.8	-5.7%	91.3 ± 3.2	86.8 ± 2.4	-4.9%
480W T=70ºC	88.5 ± 5.8	89.8 ± 2.1	+1.5%	86.0 ± 5.7	87.8 ± 2.1	+2.1%
480W T=90ºC	89.3 ± 2.3	88.8 ± 3.0	-0.6%	86.5 ± 2.2	86.8 ± 2.8	+0.4%
1080W T=50ºC	91.2 ±1.3	89.2 ± 1.5	-2.2%	88.5 ±1.7	86.7 ± 1.9	-2.1%
1080W T=70ºC	88.0 ± 0.5	86.0 ± 1.3	-2.3%	85.3 ± 0.3	83.7 ± 1.4	-2.0%
1080W T=90ºC	92.2 ± 2.0	90.7 ±1.6	-1.6%	88.5 ± 1.3	88.3 ±1.3	-0.2%

Table B.5 Average solids (TDS, VDS) solubilization parameters and their standard deviations before and after microwave pre-treatment.

Treatment Level	TDS			VDS		
	Initial (g/L)	Final (g/L)	Change (%)	Initial (g/L)	Final (g/L)	Change (%)
Untreated	46.7 ± 0.7	***n.d.***	***n.d.***	39.3 ± 0.8	***n.d.***	***n.d.***
480W T=50ºC	53.8 ± 4.6	67.6 ± 4.4	+25.5%	47.8 ± 3.7	61.0 ± 3.9	+27.5%
480W T=70ºC	56.3 ± 0.6	59.3 ±1.3	+5.2%	51.0 ± 0.6	53.3 ± 1.3	+4.4%
480W T=90ºC	63.3 ± 2.2	66.7 ± 4.6	+5.3%	57.0 ± 3.0	60.2 ± 4.6	+5.6%
1080W T=50ºC	69.8 ± 5.7	67.5 ± 6.8	-3.4%	64.0 ± 4.8	64.7 ± 6.0	+1.0%
1080W T=70ºC	65.7 ± 7.5	73.1 ± 3.6	+11.3%	59.7 ± 6.5	67.3 ± 3.4	+12.6%
1080W T=90ºC	69.0 ± 1.5	71.7 ± 3.6	+3.9%	64.0 ± 2.0	65.5 ± 3.5	+2.3%

Table B.6 BMP bottle setup based on F/M ratio of 0.5.

	Mix	Bottle No.	Seed Sludge (mL)	Substrate (mL)	DIW (mL)	Total (mL)	Expected TCOD (g/L)	Expected VS (g/L)	Theoretical Yield (mL)
F/M = 0.5	Untreated	1	300.00	13	187	500	20.1	14.5	3170
		2	300.00	13	187	500	20.1	14.5	3170
		3	300.00	13	187	500	20.1	14.5	3170
	480W T = 50ºC	4	300.00	12	188	500	20.1	15.0	3170
		5	300.00	12	188	500	20.1	15.0	3170
		6	300.00	12	188	500	20.1	15.0	3170
	480W T = 70ºC	7	300.00	12	188	500	20.1	14.6	3170
		8	300.00	12	188	500	20.1	14.6	3170
		9	300.00	12	188	500	20.1	14.6	3170
	480W T = 90ºC	10	300.00	11	189	500	20.1	14.7	3170
		11	300.00	11	189	500	20.1	14.7	3170
		12	300.00	11	189	500	20.1	14.7	3170
	1080W T = 50ºC	13	300.00	12	188	500	20.1	15.1	3170
		14	300.00	12	188	500	20.1	15.1	3170
		15	300.00	12	188	500	20.1	15.1	3170
	1080W T = 70ºC	16	300.00	12	188	500	20.1	15.0	3170
		17	300.00	12	188	500	20.1	15.0	3170
		18	300.00	12	188	500	20.1	15.0	3170
	1080W T = 90ºC	19	300.00	12	188	500	20.1	15.1	3170
		20	300.00	12	188	500	20.1	15.1	3170
		21	300.00	12	188	500	20.1	15.1	3170
	Control	22	300.00	25	175	500	20.1	11.3	3170
		23	300.00	25	175	500	20.1	11.3	3170
		24	300.00	25	175	500	20.1	11.3	3170
	Blank	25	300.00	0	200	500	15.4	11.3	2420
		26	300.00	0	200	500	15.4	11.3	2420
		27	300.00	0	200	500	15.4	11.3	2420

Table B.7 COD balance, TCOD removal efficiency, and pH of BMP bottles

Mix	Bottle #	TCOD of Bottles				ΔTCOD		CH_4		COD Balance	TCOD Removal Efficiency	Initial pH	Final pH
		Initial (mg/L)	Final (mg/L)	Initial (mg)	Final (mg)	mg/L	mg	NmL	mgCOD				
Untreated	1	19920	14259	8964	6416	5662	2548	716	2046	94%	72%	7.06	7.93
	2	19622	13772	8830	6198	5849	2632	731	2088	94%	70%	7.15	8.14
	3	19230	14076	8653	6334	5153	2319	817	2333	100%	73%	7.17	8.05
480W T = 50°C	4	18639	14043	8387	6319	4596	2068	662	1892	98%	75%	7.17	7.95
	5	18592	14664	8367	6599	3928	1768	682	1949	102%	79%	7.04	7.89
	6	18625	13826	8381	6222	4799	2160	741	2116	99%	74%	7.03	7.88
480W T = 70°C	7	18340	14022	8253	6310	4318	1943	653	1865	99%	76%	7.17	8.05
	8	18181	13752	8181	6188	4429	1993	666	1903	99%	76%	7.14	7.97
	9	18572	14049	8358	6322	4523	2035	734	2096	101%	76%	7.13	7.94
480W T = 90°C	10	18486	13637	8319	6137	4849	2182	657	1877	96%	74%	7.13	7.90
	11	18625	14151	8381	6368	4475	2014	634	1811	98%	76%	7.14	7.92
	12	18054	13820	8125	6219	4235	1906	638	1823	99%	77%	7.16	7.90
1080W T = 50°C	13	18517	13820	8333	6219	4697	2114	684	1954	98%	75%	7.17	8.10
	14	18806	13718	8463	6173	5087	2289	714	2040	97%	73%	7.16	7.98
	15	18950	13894	8528	6252	5056	2275	712	2035	97%	73%	7.16	8.08

Table B.8 COD balance and TCOD removal efficiency for BMP bottles (continued)

Mix	Bottle #	TCOD of Bottles				ΔTCOD		CH_4		COD Balance	TCOD Removal Efficiency	Initial pH	Final pH
		Initial (mg/L)	Final (mg/L)	Initial (mg)	Final (mg)	mg/L	mg	NmL	mgCOD				
1080W T = 70ºC	16	18976	14002	8539	6301	4974	2238	670	1915	96%	74%	7.09	8.16
	17	19232	14778	8655	6650	4454	2004	649	1853	98%	77%	7.12	8.26
	18	19042	15109	8569	6799	3933	1770	716	2046	103%	79%	7.13	8.12
1080W T = 90ºC	19	18780	15043	8451	6769	3736	1681	677	1933	103%	80%	7.20	7.98
	20	18556	15249	8350	6862	3308	1489	659	1882	105%	82%	7.17	8.04
	21	18609	14911	8374	6710	3698	1664	717	2047	105%	80%	7.16	8.01
Control	22	19659	14380	8846	6471	5278	2375	914	2610	103%	73%	7.19	8.22
	23	19409	14805	8734	6662	4605	2072	936	2674	107%	76%	7.06	7.93
	24	-	-	-	-	-	-	-	-	-	-	-	-
Blank	25	15104	13971	6797	6287	1133	510	228	650	102%	93%	7.17	7.72
	26	15866	13574	7140	6108	2292	1031	248	708	95%	86%	7.19	7.80
	27	15566	13490	7005	6070	2077	935	269	769	98%	87%	7.30	7.84

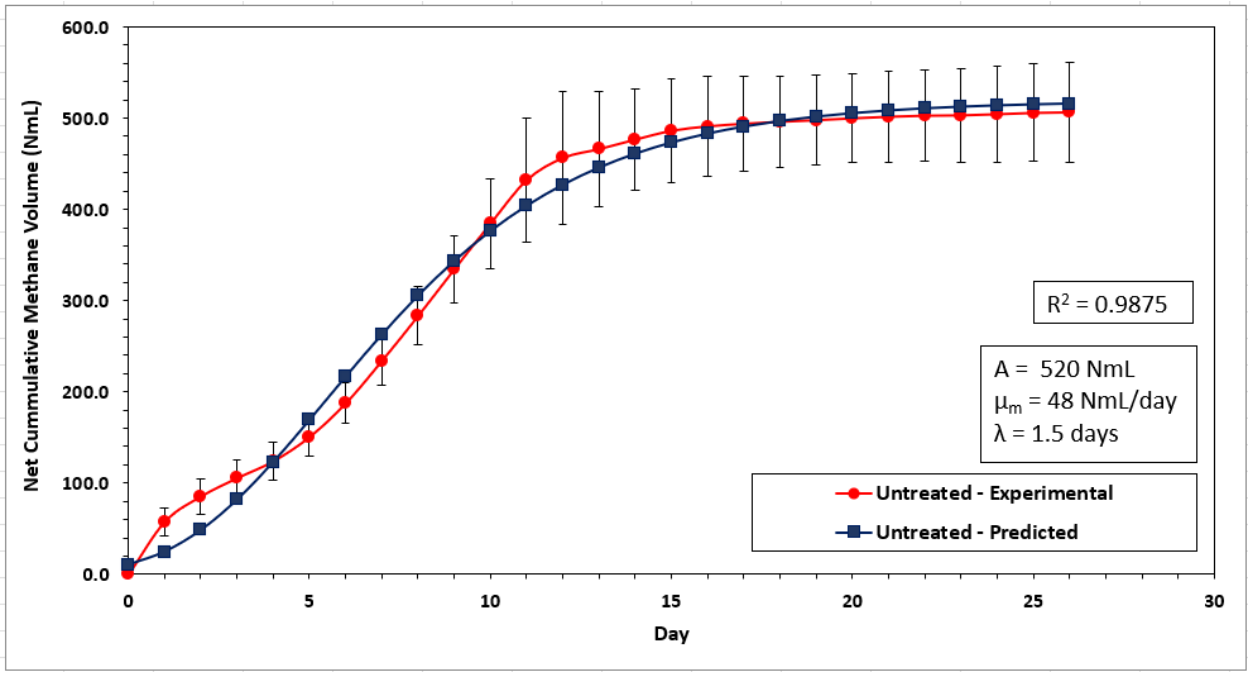

Figure B.2 Curve fitting for average net cumulative methane volume of untreated assays. Respective standard deviations of assays are plotted.

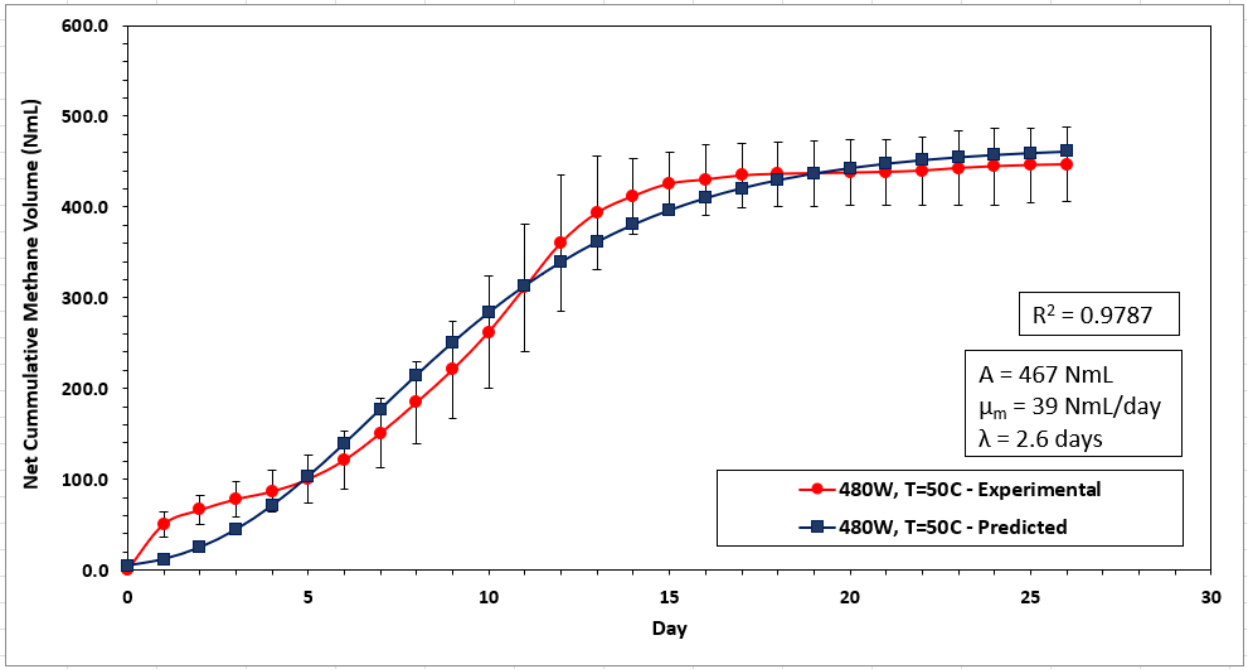

Figure B.3 Curve fitting for average net cumulative methane volume of 480W, T=50ºC treatment assays. Respective standard deviations of assays are plotted.

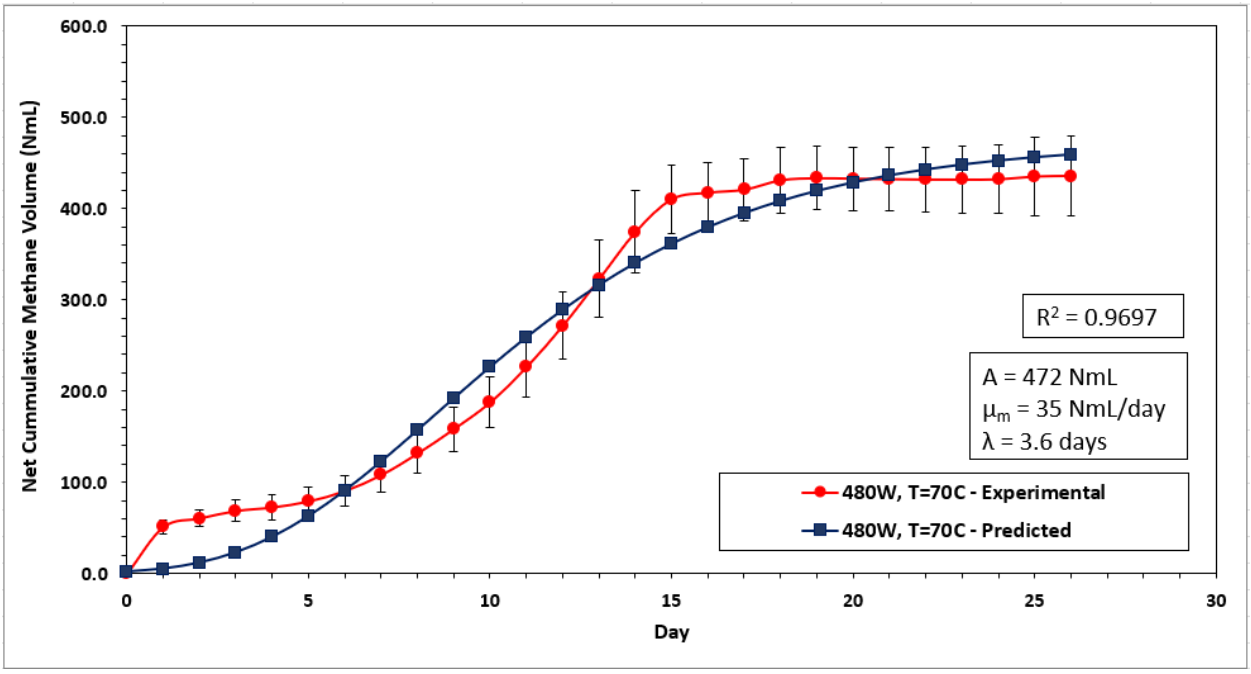

Figure B.4 Curve fitting for average net cumulative methane volume of 480W, T=70ºC treatment assays. Respective standard deviations of assays are plotted.

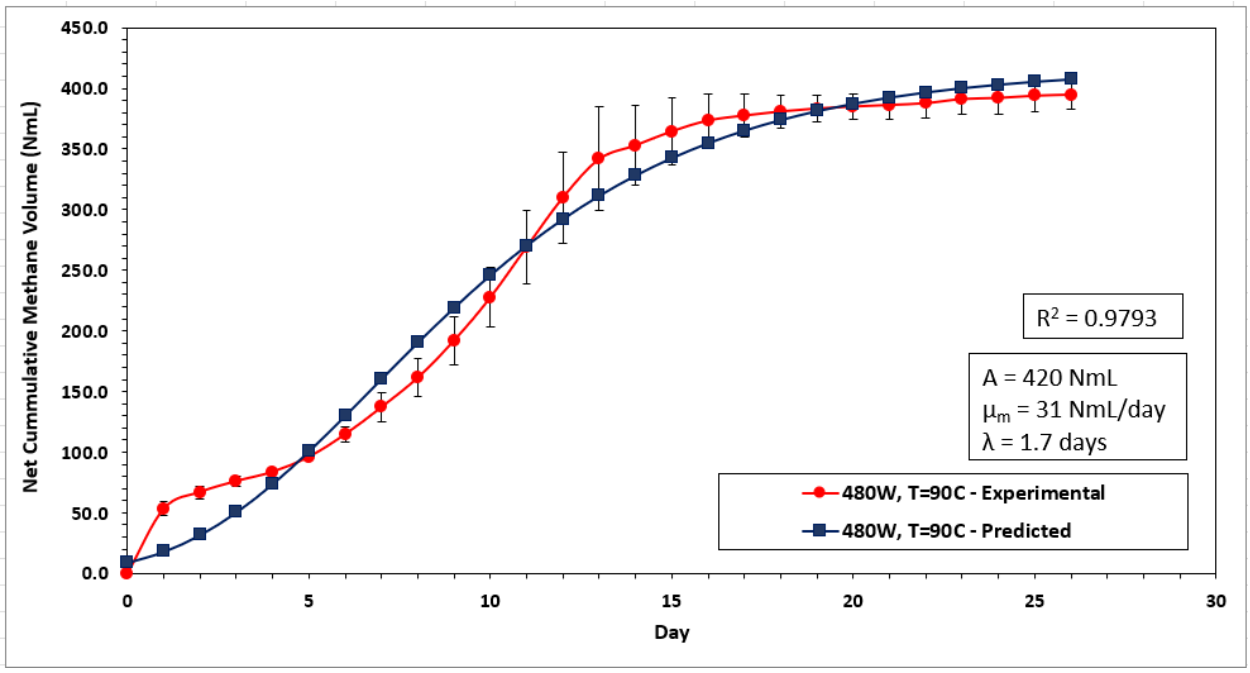

Figure B.5 Curve fitting for average net cumulative methane volume of 480W, T=90ºC treatment assays. Respective standard deviations of assays are plotted.

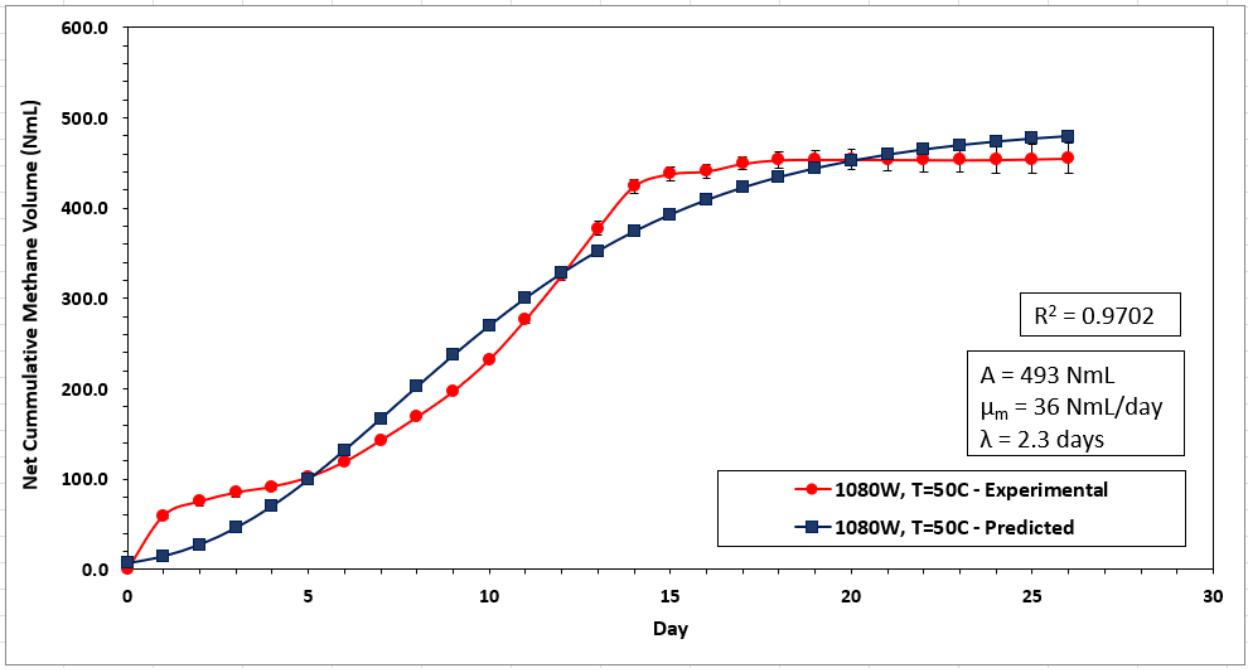

Figure B.6 Curve fitting for average net cumulative methane volume of 1080W, T=50ºC treatment assays. Respective standard deviations of assays are plotted.

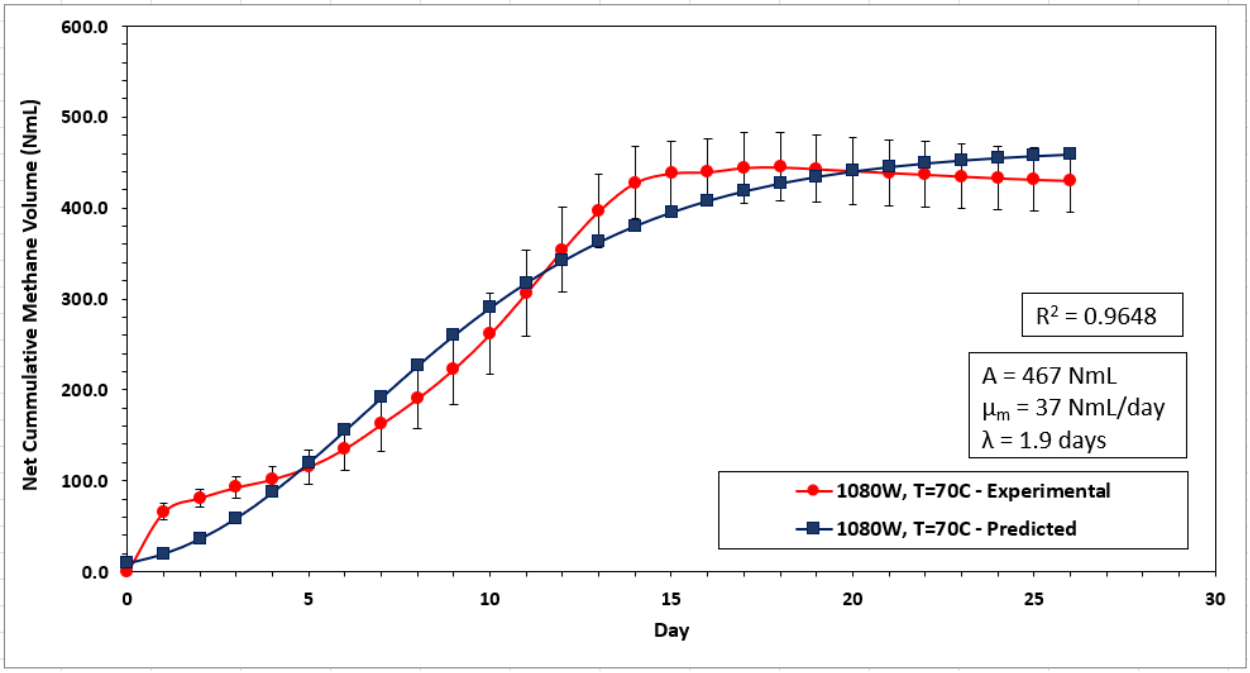

Figure B.7 Curve fitting for average net cumulative methane volume of 1080W, T=70ºC treatment assays. Respective standard deviations of assays are plotted.

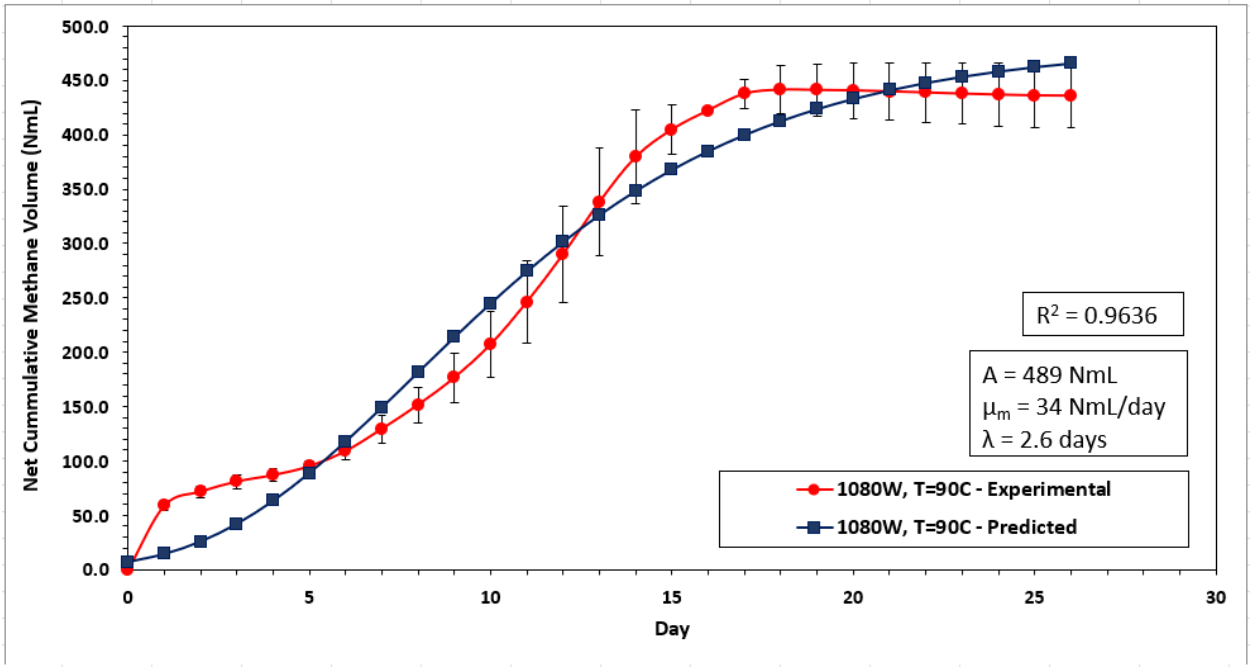

Figure B.8 Curve fitting for average net cumulative methane volume of 1080W, T=90ºC treatment assays. Respective standard deviations of assays are plotted.

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