

**VOLATILE FATTY ACIDS AND METHANE PRODUCTION FROM ORGANIC
WASTES USING ANAEROBIC DIGESTION**

by

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ABSTRACT

The abundance of organic waste poses environmental issues as it is disposed of into landfills resulting in the emission of greenhouse gases. Industries have been seeking environment-friendly solutions, as waste handling is expensive due to the bulk volume of the waste. One of such solutions is resource recovery using anaerobic digestion (AD) process. AD process can be used to produce methane and volatile fatty acids (VFAs). AD process performance can be enhanced using thermal hydrolysis pretreatment (THP). The effect of solubilization of waste potato starch due to THP was studied on methane and VFAs production. A relationship was established between THP temperature and potato starch solubilization ($R^2 = 0.74-0.90$). The AD reactors were fed with raw starch and starches pretreated at 100 (THP_100) and 140 °C (THP_140) for VFAs production. VFAs production from THP_140 starch fell significantly, but raw starch and THP_100 samples showed no changes. A maximum VFAs yield of 0.482 mg-VFAs/mg-COD was obtained at an organic loading rate of 9 kg-COD/m³·day and at hydraulic retention time (HRT) of 2 days for THP_100 starch, 6% higher than VFAs yield using raw starch. The methane production was carried out using raw starch, THP_70, THP_100, and THP_140 starches. The highest methane yield of 0.32 mL-CH₄/mg-COD was obtained from THP_70 starch, in comparison to 0.33 mL-CH₄/mg-COD for the co-digestion of starch and pre-hydrolysis liquor obtained from the dissolving pulp mill. The alkalinity requirements went down by 25% during co-digestion compared to single substrate digestion. The food waste was found to be a good source of methane production. A continuous lab-scale bioreactor was used to evaluate VFAs production. The optimal HRT of 0.26 d provided the maximum VFAs production of 0.78 mg-VFAs/mg-COD.

DEDICATION

I dedicate this thesis to my parents Neelam Bhargava and Vijai Bhargava and mentor Dr. Lalit Agrawal, as well as to my brother Mayank Bhargava for their constant support, help, and belief throughout the length of the project.

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List of Symbols, Nomenclature or Abbreviations

AD – Anaerobic Digestion

VFAs – Volatile Fatty Acids

THP – Thermal Hydrolysis Pretreatment

COD – Chemical Oxygen Demand

tCOD – Total Chemical Oxygen Demand

fCOD – Filtered Chemical Oxygen Demand

sCOD – Soluble Chemical Oxygen Demand

OLR – Organic Loading Rate

BOD – Biochemical Oxygen Demand

PS – Primary Sludge

WAS – Waste Activated Sludge

MLSS – Mixed Liquor Suspended Solids

MLVSS – Mixed Liquor Volatile Suspended Solids

TS – Total Solids

TSS – Total Suspended Solids

VSS – Volatile Suspended Solids

GC – Gas Chromatography

HRT – Hydraulic Retention Time

1. Introduction

1.1 Background and problem statements

Wastewater treatment methods have been traditionally aimed at the removal of pollutants from water. The primary goal of these treatment methods has been the production of clean water that can be returned to the environment without adverse effects either on the environment or on human health. Because of stringent environmental regulations over the years related to wastewater and solid waste disposal, food processing industries and food retailers have been seeking environment-friendly economical solutions, one of which is resource recovery.

Resource recovery is an attractive and sustainable waste management approach aimed at selective extraction of value-added products from low-value organic waste streams using anaerobic digestion (AD) process. AD process is a mature and established technology that reduces the amount of waste generated by increasing its utilization to produce bio-products. These bioproducts can have a significant positive impact on the environment. One of the examples of this process is the production of biogas containing methane which is a result of waste stabilization as compared to conventional aerobic treatment methods. Other advantages of the AD process include low production of biomass, no oxygen requirement, the ability of the reactor to handle higher organic loading and low nutrient requirements (McCarty, 1964).

The AD process has been recognized as a sustainable technology for the future and has full-scale installations for biogas production across the globe. Biogas production using organic waste can help in minimizing human dependence on non-renewable energy

resources such as coal and natural gas, which are also the primary raw material for commercial production of volatile fatty acids (VFAs). Biogas usually contains methane (CH_4), carbon dioxide (CO_2), hydrogen sulfide (H_2S), and ammonia (NH_3), but the contents vary with the composition of the substrate. Production of methane is an attractive process as it can ultimately be used for the generation of either heat or electricity using combined heat and power units, like captive power plants. The energy generated using methane is eco-friendly as the emission of greenhouse gases (GHGs) is minimized. GHGs are a significant contributor to global warming leading to climate change. In addition to that, this energy generation also helps in offsetting some of the operational expenditure incurred by the industries.

The attractiveness of biogas production using the AD process is often questioned as higher economic value intermediate products such as VFAs including acetic acid ($\text{C}_2\text{H}_4\text{O}_2$), propionic acid ($\text{C}_3\text{H}_6\text{O}_2$), butyric acid ($\text{C}_4\text{H}_8\text{O}_2$) and valeric acid ($\text{C}_5\text{H}_{10}\text{O}_2$) are formed during the digestion of organic matter. Acetic acid is the most useful VFA for supporting denitrification and enhancing biological phosphorus removal (Shanableh and Jones, 2001). These higher-value intermediate products called for further research and development of the existing AD process used for biogas production. In addition to these VFAs, the production of hydrogen (H_2) and nutrient recovery in the form of nitrogen (N) and phosphorus (in form of struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$)) are potential value-added products which have received considerable interest over the years (Ucisik and Henze, 2008). The production of VFAs remains a lucrative option considering their vast application as chemical reagents in the field of chemistry (Zacharof and Lovitt, 2013).

Several different pretreatment methods, such as mechanical grinding, thermal, chemical (acid/alkaline), biological, or combination of two, such as thermal chemical or thermal biological pretreatments, have been employed by the researchers over the years to enhance the production of these value-added products. These pretreatment methods are used upstream of the AD process, and their main goal is to increase the availability of the organic waste to the microorganisms responsible for AD. These pretreatment methods, at specific operating conditions, have yielded promising results for AD process in terms of biogas production (volume and rate), reduction in effluent chemical oxygen demand (COD), biosolids production but these pretreatment methods may require energy efficiency optimization to reduce its environmental burden (Carballa et al., 2011).

The pretreatment method used for this study was thermal hydrolysis pretreatment (THP).

Over the years, the number of studies related to THP of sludge has increased. This pretreatment leads to partial or full solubilization of sludge as it disintegrates the cell structure and releases the trapped water. THP gained widespread popularity for sludge management because of its ability to increase the anaerobic digester capacity, generation of class A biosolids (free of pathogens) (Oosterhuis et al., 2014), and reducing overall treatment costs for wastewater treatment. A few industries such as Cambi™ and Bio Thelys™ from Veolia Water Technologies commercialized THP. These industries have tested these technologies for years at industrial-scale operations, further proving their performance and reliability. The pretreatment is usually carried out using steam by heating sludge to around 130-180 °C for 30-60 minutes.

The organic waste required to carry out the AD process is abundantly available in Canada. The potato processing industry in New Brunswick, Canada utilizes 56% of the

potatoes cultivated on approximately 200 million m² of land in the province to manufacture a range of potato products (New Brunswick Potatoes, 2017). In addition to the potato waste, over six million tons of solid food waste was reported between the retail level and the consumer level across Canada in 2007 – equivalent to 183 kg per person (Statistics Canada, 2008). These losses do not include losses at the production level or during food processing. These abundant wastes pose significant challenges for industries including bulk volume and disposal costs (tipping fees). Similarly, the challenge faced by the municipalities include stress on the water infrastructure as in some cases, food waste ends up down the drains. These wastes can be recycled as compost or as animal feed, but demand for them is usually low because of their poor quality. The typical disposal method is to move these wastes to landfills, giving rise to several environmental issues such as leachate production, and emission of GHGs like CH₄ and CO₂ (Abichou et al., 2006), in addition to limited landfill space and logistics costs. These issues are a result of high moisture content and organic matter present in the wastes, which makes these wastes an ideal substrate for AD.

Considering the abundance of organic waste in the province of New Brunswick, Canada and the application of the pretreatment method to enhance the AD process, this work explored the possibility of harvesting valuable products such as methane and VFAs from organic wastes by analyzing the effect of pretreatment methods on the AD process. The AD process can provide sustainable solutions for mutual benefit for the environment and the concerned industries and at the same time, has the potential to change the outlook of organic wastes.

1.2 Objectives

This research project focused on investigating the impact of THP and its operating parameters (independent variables) on the AD of waste potato starch obtained from a local potato processing plant and comparing the methane yield and VFAs yield. Simultaneously, co-digestion of starch and pre-hydrolysis liquor (PHL), and the anaerobic treatability study of food waste was conducted for methane production. The food waste and PHL were obtained from a local grocery store and a local pulp mill respectively.

The specific objectives of the study are as follows:

1. To evaluate the relationship between THP operating parameters, i.e., THP temperature and COD solubilization, measured as filtered COD (fCOD) and soluble COD (sCOD). This is necessary to determine the maximum solubilization attainable for waste potato starch. Other THP variables such as agitator rotation speed and experimental run time are kept constant at 150 rpm and 30 minutes, respectively.
2. To evaluate the relationship between COD solubilization on VFAs yield, measured as mg-VFAs/mg-COD.
3. To evaluate the relationship between COD solubilization on methane yield, measured as mL-CH₄/mg-COD.
4. To evaluate the reactor performance using COD balance for co-digestion of potato starch and PHL.
5. To evaluate the anaerobic digestibility of food waste.

6. To evaluate the feasibility of continuous bioreactor (anaerobic trickling filter - ATF) to produce VFAs using sucrose and potato starch as substrate.

The ultimate objective is to determine if the substrate is suited for methane production and the mode of reactor operation, i.e., batch mode or continuous mode for VFAs production.

1.3 Scope of the project

The study employed three different reactor systems for six different phases of the project, as mentioned below:

1. Parr reactor – THP
2. Respirometric Reactor – Batch mode of operation
3. Anaerobic Trickling Filter (ATF) – Continuous mode of operation

More details regarding the reactor systems are available in Chapter 3.

THP was carried out for three different starch concentrations (10 g/L, 15 g/L and 25 g/L) at six different temperatures, i.e., 50, 70, 80, 100, 120 and 140 °C. A relationship was established between THP temperature and solubilization of thermally pretreated potato starch. These objectives were accomplished through the application of THP on a given potato starch solution and observing its effect on total COD (tCOD), fCOD, sCOD, total solids (TS), total suspended solids (TSS), volatile suspended solids (VSS) and pH. These sets of experiments were followed by a respirometric study for methane and VFAs production to determine the effect of THP.

The effect of solubilization due to THP temperatures of 100 and 140 °C was determined on VFAs production using thermally shocked mixed culture (102 °C for 2.5 hours). Next, the effect of solubilization due to THP temperatures of 70, 100, and 140 °C on methane production was determined. Specific methane production on anaerobic seed sludge was determined before the methane production study. The effect of THP was not determined for the co-digestion study of potato starch and PHL and food waste. The methane production obtained through the co-digestion of potato starch and PHL and treatability of food waste study were compared to methane production using thermally pretreated starch to determine which substrate offers the highest methane yield. The ATF was the final part of the study to quantify VFAs production. AD process was carried out at 35 °C, i.e., mesophilic conditions.

1.4 Organization of the thesis

The thesis is organized in five chapters as follows:

Chapter 1 presents the background and importance of resource recovery, the problem statement, research objectives, and scope of the study followed by the organization of the thesis.

Chapter 2 presents the literature review which contains information related to the basics of AD, its four sequential steps followed by the AD process diagram. The second section of the literature review discusses the effect of THP on increasing anaerobic conversion for methane and VFAs production. The knowledge gaps were identified through literature

review and it was determined how this research attempts to contribute to the existing scientific knowledge base in the area of anaerobic biotechnology.

Chapter 3 describes the methodology and materials used. This section provides information related to reactor set-ups used for AD and analytical procedures. This chapter also provides information related to variables and the experimental design.

Chapter 4 consists of results and discussion. This section contains information including tables and figures related to the effect of THP on the solubilization of potato starch. AD process was carried out under batch mode of operation to quantify methane yield and VFAs yield, and VFAs production profile was developed. The co-digestion of PHL and potato starch was carried out along with treatability studies for food waste. The research findings for the effect of THP on waste potato starch, i.e., solubilization and its effect on AD was presented at the Canadian Society for Civil Engineering (CSCE) conference, Fredericton, New Brunswick. These research findings¹ were also submitted for the peer-reviewed Journal of Environmental Engineering, American Society of Civil Engineers (ASCE). The research finding related to VFAs production using different modes of reactor operation was presented at the World Congress on Anaerobic Digestion AD16, Delft, Netherlands.

Chapter 5 consists of conclusions and recommendations. Conclusions were drawn based on the results obtained for THP and determining the substrate suited for methane and VFAs production. Recommendations include research to build on the existing work.

¹ Journal paper title: Volatile fatty acid production from thermally pretreated potato starch using anaerobic digestion. Authored by Mohit Bhargav, M.Sc.E. Candidate and Dr. Kripa Singh, P.Eng. (Supervisor).

2. Literature Review

2.1 Anaerobic Digestion

AD is a microbiological process where complex organic waste is converted into biogas containing methane and carbon dioxide in the absence of oxygen through four main sequential steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis (McCarty, 1964). Figure 1 (Batstone et al., 2002) shows the conversion process of complex organic molecules to gaseous products of methane (CH_4) and carbon dioxide (CO_2); which is possible owing to the syntrophic association of four different groups of microorganisms allowing them to degrade several substrates (Demirel and Scherer, 2008). The products formed by one group of microbes serve as a substrate for the next group. These microbes have established roles during AD of wastes and occur in the natural environment. The mesophilic and thermophilic bacteria are described in the literature and exhibit high activity close to $35\text{ }^\circ\text{C}$ and $55\text{ }^\circ\text{C}$, respectively.

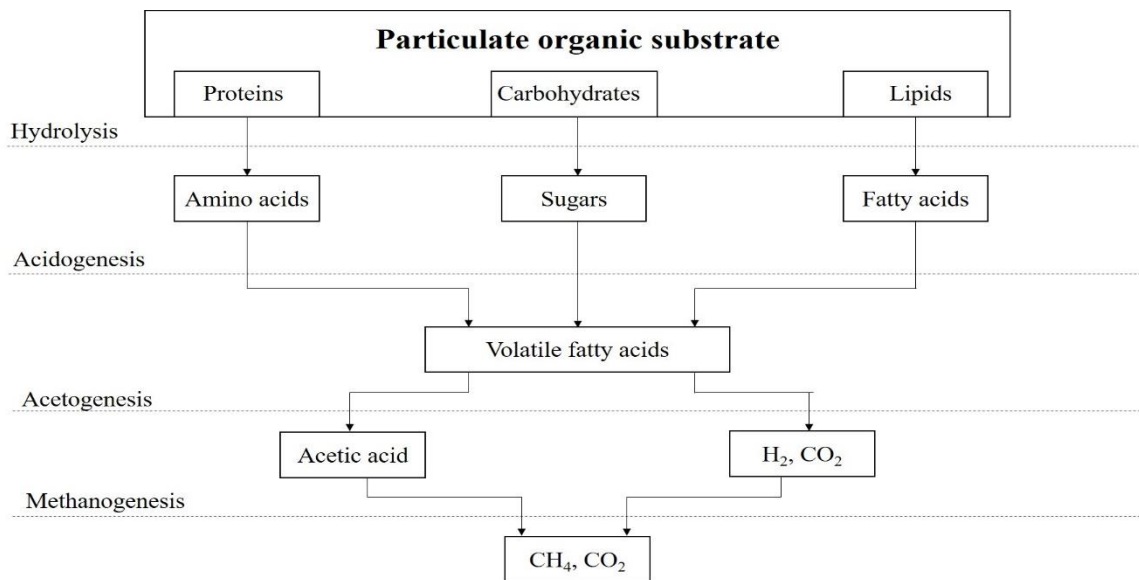


Figure 1: Anaerobic digestion pathways (Batstone et al., 2002)

The four sequential steps of the AD process are described below.

2.1.1 Hydrolysis

Hydrolysis is the first step in AD where complex organic compounds (polymers such as fats, proteins, and carbohydrates) are converted to simpler forms (dimers and monomers such as monosaccharides, fatty acids, amino acids). This conversion is possible because of the enzymatic action of hydrolytic bacteria. The rate of hydrolysis is usually affected by the physical characteristics and chemical composition of the substrate, which often makes it the rate-limiting step during AD (Izumi et al., 2010; Ma et al., 2011; Rafique et al., 2010). Pretreatment methods are employed to increase the rate of hydrolysis by partial or full solubilization (Haug et al., 1978) to overcome this limitation. The overall hydrolysis rate is generally determined by the particle surface area available for enzyme binding (Sanders et al., 2000; Veecken et al., 2000). The majority of the molecules after hydrolysis are still relatively large, which must be further be converted to small molecules such as acetic acid, so that they may be used to produce methane.

2.1.2 Acidogenesis

During this second stage, bacteria convert water-soluble chemical compounds (monomers and dimers) to higher organic acids such as butyric acid, propionic acid, and to acetic acid, hydrogen, and carbon dioxide. The higher organic acids are subsequently transferred to acetic acid and hydrogen by acetogenic bacteria. It is always not possible to draw a clear distinction between acidogenic and acetogenic reactions. Acetate and hydrogen are produced during acidification and acetogenic reactions, and both of them are substrates of methanogenic bacteria.

2.1.3 Acetogenesis

In this step, acetogenic bacteria convert the acid phase products into acetate and hydrogen, which may directly be used by methanogenic bacteria. Hydrogen is released, as a result of acidogenesis and acetogenesis, which harm these microorganisms. A symbiotic relationship is necessary for acetogenic bacteria with methanogenic bacteria using hydrogen and hence referred to as interspecies hydrogen transfer (McCarty, 1964). Interspecies hydrogen transfer plays a crucial role in maximizing organic acid yield, especially for the conversion of higher fatty acids to acetic acid. The acetogenesis phase determines the efficiency of methanogenesis as 70% of methane is a result of acetate reduction (McCarty, 1964).

2.1.4 Methanogenesis

This final phase consists of methane production from methanogenic bacteria. Methane is produced from substrates such as acetate and carbon dioxide and hydrogen. Two-thirds of the methane produced during the AD process is derived from acetate, and about one-third is derived from carbon dioxide reduction (Bajpai, 2017). During this process, H₂ is used up, which creates low partial pressure conditions for the development of acidogenic bacteria. Long-chain fatty acids (LCFAs) such as butyric acid can only be converted by these microbes when low pressure of H₂ is maintained (Demirel and Scherer, 2008). The methanogenic bacteria are crucial to the AD process because they are slow-growing and susceptible to changes in the environmental conditions, particularly changes in temperature and pH (McCarty, 1964).

2.2 Thermal Hydrolysis Pretreatment (THP)

THP dates back to the 1970s when it was initially applied to improve the dewaterability of sludge (Haug et al., 1978). This converts a complex molecule into a simpler form and increases its availability to the microorganisms, which further enhances the AD process (Bougrier et al., 2008). THP is often employed to counteract the rate limitation of hydrolysis, which is the first step of AD. The complex compounds are converted into simpler compounds, which are more readily biodegradable by anaerobic bacteria, thus increasing the rate of hydrolysis. The two variables related to THP are THP temperature and THP duration (Bougrier et al., 2008; Donoso-Bravo et al., 2011). Pressure is also an important variable during THP. However, pressure during THP usually changes with temperature. Therefore, pressure was not considered part of the work presented in this thesis. Moreover, THP temperature is considered more important in terms of solubilizing organics (Donoso-Bravo et al., 2011). Another variable was the time required to reach the desired THP temperature. Bougrier et al. (2008) found that the duration to reach the desired THP temperature varied from 25 to 60 minutes. Donoso-Bravo et al. (2011) only required 10 minutes to reach a temperature of 170 °C, while Xue et al. (2015) reported that the rise in temperature took 90 to 120 minutes.

Simultaneously, THP duration is important when assessing the effectiveness of low-temperature THP (Xue et al., 2015). Higher solubilizations can be achieved with low THP temperatures, but a longer duration of THP is required (Ariunbaatar et al., 2014). The main disadvantage of THP is energy requirement (Carballa et al., 2011). However, energy is usually recovered by coupling THP with AD to enhance the production of biogas.

THP temperatures selected for the current study were 50, 70, 80, 100, 120 and 140 °C at a fixed THP duration of 30 minutes. The time required to reach the specified temperatures was dependent on the setpoint value on the temperature controller. Hence, the THP duration does not include the time required to reach specified temperatures.

2.3 Effect of THP on VFAs production

The increase in solubilization due to THP is usually studied at several different THP temperatures and THP durations. The VFAs production was carried out using thermally pretreated food waste for 30 minutes at several different THP temperatures ranging from 100–220 °C. Yin et al. (2014) found that the Maximum solubilization (increase in sCOD) of 42.5% was achieved at 180°C with maximum VFAs yield of 0.908 g/g-VSS_{removal} achieved at 160 °C (28% higher than VFAs yield at 180 °C). The increase in sCOD is related to THP temperatures. Increase in VFAs production was a result of solubilization of organic waste, but treatments at a higher THP temperature showed increased solubilization, but no increase in VFAs production. The hydrothermal pretreatment of food waste at temperatures higher than 160 °C led to the formation of toxic and non-biodegradable products despite higher COD solubilization (Yin et al., 2014). Dewatered pig manure showed a lowered rate of biogas production when thermally pretreated at 110, 130, and 150 °C in comparison to pretreatment temperatures in the range of 70–100 °C (Rafique et al., 2010).

Consequently, higher temperatures can increase solubilization but lower AD reactor performance, but this is determined by the type of substrate used for AD. For example,

THP at 170 °C has been shown to cause the agglomeration of waste activated sludge (WAS), suggesting the formation of chemical bonds (Bougrier et al., 2006).

The effects of THP on VFAs production using several substrates are well documented, but information related to the effect of solubilization of potato starch on VFAs production using AD is scarce and requires further investigation. Hence, this study was focused on evaluating the effect of THP temperatures on starch solubilization, followed by VFAs production at different operating conditions using starch (No_THP, THP_100, and THP_140), and heat-shocked mixed culture (to inhibit methanogenesis) as an inoculum to determine if VFAs can be produced consistently under specified operating conditions.

2.4 Effect of THP on methane production

The improvements in the production of biogas containing methane are well documented (Donoso-Bravo et al., 2011; Xue et al., 2015). Particle size is inversely proportional to the substrate utilization rate as discussed by Kim et al. (2000). Similarly, Esposito et al. (2011) inferred that lower methane production was a result of a large particle radius. Ma et al. (2011) reported 24% increase in methane production when kitchen waste was thermally treated at 120°C for 30 minutes, whereas Liu et al. (2012) reported a decrease in methane production by approximately 8% and 12% from thermally treated kitchen waste (KW) and vegetable/fruit residue (VFR) respectively at 175°C for 60 minutes. Treatments at a higher temperature (above 150°C) usually showed increased solubilization but no increase in methane production because of the Maillard reaction (Dwyer et al., 2008). Therefore, it can be inferred that enhanced biogas production was

mostly due to solubilization of particulates and any temperature higher than 150°C might lower the performance of AD process digester due to the formation of melanoidins (Dwyer et al., 2008).

Moreover, the effect of any pretreatment can be easily visualized for biogas production because biogas, a gaseous product, separates itself from the aqueous phase. However, the same analogy cannot be applied for VFAs which remain in the aqueous state and are essential as an energy and carbon source for microorganisms (Parawira et al., 2004).

As such, the THP process was the focus of this project. The temperatures selected in this study were 70, 100, and 140 °C for methane production. For each THP temperature, the duration employed was 30 minutes.

2.5 Co-digestion of potato starch and PHL

As discussed before, AD is considered as waste-to-energy technology and has been used for digestion of different organic wastes (Haug et al., 1978; Izumi et al., 2010; Rafique et al., 2010). Anaerobic co-digestion technology allows the simultaneous digestion of various organic wastes. The co-digestion allows overcoming a deficiency of one substrate with another with complementing properties or characteristics. The advantages of co-digestion are well documented and include the reduction in the concentration of toxic compounds, the fulfillment of nutrient deficiency, improved biodegradable substrate loading, increased biogas production via enhanced rate of digestion (Sosnowski et al., 2003) and lower cost. All these benefits are essential for stable AD reactor operation and performance (Esposito et al., 2012). This co-digestion method can provide impressive

results because of the synergistic impact between different organic substrates when processed simultaneously (Rao and Baral, 2011). The above statement is only valid if the co-substrates are used in the appropriate ratio.

In the present context of the province of New Brunswick, Canada, the two specific resources that that can be used for co-digestion are pre hydrolysis liquor (PHL) from paper mills and waste potato starch from potato processing industries. PHL is a waste product consisting mainly of carbohydrates, lignin, acetic acid, and furfural with a significant amount of reducing sugars (Kale et al., 2009). Potato starch is a polymer of glucose which contains amylose (single chained) and amylopectin (highly branched) as building blocks. The batch respirometric studies at 35 °C showed a decrease in methane production with an increasing concentration of PHL (from 20 to 100 g/L as COD) in the feed (Kale et al., 2009). The presence of slowly biodegradable substrates (furfural and lignin) in the feed was suspected of causing such behavior (Debnath et al., 2013). The hydrolysis of potato starch during AD is considered a rate-limiting because of its particulate nature and insolubility in water (Snowdon et al., 2019).

The PHL requires dilution to minimize the effect of slowly biodegradable substrates such as furfural and lignin, whereas hydrolysis of starch to glucose should be accelerated in order to minimize the rate limitation of hydrolysis. The conversion of starch to glucose is usually carried out by an important chemical process using acids, known as acid pretreatment or hydrolysis. The acid usually used for this conversion process is sulfuric acid. Sulfuric acid remains a better hydrolyzing agent studied across the literature, but the sulfate reducers (a subcategory of methanogens) are known to compete for precursors

such as VFAs and hydrogen leading to the production of hydrogen sulfide, instead of methane.

The PHL should be preferred as a hydrolyzing agent because it contains acetic acid, which is also a precursor for methane production. Although there is a deeper understanding of the effect of acid hydrolysis on starch solubility, its co-digestion with PHL (containing acetic acid) using AD remains to be investigated. Hence, three different concentrations (based on COD) using potato starch and PHL were considered for this study to analyze the effect of co-digestion.

2.6 Anaerobic treatability study of food waste

In the present context of North America, the amount of food waste in the United States has increased by 50% since 1974, and currently, about 38 million tons of food waste are discarded annually, with 76.3% landfilled. There are very few plants that solely digest food waste, or Source-Separated Organics (SSO) as it is termed in Canada. Food waste can be generated from several sources, including the production, processing, distribution, storage, sale, preparation, cooking, and serving of food. To keep the explanation simple, only food waste from grocery stores was considered during this literature review. On average, developed countries generate 100–170 kg of food waste per capita per year, more than twice that of developing countries (Dung et al., 2014). Some developing countries, such as China and India, also face challenges in food waste disposal due to the large total population.

Recovering energy and nutrients from food waste not only constitutes substantial economic opportunity but is also an essential requirement for the sustainable development of human society. AD of food waste is facing technical challenges such as lack of concise process control and optimization, production of harmful intermediate compounds, or causing low methane yield or foaming (Grimberg et al., 2015). One common type of system instability is caused by the rapid conversion of the easily digestible food waste to VFAs at an early stage of the digestion process, resulting in a drastic pH drop if sufficient buffering capacity is not present. The high protein and lipids contents in food waste can also easily lead to inhibitory levels of ammonia, hydrogen sulfide, and long-chain fatty acids. As a result, AD of food waste often has to be performed at low organic loading rates (OLRs) of 2–3 kg-COD/m³·day to prevent process failure (Hecht and Griehl, 2009). Increasing food waste loading and system stability remains a challenge and is crucial for the economic viability of AD. Hence, anaerobic treatability of food waste was carried out to determine the digestibility of food waste.

3. Materials and Methodology

3.1 Reactor configuration and operation

3.1.1 Thermal Hydrolysis Pretreatment (THP)

A Parr Reactor, with a working volume of 400 mL and a total volume of 600 mL, was employed in the study. It was initially used to treat starch solution at three different starch concentrations (10 g/L, 15 g/L and 25 g/L) at six different THP temperatures (50 °C, THP_50; 70 °C, THP_70; 80 °C, THP_80; 100 °C, THP_100; 120 °C, THP_120 and 140 °C, THP_140) to enhance solubilization. The temperature was controlled using an external heater. All the experiments were conducted at the same agitator rotation speed and experimental run time, i.e., 150 rpm and 30 min, respectively. The time required to reach the specified temperatures was dependent on the setpoint value entered on the temperature controller. The experimental run time does not include the time required to reach specified temperatures. A schematic and a photo of the Parr reactor (Series 4560, Parr Instrument Company, Illinois, USA) is shown in Figure 2.

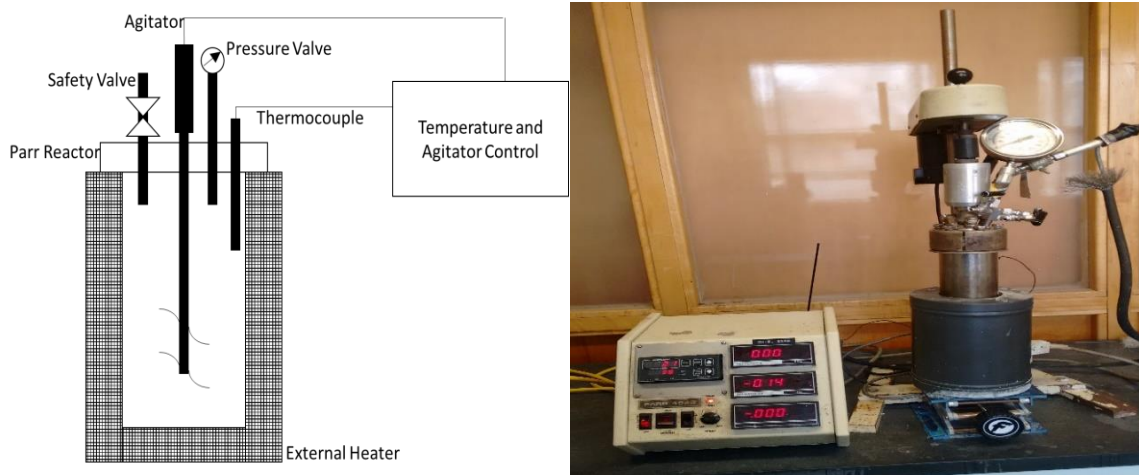


Figure 2: Parr reactor

3.1.2 Batch anaerobic respirometer system

3.1.2.1 VFAs production

Identical reactors, with a working volume of 250 mL and a total volume of 310 mL, were employed in the study. The reactors were operated in batch mode. Two reactors each were fed with starch with no thermal hydrolysis pretreatment (No_THP), starch after thermal hydrolysis pretreatment at 100 °C (THP_100) and starch after thermal hydrolysis pretreatment at 140 °C (THP_140) were used. Each reactor was stirred at a constant speed of about 150 rpm. The temperature was controlled at 35±1 °C using a water bath connected to a water recirculator. Nutrients were supplied along with substrate (Young and Cowan, 2004) as discussed in Section 3.7.8. Sodium bicarbonate (NaHCO₃) was used as a pH buffering reagent to maintain the pH between 6.5-6.8. Reactors were flushed with nitrogen gas to create anaerobic conditions. The arrangement for the batch anaerobic respirometric system (Challenge AER-208, Arkansas, USA) is shown in Figure 3.

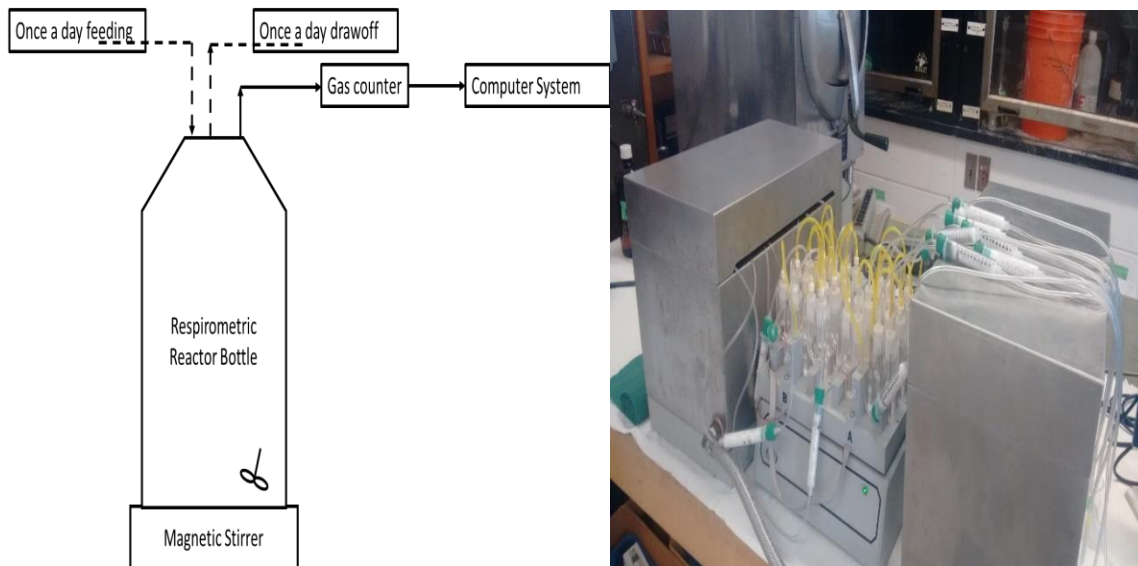


Figure 3: Batch anaerobic respirometric reactor

3.1.2.2 Methane production

The same reactor system as described in Section 3.1.2.1, was used for methane production. Two reactors each were fed with starch with no thermal hydrolysis pretreatment (No_THP), starch after thermal hydrolysis pretreatment at 70 °C (THP_70), starch after thermal hydrolysis pretreatment at 100 °C (THP_100), and starch after thermal hydrolysis pretreatment at 140 °C (THP_140) were used. The pH was maintained between 6.8-7.2 using sodium bicarbonate.

3.1.3 Continuous Anaerobic Trickling Filter (ATF)

Anaerobic Trickling Filter (ATF) using Kaldnes K1 as specialized growth media was used for a continuous mode of operation. A laboratory-scale reactor with an effective volume of 8.64 L was designed in-house for a continuous mode of operation. The reactor was made of plexiglass and was designed as a cylindrical unit. Feeding was through the top inlet using a peristaltic pump (P1), and the effluent was withdrawn from the bottom of the reactor using pump P2. A pipe from the reactor was connected to a tipping bucket gas meter to measure the total biogas production. The arrangement for the continuous study and the media i.e., Kaldnes K1, is shown in Figure 4 and Figure 5, respectively.

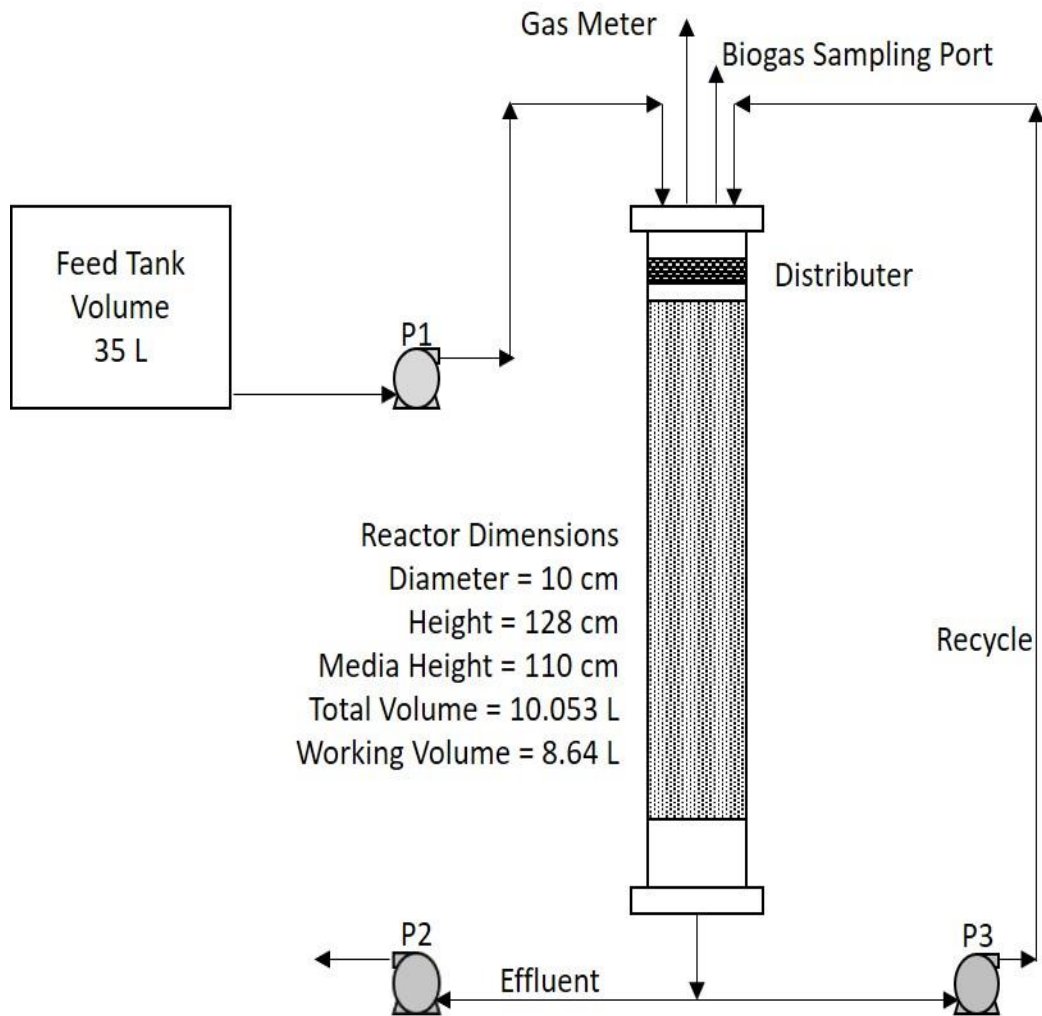


Figure 4: Continuous Anaerobic Trickling Filter (ATF)



Figure 5: Kaldnes K1 media

3.2 Influent characterization

Potato starch was obtained from a local potato processing industry. Starch solutions were made using oven-dried (105 °C for 4 hours) starch and adding distilled water to obtain the desired concentrations. The potato starch obtained from the industry had big chunks of starch which was ground to a finer powder using a pestle and mortar, as shown in Figure 6 and Figure 7.



Figure 6: Potato starch

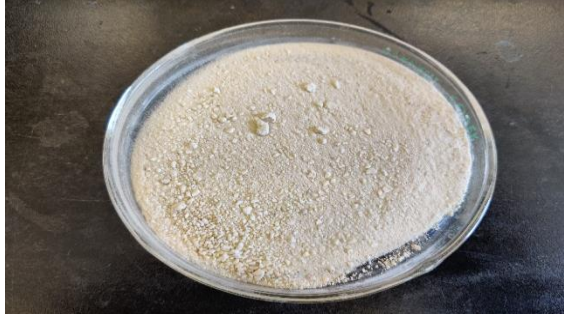


Figure 7: Potato starch after grinding

Figure 8 represents the AD reactor feeding strategy to determine the effect of THP on VFAs production. The same feeding strategy, as shown in Figure 8 was used for methane production, but the anaerobic sludge was not thermally pretreated at 102 °C for 2 hours.

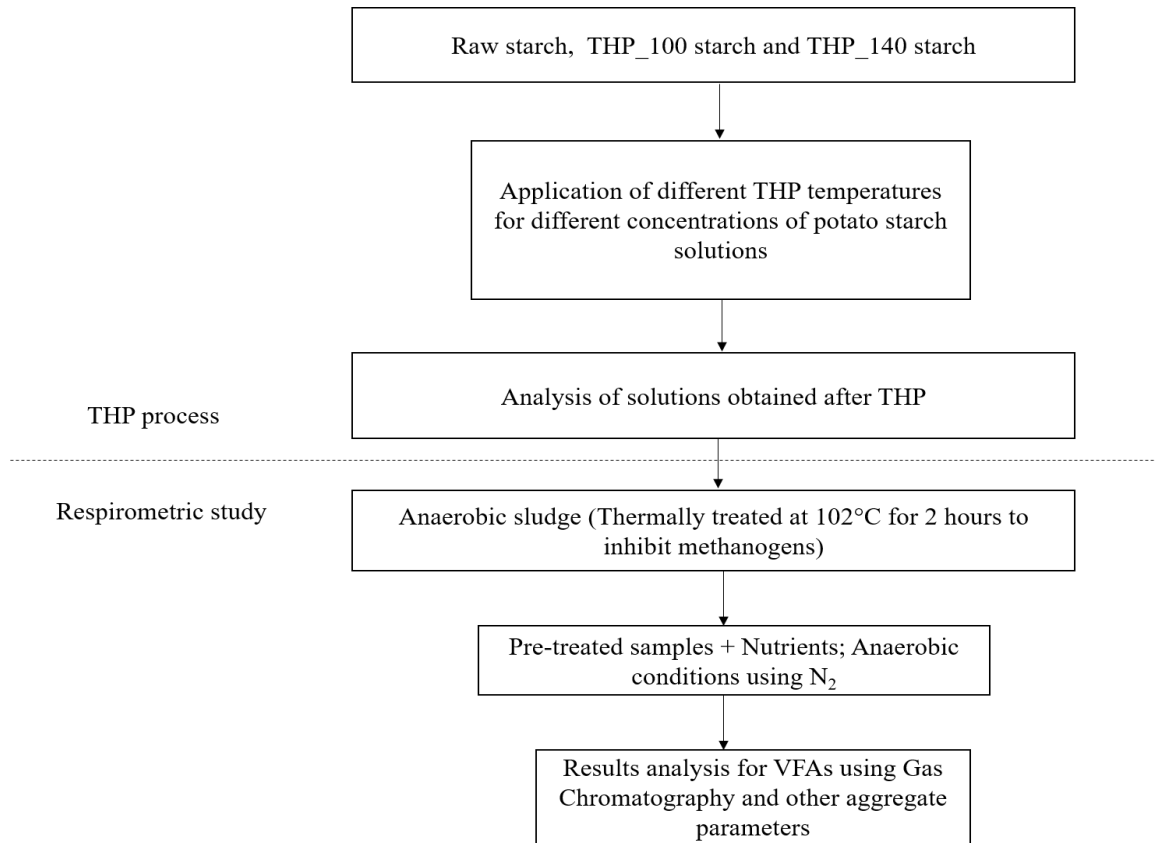


Figure 8: AD reactor feeding strategy for VFAs production

The potato starch used in the co-digestion study (with PHL) was oven-dried as well. PHL exists in aqueous form. The substrates were mixed to provide three different concentrations of PHL and potato starch, namely i) 100 % PHL, ii) 100 % potato starch and iii) 50% PHL and 50% potato starch.

The food waste was obtained from a local grocery store. It primarily consisted of a mixture of produce or vegetable waste and meat waste. The food waste contained 2.79 kg of green leafy waste including lettuce and spinach (qualitative resemblance to the salad), along with 3.69 kg of meat waste including chicken, steak, and beef. The waste was homogenized using a blender and passing through 1.6 mm sieve before feeding to the AD

reactors as depicted in Figure 9 and Figure 10. The blender seemed to work fine with produce waste but was not able to ground the meat chunks into smaller portions. Hence, the meat wastes were discarded for this study.



Figure 9: Food waste

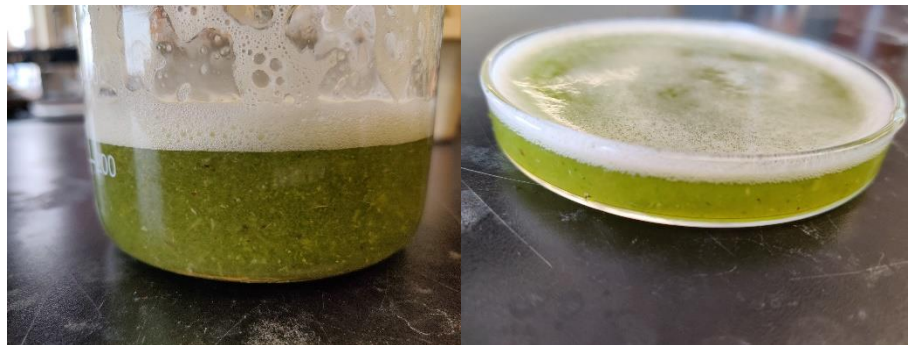


Figure 10: Food waste after grinding

All the reactor systems were run based on the aggregate composition of the substrates, including COD and BOD. Other analyses included alkalinity and pH. Figure 11 represents the AD reactor feeding strategy to determine methane production from the co-digestion of PHL and potato starch, and food waste.

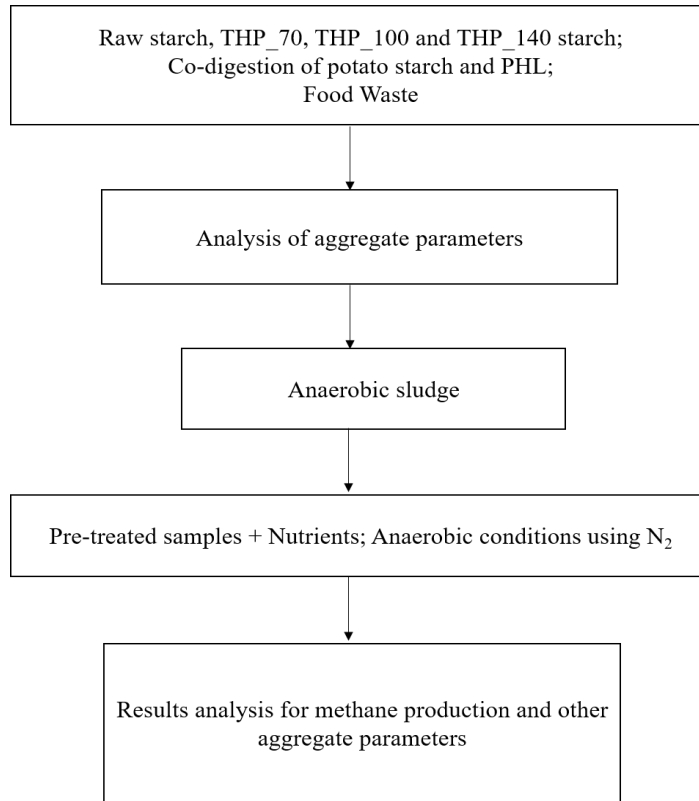


Figure 11: AD reactor feeding strategy for methane production

3.3 Master culture

The master culture reactor was set up at 35 °C to allow acclimatization of biomass to the substrate. The anaerobic seed sludge was obtained from ADI Systems Inc. pilot reactor used for the treatment of sugar and starch-based synthetic wastewater and was used as inoculum. The inoculum was used to seed the master culture reactor for biogas production and later, this seed sludge was used to run the respirometric study for biogas production using potato starch, co-digestion of starch and PHL, and anaerobic treatability study of food waste. The seed sludge from the master culture reactor was heat-shocked at 102 °C for 2.5 hours to limit methanogenesis for VFAs production. The master culture reactor was operated in batch mode of operation with once a day feeding and once a day

draw off mode. Initially, the reactor was fed with sucrose and later was switched to starch-based synthetic wastewater. Micronutrients were added as discussed in Section 3.7.8 Nutrients and buffer formulation. Daily gas production and effluent pH were monitored.

3.4 Experimental Design for THP

The experimental design for the THP process is available in Table 1.

Table 1: Experimental design for THP process

Group	Observation	Pretreatment Method	Observation
Group 1	COD, pH, Solids analysis	Thermal hydrolysis pretreatment (THP)	COD, pH, Solids analysis
Group 2	COD, pH, Solids analysis	-	COD, pH, Solids analysis

The above experimental design is based on the pretest-posttest control group design. This design allows determining the change in the independent variable, i.e. soluble COD with a change in the independent variable, i.e. THP temperatures.

The experimental design for THP is provided in Table 2.

Table 2: Independent variables for THP process

Experimental Run	Starch concentration (mg/L)	THP temperature (°C)
1 – 3		50
4 – 6		70
7 – 9		80
10 – 12	10, 15, 25	100
13 – 15		120
16 – 18		140

Initially, it was proposed that experimental runs would be carried out for starch-based wastewater concentration (as COD) at 15, 25, and 40 g/L. However, the volume of the filtrate obtained through glass fiber and membrane filter papers after preliminary THP experimental runs using these starch concentrations was too low to carry out any analysis. Hence the experimental design was changed, and starch concentration to be studied was brought down to 10, 15 and 25 g/L.

3.5 Experiment design for methane production

After studying the impact of THP on solubilization, the effect of solubilization was evaluated on methane production. The AD reactors were operated until they reached the steady-state condition (methane volume $\pm 10\%$). The experimental design is available in Table 3. The reactor pH was kept between 6.5 – 7.1 and the reactors were stirred at 150 rpm. The reactors were operated in once-a-day feeding and once-a-day draw-off mode.

The micronutrients were added as discussed in Section 3.7.8 Nutrients and buffer formulation, and pH was maintained by dosing sodium bicarbonate (NaHCO₃).

Table 3: Experimental design for methane production

Group	Observation	Respirometric Study	Observation
Group 1	Substrate (after THP)	Sample added to	Biogas production rate,
Group 2	Substrate (No_THP)	AD reactors	Effluent COD, pH

3.6 Experimental design for VFAs production

The experimental design used for VFAs study using batch mode and continuous mode is available in Table 4.

Table 4: Experimental design for VFAs production

Mode of Operation	Run	Temperature (°C)	pH	HRT (days)	OLR (kg-COD/m³·day)	COD (g/L)
Batch Mode	1	35	6.5-6.7	0.5	6	3
	2			1	6	6
	3			2	6	12
	4			0.5	9	4.5
	5			1	9	9
	6			2	9	18
Continuous Mode	1	35	6.5-6.7	0.28	3.6	1
	2			0.28	5.5	1.50
	3			0.28	9.5	2.65

Independent variables such as organic loading rates (OLRs) and hydraulic retention times (HRTs) were changed continually by changing the concentration after the reactor reached a steady-state (VFAs concentration $\pm 10\%$). Reactors were operated in once-a-day feeding and once-a-day draw-off mode. Reactor pH was maintained around 6.5 to limit methanogenesis and the reactors were stirred at 150 rpm.

3.7 Analytical methods

Several analytical methods were used following Standard Methods (APHA, 2005) and mentioned below:

3.7.1 Chemical Oxygen Demand (COD)

The COD is used as an indicator of pollutants in wastewater and is a measure of the oxygen required for the degradation of biodegradable and non-biodegradable components present in the given sample. The COD is categorized into three subcategories as mentioned below:

Total COD (tCOD): It is due to the presence of organic matter which is the combination of suspended solids and dissolved organic compounds. It was measured using the given sample after the required dilution.

Filtered COD (fCOD): It is due to the dissolved organic compounds only. It was measured using the portion of the sample that passes through 1.2 μm glass fiber filter paper (Whatman 934-AH, UK).

Soluble COD (sCOD): It is due to the presence of dissolved particles (completely soluble) and a portion to the presence of colloidal particles. It was measured using the

portion of the sample obtained from filtered COD and passing through 0.45µm membrane filter (Whatman WCN type, UK).

All COD were triplicated and conducted before and after every experimental run.

3.7.2 Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD) is the biodegradable component of organic waste and is an important analysis to determine the biodegradability of organic waste. Influent BOD was measured using the 5-Day BOD Test. The final and initial dissolved oxygen (DO) concentrations were measured using a YSI DO Meter (Model No. 58, USA) calibrated at the time of usage at ambient temperature.

3.7.3 pH

The pH was measured using the Fisher Scientific AB15 pH Meter. The pH probe was standardized against standard solutions of pH 4.0 and 7.0.

3.7.4 Solids analysis

The influent Total Solids (TS), Total Suspended Solids (TSS), and Volatile Suspended Solids (VSS) were measured using the gravimetric method. TS was measured using a select sample volume and drying the sample at 102 °C. TSS was measured using a select sample volume that was filtered through 1.2 µm glass-fiber filter paper (Whatman 934-AH, UK) before drying in the oven at 102 °C, followed by igniting the samples at 550 °C for VSS.

3.7.5 Biogas analysis

Varian CP-3800 gas chromatograph (GC) was used for biogas analysis. The GC is fitted with an Alltech CTR 1 concentric packed column and a thermal conductivity detector (TCD). Helium is used as a carrier gas at a flow rate of 30mL/min.

3.7.6 VFAs measurements

VFA samples, before being analyzed using GC, were centrifuged at 4750 RPM for 30 minutes followed by filtering the sample solution through a 0.45 μ m membrane filter (Whatman WCN type, UK) and then adding 5% v/v phosphoric acid. VFAs were analyzed with Varian CP-3800 Gas Chromatograph. It is equipped with a wall coated open tubular fused silica column (0.32 mm inner diameter), a wall thickness of 1.2 μ m, and a flame ionization detector (FID). Helium (He) is used as a carrier gas at a flow rate of 5 mL/min.

3.7.7 Alkalinity

The total alkalinity of the reactor was measured using the titration method. A selected sample size of effluent had a standard solution of sulfuric acid added until reaching a pH of 4.3. The pH of the solution during the titration process was monitored using the Fisher Scientific AB15 pH Meter.

3.7.8 Nutrients and buffer formulation

The micro-nutrients and buffer used are formulated in four categories in Table 5 (Young and Cowan, 2004).

Table 5: Nutrient and buffer formulation

Category	Nutrients	Amount	Elemental amount
Mineral Base 1	CoCl ₂ .6H ₂ O	0.25	0.062 mg-Co/mL
	FeCl ₂ .4H ₂ O	4.00	1.126 mg-Fe/mL
	MnCl ₂ .4H ₂ O	0.05	0.0139 mg-Mg/mL
	H ₃ BO ₃	0.025	0.0044 mg-B/mL
	ZnCl ₂	0.025	0.0119 mg-Zn/mL
	Na ₂ MoO ₄ .2H ₂ O	0.005	0.0020 mg-Mo/mL
	NiCl ₂ .6H ₂ O	0.025	0.0062 mg-Ni/mL
	Na ₂ SeO ₄	0.025	0.0104 mg-Se/mL
Mineral Base 2	CuCl ₂ .2H ₂ O	0.007	0.0026 mg-Cu/mL
	CaCl ₂	15	5.4 mg-Ca/mL
	MgCl ₂ .6H ₂ O	20	2.36 mg-Mg/mL
Nutrient Base	NH ₄ Cl	53	13.9 mg-N/mL
Buffer Base	NaHCO ₃	60	-

For every 20g-COD/L of the substrate, 20mL/L each of mineral base 1, mineral base 2, the nutrient base were added when solutions were made. Sodium bicarbonate (usually 6 g/L) was added separately to maintain the pH as required. It was assumed that this nutrient composition provided all the nutrients for microbial activity in sufficient amounts, i.e. without any nutrient deficiencies.

3.7.9 Data collection

The variables and their frequency of measurement, for THP study and AD study, are available in Table 6.

Table 6: Data collection frequency

Variables	Data collection frequency (weekly)	
	THP study	AD process study
THP temperature	3 times	-
THP agitator rpm	3 times	-
THP duration	3 times	-
Total COD	3 times (before and after)	2 times for influent; 5 times for effluent
Filtered COD	3 times (before and after)	-
Soluble COD	3 times (before and after)	-
Total Solids	3 times (before and after)	2 times for influent
TSS	3 times (before and after)	2 times for influent
VSS	3 times (before and after)	2 times for influent
pH	3 times (before and after)	2 times for influent
VFAs - GC analysis	Once every week	5 times for effluent
BOD	-	Once a month for influent
Biogas volume	-	5 times for biogas
AD temperature	-	5 times for AD study
Biogas - GC analysis	-	5 times for biogas
Alkalinity	-	2 times for effluent

4. Results and Discussion

4.1 Effect of THP on starch solubilization

The starch samples were thermally pretreated in the Parr reactor. Before THP, the fCOD and sCOD values ranged from 180–225 mg/L and 130–165 mg/L, respectively, which is only 0.5–2% of the tCOD, irrespective of the starch concentration. Numerically, the COD values of the starch samples resemble the COD values obtained for primary sludge (PS) and WAS, which range from 18,000 mg/L to 20,000 mg/L, respectively. However, the sCOD values were merely a fraction of the tCOD values (Shanableh and Jones, 2001; Wu et al., 2009). Figure 12 compares the values of tCOD, fCOD, and sCOD for the starch treated by THP at different temperatures.

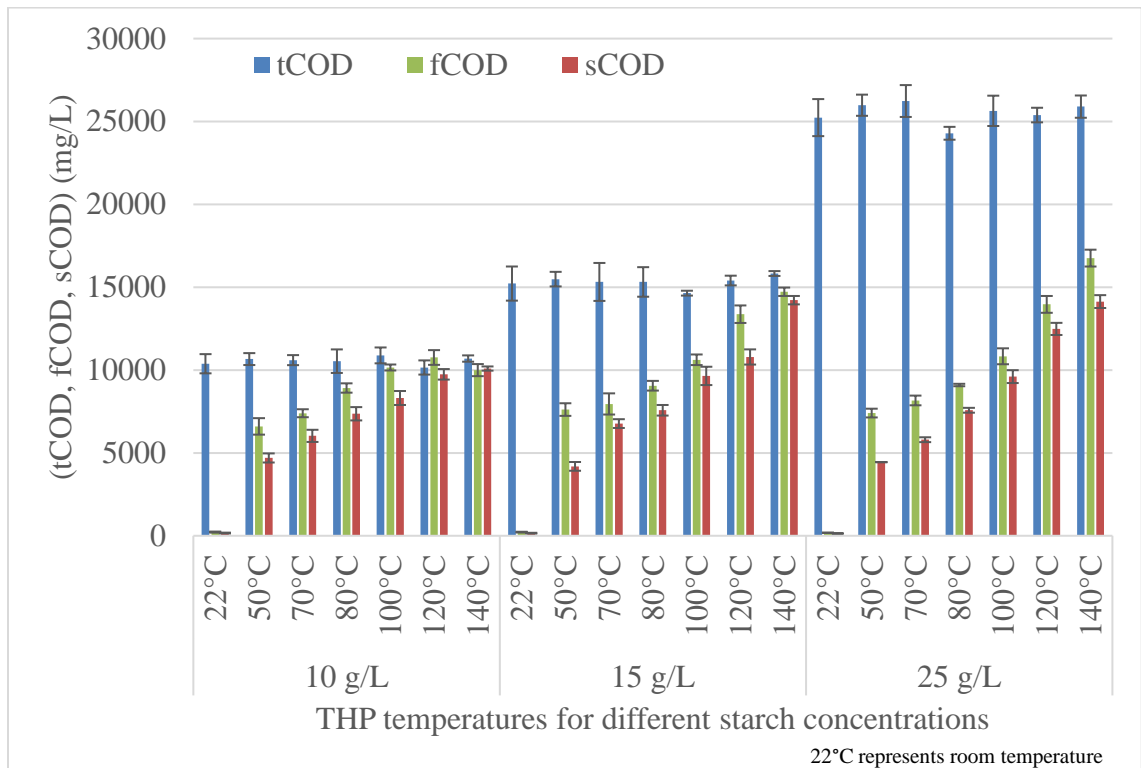


Figure 12: COD measurements of starch samples at different THP temperatures

The tCOD and TS have similar values before and after THP, showing the variabilities that range from only 5–6% and 1–10%, respectively, at all concentrations. The solution was allowed to cool inside the reactor after pretreatment and only taken out once it had reached 30–35°C, which resulted in higher numerical values after THP for all starch concentrations and all THP temperatures because there was loss of some water as the solution cooled to room temperature. However, the percentage difference between the tCOD values before and after THP was less than 2%.

Figure 13 shows that the solubilization increases with the pretreatment temperatures. The solubilization is a function of the THP temperature because of sCOD increases with increasing temperature. The increase in sCOD with THP shows a linear increase with R^2 values ranging from 0.74–0.90, with the maximum solubilization achieved at 140 °C for all concentrations.

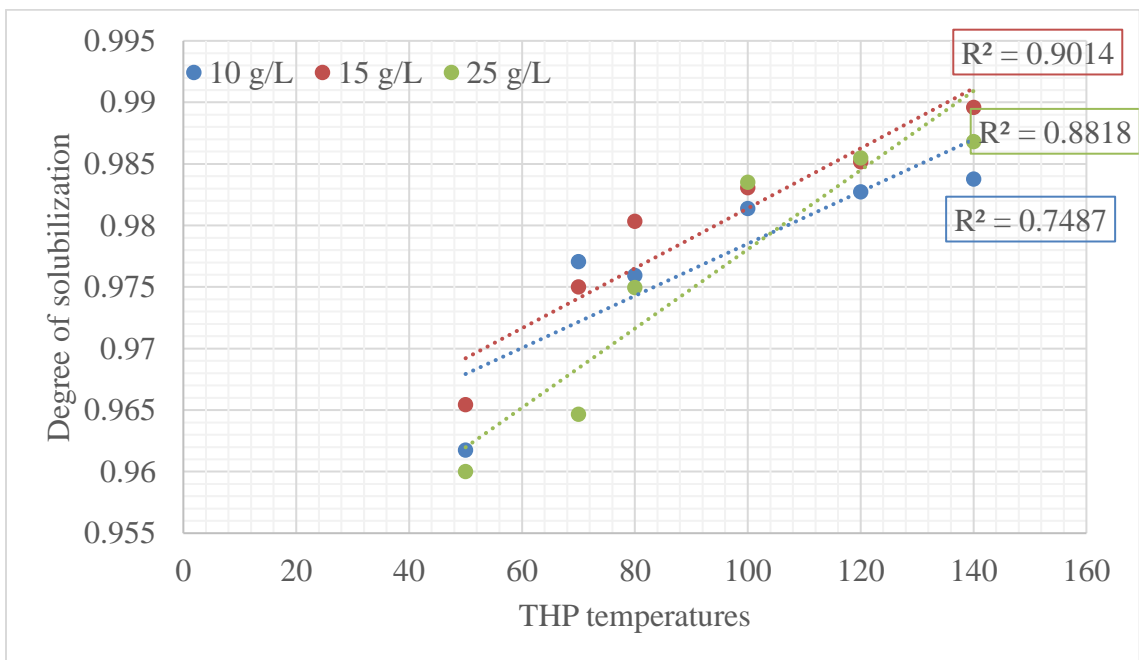


Figure 13: Solubilization at different THP temperatures

After THP, the solutions having starch concentrations greater than 15 g/L were filtered at a slow rate through a glass fiber and membrane filter, which was attributed to the increase in the size of the starch granules induced by THP. The increase in solubilization, i.e., the increase in sCOD and reduction in TSS, were calculated using equation (1) and (2).

$$\Delta(sCOD) = \frac{sCOD_f - sCOD_i}{sCOD_f} \times 100 \quad (1)$$

$$\Delta(TSS) = \frac{TSS_f - TSS_i}{TSS_f} \times 100 \quad (2)$$

Here, the results are given in percentage units; $sCOD_i$ and $sCOD_f$ are the sCOD values before and after THP treatment, respectively; and TSS_i and TSS_f are the TSS values before and after treatment, respectively. The above equations were also used to calculate the increase in fCOD and reduction in VSS.

Because of solubilization, increases in fCOD and sCOD were observed with a simultaneous reduction in TSS and VSS in comparison to the No_THP starch sample. The results obtained for the increase in solubilization, i.e., increase in fCOD and sCOD, were comparable to the reduction in TSS and VSS. Figure 14 compares the reduction in TSS and VSS at six different THP temperatures.

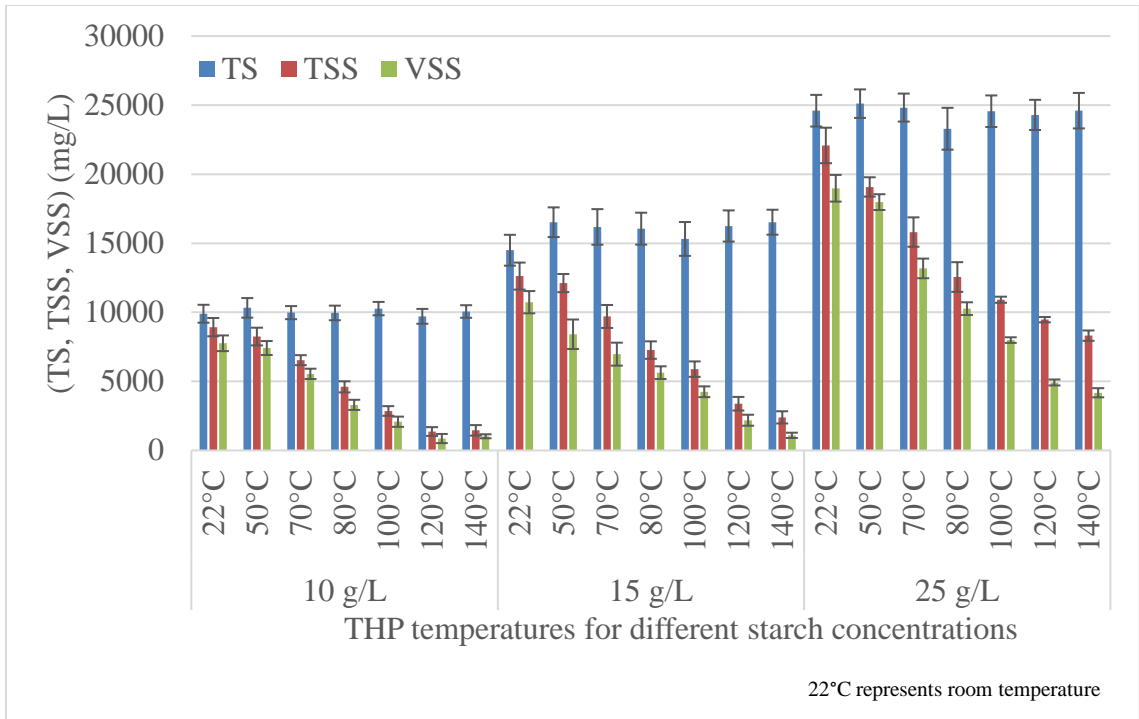


Figure 14: Solids measurements for starch samples at different THP temperatures

The average pH for the No_THP starch sample ranged from 7.5–8.1, which is slightly basic, as expected. The pH decreased after THP, falling to 6.25 and 5.16 for THP_100 and THP_140, respectively, because the solutions were not buffered. The same finding was reported by Marchal et al. (1999). As the pH decreased with increasing THP temperature, VFA analysis was performed on the samples; however, no VFAs were detected.

4.2 VFAs production from AD reactors fed with starch (No_THP and THP_100)

The waste potato starch was thermally pretreated and fed to anaerobic respirometric reactors at organic loading rates (OLRs) of 6 and 9 kg-COD/(m³·day) and HRTs of 0.5, 1, and 2 days by continually changing the thermally pretreated starch concentrations. The reactors were operated at (35 ± 1) °C. The pH was maintained between 6.5 and 6.7 using NaHCO₃ to limit methanogenesis, as the desired pH for methanogenesis is between 6.8 and 7.6 (McCarty, 1964). The effluent COD and pH were measured every day. The VFAs were also quantified by GC every day, and the reactor performance was evaluated in terms of VFA yield (measured as mg-VFAs/mg-COD_{applied}) and COD balance. The sample COD calculations are available in Appendix A. The tCOD of the influent remained constant throughout the experiment but varied slightly (by 6%–10% for all substrate concentrations) as new solutions were fed into the reactor frequently. The headspace of the reactors was purged with nitrogen gas to create anaerobic conditions. The concentration of starch inside the reactors fed with No_THP starch showed 5%–10% variability possibly because of the heterogeneity of starch, i.e., its particulate nature and insolubility in water. The individual reactors were assumed to be in a steady state when the total VFAs concentration in the effluent reached a constant value (±10%). Minimal quantities of methane were generated during the AD process for reactors fed with No_THP, THP_100, and THP_140 starch samples.

Figure 15 compares the VFAs profiles for reactors fed with No_THP and THP_100 starch at an OLR of 6 kg-COD/(m³·day) with HRTs of 0.5, 1, and 2 days. Initially, the concentration of individual VFAs increased significantly, reaching a steady-state after 15 and 9 days for the reactors fed with No_THP and THP_100, respectively, at an HRT of

0.5 days. Similar observations were made for the reactors fed with THP_100 starch as the reactors were able to reach steady-state faster when operational condition i.e. HRT was changed from 0.5 to 1 and then 2 days in comparison to reactors fed with No_THP starch. This was a result of the higher sCOD of the substrate pretreated at 100 °C in comparison with that of starch not thermally pretreated. The total VFAs concentration increased with increasing HRT, from 0.53 g/L to 1.48 g/L, and finally to 3.30 g/L. The maximum VFAs yield of 0.308 mg-VFAs/mg-COD_{applied} was obtained at an HRT of 2 days, which was 12.1% higher than the maximum VFAs yield of 0.271 mg-VFAs/mg-COD_{applied} obtained for the reactor fed with No_THP. The GC analysis for VFAs speciation showed the presence of several VFAs, including acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, and valeric acid. It is difficult to predict the product spectrum with changing operating conditions, and hence, the analysis of individual VFAs was necessary. Banerjee et al. (1999) reported the higher concentration of butyric acid was a result of using starch as a substrate. Irrespective of the HRT, acetic acid and butyric acid together represent approximately 79%–83% of the product spectrum at an OLR of 6 kg-COD/(m³·day), whereas propionic acid was only 8-13% of the product spectrum. The concentrations of iso-butyric acid (iso-BA), iso-valeric acid (iso-VA), and valeric acid (VA) were minimal for all operating conditions. The higher quantities of these VFAs are primarily associated with the digestion of organic waste that is high in protein (Elefsiniotis and Oldham, 1994).

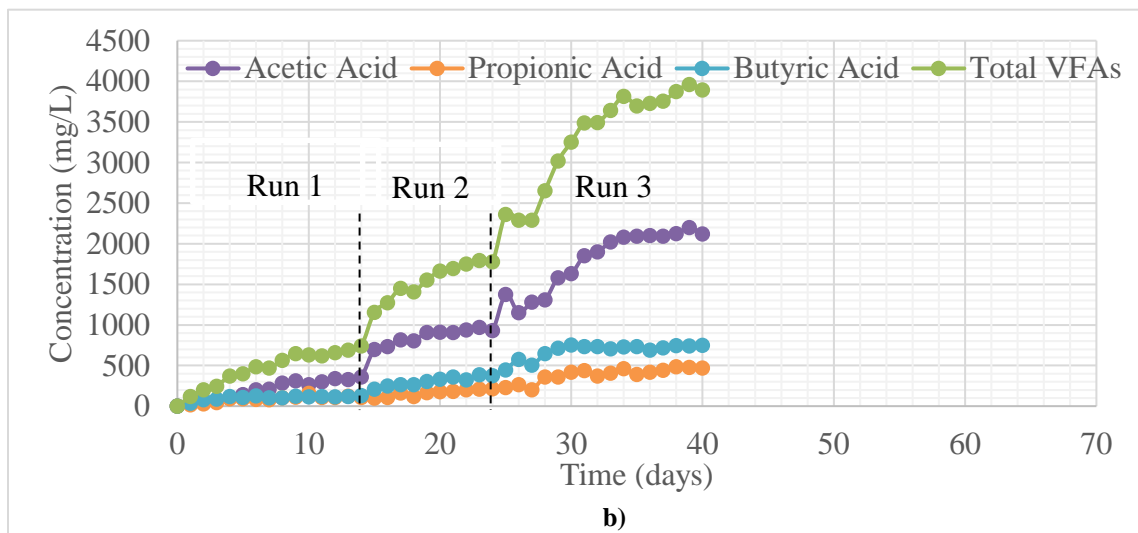
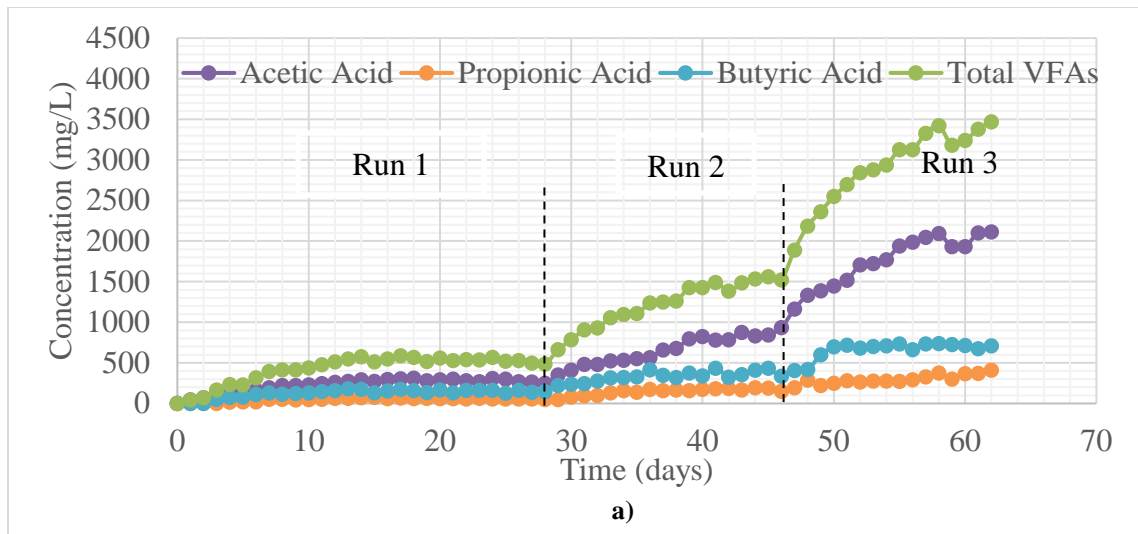


Figure 15: VFAs profile at OLR of 6 kg-COD/m³·day a) No_THP and b) THP_100

The pH can influence VFAs production because it affects the amount of acid compounds formed during hydrolysis, i.e., during and after the breakdown of complex molecules.

Even with increasing VFAs concentration, there was no change in the VFAs profile, and single-chain VFAs such as acetic acid was the dominant product, followed by butyric and propionic acid. Lee et al. (2014) reported that the VFAs profile is dependent on the pH

level and the substrate used. The concentration of acetic acid was the highest and can be explained by the pH values, which ranged from 6.5–6.7 (Horiuchi et al., 2002). Within this pH range, the reactors fed with No_THP and THP_100 yielded the same VFAs profile, as also observed by Parawira et al. (2004) at solid potato loads of 500 g. Thus, THP did not have any significant effect on the VFAs profile. Small quantities of VFAs such as iso-BA, iso-VA, and VA were formed.

The application of higher HRT proved beneficial because the concentration of VFAs increased with increasing HRT. A higher HRT results in more contact time between the microorganisms and substrate, leading to greater VFAs production. The same observations were made for No_THP and THP_100 operated at an OLR of 9 kg-COD/(m³·day) at multiple HRTs. The only effect of THP was the solubilization of particulate starch, i.e., an increase in sCOD, which helped in achieving the steady-state conditions approximately 45% faster, i.e., 7 days for the reactors fed with THP_100 instead of 13 days for reactors fed with No_THP at an HRT of 0.5 days, which is evident from Figure 16.

At 9 kg-COD/(m³·day), acetic acid remained the dominant product, followed by butyric acid, together representing 66%–75% of the product spectrum, irrespective of HRT, and there was no significant solubilization effect arising from THP, apart from a reduction in reactor startup time. The lesser time required to reach steady-state performance signified the importance of using a simple substrate (solubilized starch – high soluble COD) for reactor startup rather than a particulate starch. The maximum VFAs yield was found to be 0.297 mg-VFAs/mg-COD_{applied} for the reactors fed with the THP_100 starch samples,

which was 3% higher than the 0.289 mg-VFAs/mg-COD_{applied} for the reactors fed with the No_THP starch samples.

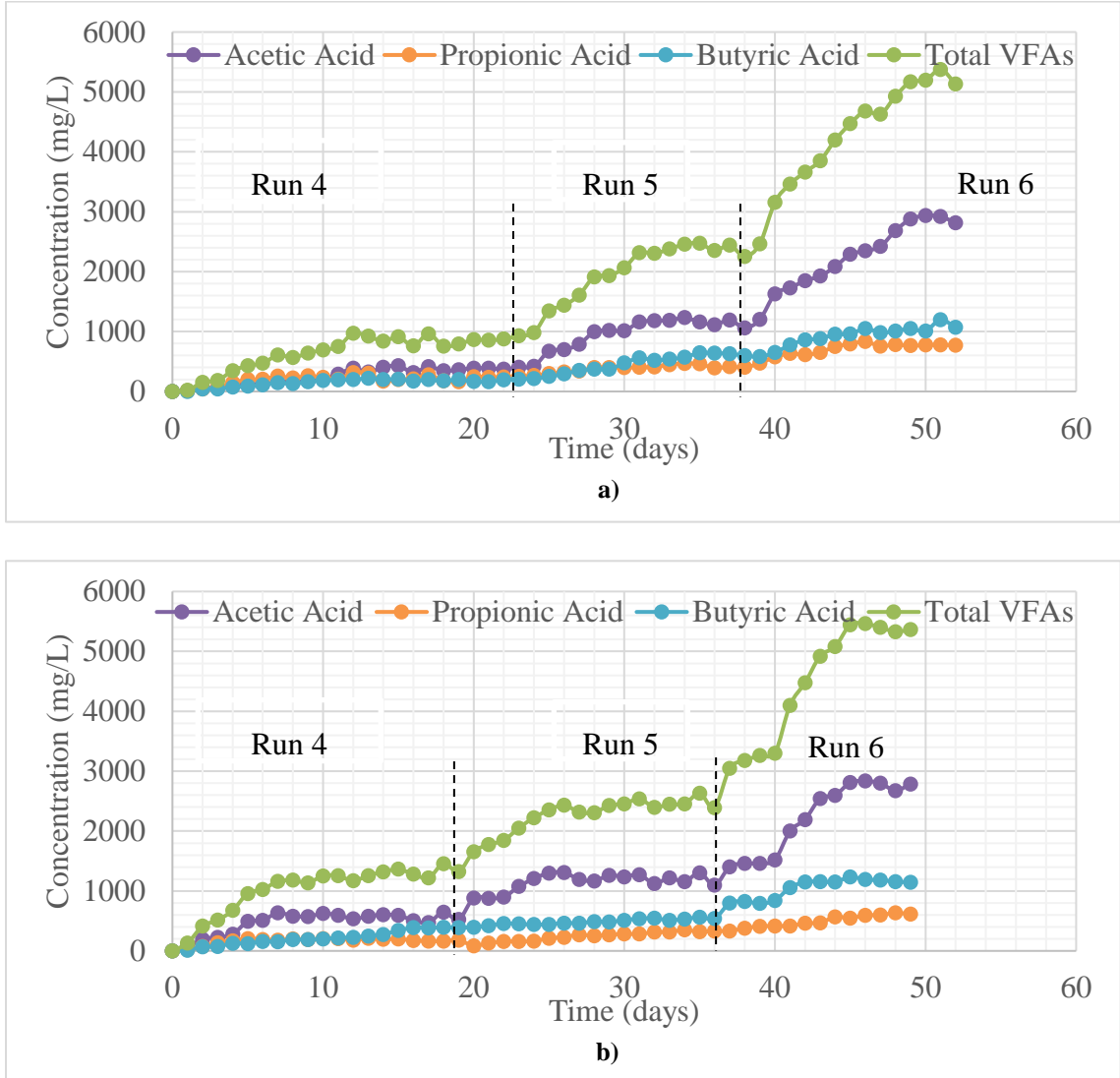


Figure 16: VFAs profile at OLR of 9 kg-COD/m³·day a) No_THP and b) THP_100

A separate experiment was performed using No_THP starch at an HRT of 10 days and an OLR of 1.5 kg-COD/(m³·day) to quantify the effect of a higher HRT on the VFAs profile, as shown in Figure 17. The VFA yield increased to 0.546 mg-VFAs/mg-COD_{applied},

which was 45.6% higher than the 0.297 mg-VFAs/mg-COD_{applied} achieved with an OLR of 9 kg-COD/(m³·day) at an HRT of 2 days for the reactors fed with THP_100 starch. Apart from VFAs, there were several other fermentation end products of carbohydrate metabolism, which may have contributed to the COD. The other end products, such as hydrogen, methanol, ethanol, and propanol, were not investigated in this study.

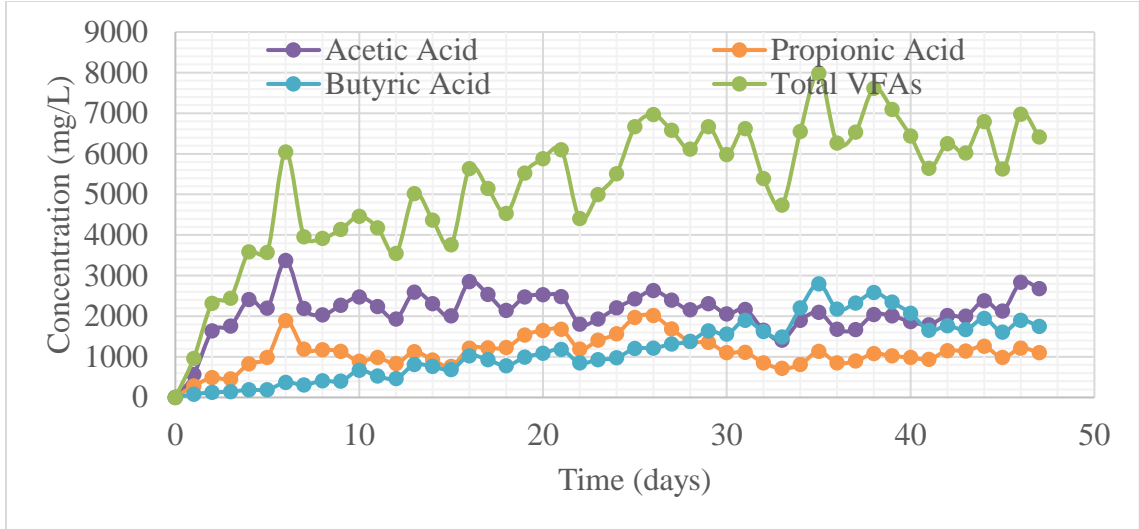


Figure 17: VFAs profile at HRT of 10 days

4.3 VFAs production from AD reactors fed with starch (THP_140)

Figure 18 shows the variation in the VFAs concentration at OLRs of 6 and 9 kg-COD/(m³·day) at different HRTs. The individual VFAs concentrations increased with increasing HRT at the beginning of each experimental run but were lower than the concentration achieved for the reactors fed with No_THP and THP_100. The VFAs production from the reactors fed with THP_140 starch showed reduced reactor performance. The maximum VFAs volumes of (1717.2 ± 46.4) mL (0.150 mg-VFAs/mg-

COD_{applied}) and (2668.7 ± 58.8) mL (0.138 mg-VFAs/mg-COD_{applied}) were obtained at OLRs of 6 and 9 kg-COD/(m³·day), respectively, and an HRT of 2 days. This steady-state production of VFAs was 45% and 52% lower than those of the reactors fed with No_THP starch at OLRs of 6 and 9 kg-COD/(m³·day), respectively, and an HRT of 2 days. The maximum solubilization was achieved at 140 °C as the ratio of sCOD/tCOD reached 92.8% for 10 g/L, 88.9% for 15 g/L, and 54.0% for 25 g/L. However, the reduced reactor performance was a result of the decreased biodegradability of the substrate because of overheating at high temperatures. Thus, increasing the solubilization would not necessarily improve the reactor performance, which is consistent with the results of thermal pretreatment at high temperatures for different substrates. The overcooking of starch at high temperatures, in this case, at 140 °C, may have led to the formation of complex compounds.

Moreover, low-temperature THP was found to be favorable for substrates rich in carbohydrates (Yeshanew et al., 2016). Soluble monomers were available for the AD process without overcooking the substrate at a low-temperature THP. One of the reasons for the substrate being overcooked is the absence of sufficient water for the starch to absorb during THP, leading to the formation of complex compounds at a high THP temperature. These complex compounds should be considered a function of the ratio of potato starch to water used to make solutions for THP. VFAs profile remained the same as for reactors fed with starch (No_THP and THP_100) as acetic acid was the dominant product followed by butyric acid and propionic acid, while minimal quantities of iso-BA, iso-VA, and VA were formed during the entire run.

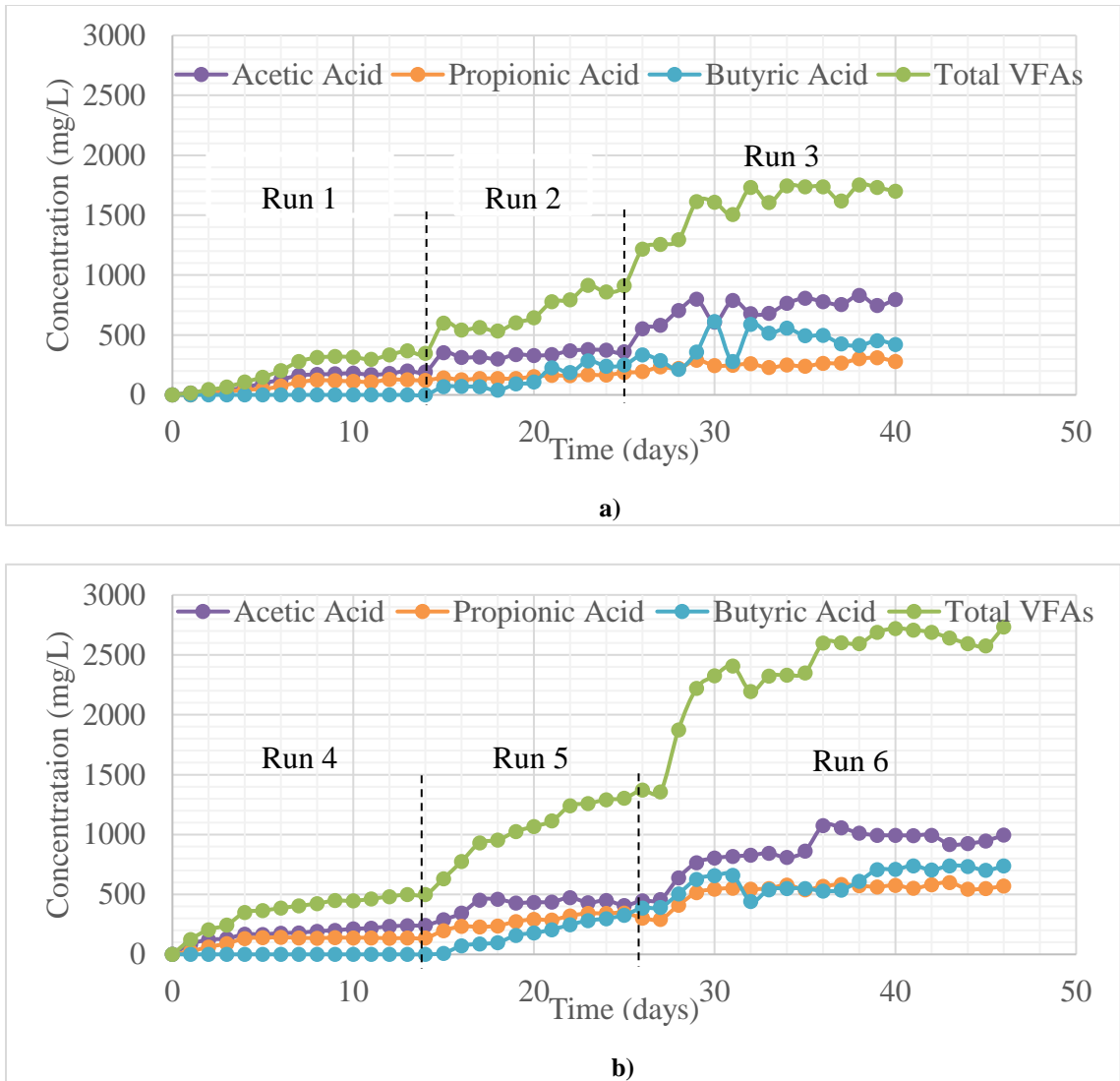


Figure 18: VFAs profile at OLR of a) 6 kg-COD/m³·day and b) 9 kg-COD/m³·day

The time required to reach the desired THP temperature must be considered to avoid overcooking the substrate because this can have a significant impact on the solubilization as well. This variable has not been consistent between studies. Xue et al. (2015) found that the temperature increase required 90–120 min, whereas Bougrier et al. (2008) reported the variation to be between 25 to 60 min. Moreover, because the time required to

reach the desired temperature was often not consistent, different results were frequently obtained. A steady increase in the temperature and subsequently pressure, which is related to solubilization, can provide better results for solubilization and allows the optimization of THP to reduce energy costs. The VFAs yields under different operating conditions are shown in Figure 19.

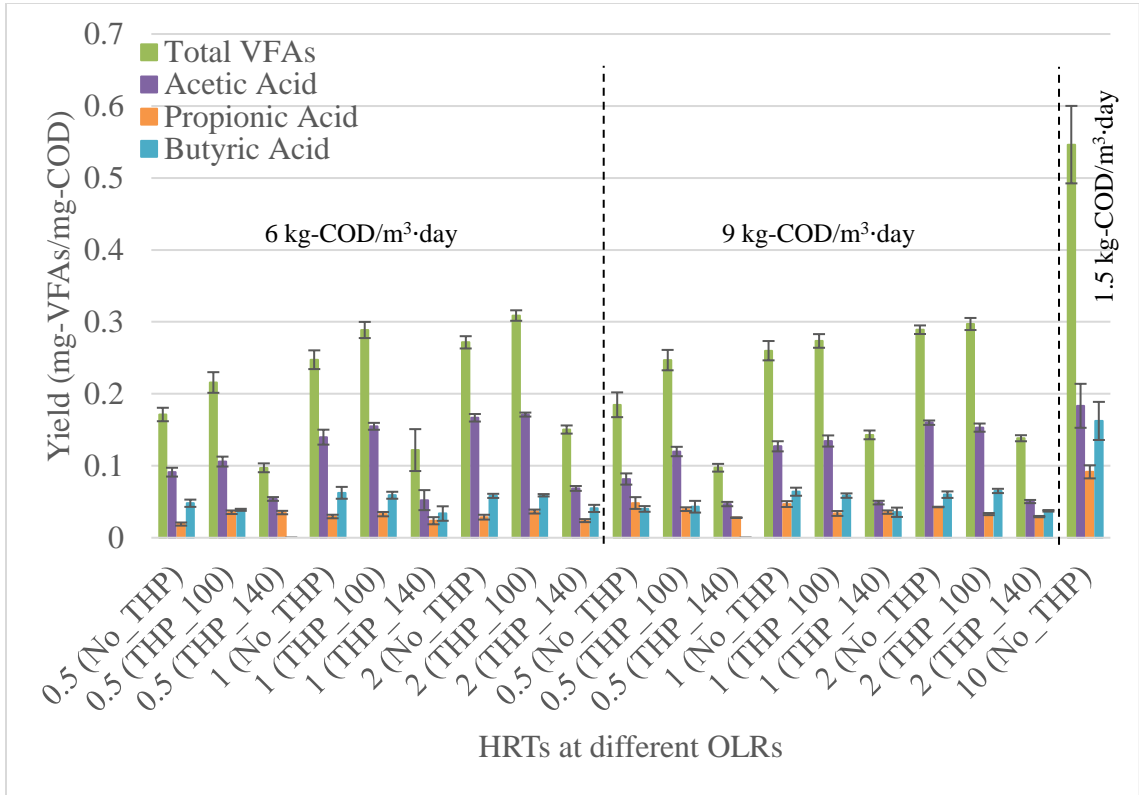


Figure 19: Reactor performance at different mentioned operating conditions

4.4 Effect of THP on methane production

The AD reactors were operated at several different operating conditions during VFAs production from potato starch, and no significant difference was observed in terms of reactor performance. The VFAs production potential of reactors hardly changed and the

only noticeable effect was during the startup of the reactor. To understand the effect of THP on methane production in a clear and distinct way, the AD reactors were operated at only one operating condition, i.e. influent COD and OLR of approximately 10 g/L and 4 kg-COD/m³·day respectively at an HRT of 2.5 days. HRT of 2.5 days was chosen to ensure a similar experiment as that of VFAs production and the results can be compared. This was done to analyze the effect of solubilization on methane production. Before the starch solution was fed to AD reactors, the solution was thermally pretreated at three THP temperatures, i.e. 70 °C, 100 °C, and 140 °C. The results were consistent with the findings of the THP experiment carried out during VFAs production. The higher COD solubilization (sCOD) after each THP compared to raw starch indicates that the THP significantly improved the starch solubilization by promoting the breakdown of particulates into soluble compounds. The effects of THP on solubilization has already been discussed in Section 4.1. For the given tCOD of 10 g/L, tCOD and TS remained conserved. The increase in fCOD and sCOD could only be attributed to the simultaneous reduction in TSS and VSS. A linear correlation existed, as shown in Figure 20, between the increase in fCOD and sCOD with a simultaneous reduction in TSS and VSS with R² values of 0.8291, 0.947, 0.9631 and 0.9434 respectively.

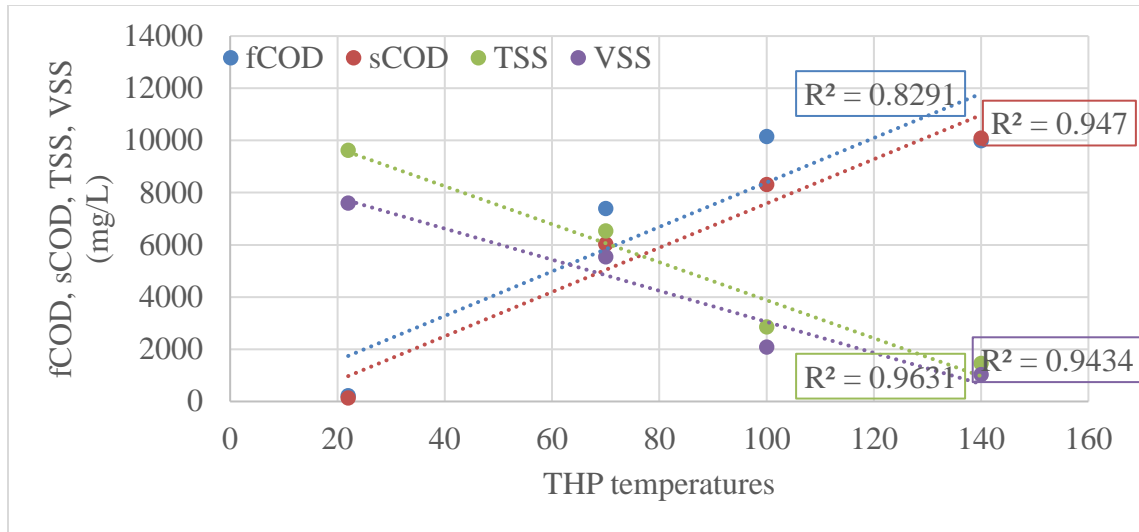


Figure 20: Effect of different pretreatment temperatures on solubilization

THP temperature had a direct impact on the increase in soluble COD. The next phase of this experiment was to determine the effect of solubilization on methane production and evaluating the relationship between COD solubilization and methane production.

Figure 21 shows the methane production over 120 days of reactor operation. Reactors reached a steady-state faster for methane production using THP_70 starch. The reactors fed with No_THP and THP_100 starch reached steady state around the same time, but as evident from Figure 21, the difference was minor. The same observation, i.e. the steady-state VFAs production was achieved faster for thermally pretreated starch in comparison to reactors fed with No_THP starch. There was a drastic increase in methane production for all the reactors fed with No_THP, THP_70, and THP_100 starch. The amount of biogas produced from reactors fed with starch THP_140 starch only amounted to $237.77 \text{ mL} \pm 21.17$, approximately 71% lower than methane production from reactors fed with starch No_THP starch. A higher VFAs production was expected with increasing THP

temperature due to increase in solubilization, whereas the reactor performance for reactors fed with THP_140 starch went down.

During high-temperature THP, caramelization and Maillard reaction are known to result in the formation of various less biodegradable compounds from the carbohydrates (Bougrier et al., 2008; Liu et al., 2012). Caramelization is the non-enzymatic browning of sugar at high temperatures. Maillard reaction is a result of the reaction of carbohydrates with an amine group at high temperatures. Also, the formation of these less biodegradable compounds was confirmed by the decrease in methane production at higher THP temperatures. Tampio et al. (2014) suggested that these compounds are not only less biodegradable but at the same time inhibit the degradation of other organics. These compounds are heterogeneous polymers characterized by a high-molecular-weight that are not only difficult to degrade but can also inhibit the degradation of other organics by suppressing the methanogenic activity (Tampio et al., 2014). After reaching steady-state for reactors fed with No_THP, THP_70, and THP_100 starch, the average methane percentage in biogas was stable at approximately 50% which indicated stable and effective performance for the reactors. No inhibition was observed due to VFAs accumulation.

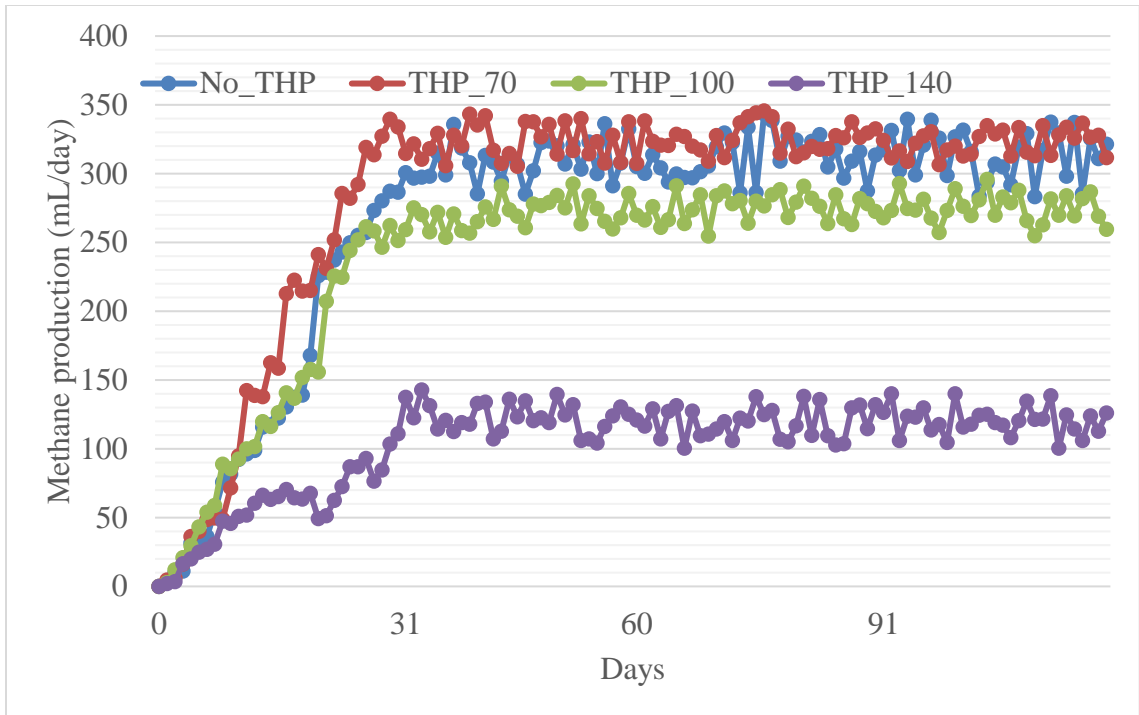


Figure 21: Methane production

Theoretical methane production was calculated based on the COD equivalence of methane at 35 °C (Speece,1996). At 35 °C, one gram of COD consumed is equivalent to 0.395 L of methane production. So, for example, if 100 mL of 10 g-COD/L of a solution is fed daily, theoretically the reactor should produce (0.395 L/g-COD × 10 g-COD/L × 100 mL/day) 395 mL of methane gas daily. Table 7 shows the effect of THP on methane production. Interesting fact is the slope of the methane production volume curve in Figure 21. The steeper slope for reactors fed with THP_70 starch is indicative of the fact that the reactors could produce more methane with a simpler and soluble substrate. The carbohydrate solubilization was more favored at lower temperature pretreatment, i.e., 80°C (Yeshanew et al., 2016). Indeed, at higher temperatures, it could be hypothesized to

have a larger amount of soluble monomers of carbohydrates. Vavouraki et al. (2013) reported that during THP of food waste, glycoside bonds of carbohydrate polymers are broken down and result in an increase in the concentration of mono sugars (glucose and fructose). The methane production potential remains the same for reactors fed with No_THP and THP_70 starch. It went slightly down for the reactors fed with THP_100 starch. The methane conversion efficiency was defined as a percentage, as the rate of experimental methane production with respect to theoretical methane production at 35°C.

Table 7: Effect of THP on methane production

Substrate Starch	No_THP		THP_70		THP_100		THP_140	
Gas composition	CH ₄	CO ₂	CH ₄	CO ₂	CH ₄	CO ₂	CH ₄	CO ₂
%	51.55	37.93	51.08	43.91	50.79	39.21	50.79	49.21
Theoretical CH₄ production (mL/day)	395		395		395		395	
Experimental CH₄ production (mL/day)	312		323		275		118	
CH₄ Conversion Efficiency (%)	78.98		81.77		69.62		29.87	

Figure 22 shows the methane yield (mL-CH₄/mg-COD_{applied}) and biogas yield (mL-biogas/mg-COD_{applied}) with COD removal efficiency. The reactors fed with starch (THP_70) had the highest methane yield of 0.317 mL-CH₄/mg-COD_{applied}, 2.8% and 15.4% higher than methane yield achieved for reactors fed with No_THP and THP_100 starch respectively. A higher methane yield was obtained by (Ariunbaatar et al., 2014) after THP of food waste at 80 °C for 90 minutes. Simultaneously, the COD removal efficiency of 91.8% was achievable for THP_70 starch, which was approximately 6% and 18% higher than No_THP and THP_100 starch. The THP_70 starch seemed to be the most favorable for the AD process.

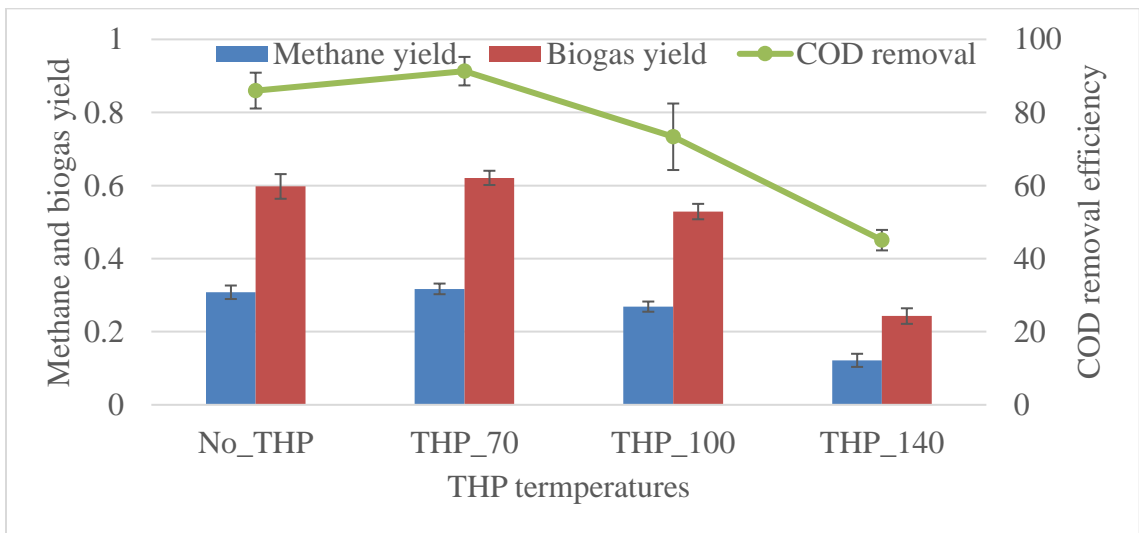


Figure 22: Methane and biogas yield from potato starch

Figure 23 shows the COD balance of reactors fed with starch after THP at different temperatures. The COD balance has been calculated in order to determine the amount of methane gas from COD (CH₄ as COD) and to compare it with the actual amount of methane gas produced from the reactors. There was not a significant difference in the

reactor performance for reactors fed with No_THP and THP_70 starch as 80.7 and 82.6 % of influent COD contributed towards methane production. The reactors fed with THP_100 starch showed only 68.98 % of influent COD contribution towards methane production. The results for reactors fed with THP_140 starch showed reduced reactor performance as only 31.44 % of influent COD contributed towards methane production, and 43.56 % was found to be in effluent COD. This was attributed to high-temperature pretreatment of the starch molecules at higher temperatures. A finding similar to VFAs production using THP_140 starch. The results obtained, for the COD balance of the reactors fed with starch No_THP and THP_70 starch, were comparable to the highest biogas and methane yields obtained at those temperatures. THP temperatures higher than 70 °C brought down reactor performance. Marin et al. (2010) obtained an improvement of starch hydrolysis at a temperature of 60-70°C for kitchen waste. THP of substrates rich in carbohydrate was more effective at lower temperatures (Yeshanew et al., 2016), accelerating the breakdown of complex compounds and increasing the release of soluble monomers. Also, the low-temperature i.e. THP_70 maintained the bioavailability of these soluble monomers for the AD process without overcooking the substrate. Similarly, Krishna and Kalamdhad (2014) highlighted that the positive effects of THP were strongly dependent on both THP temperature and substrate composition.

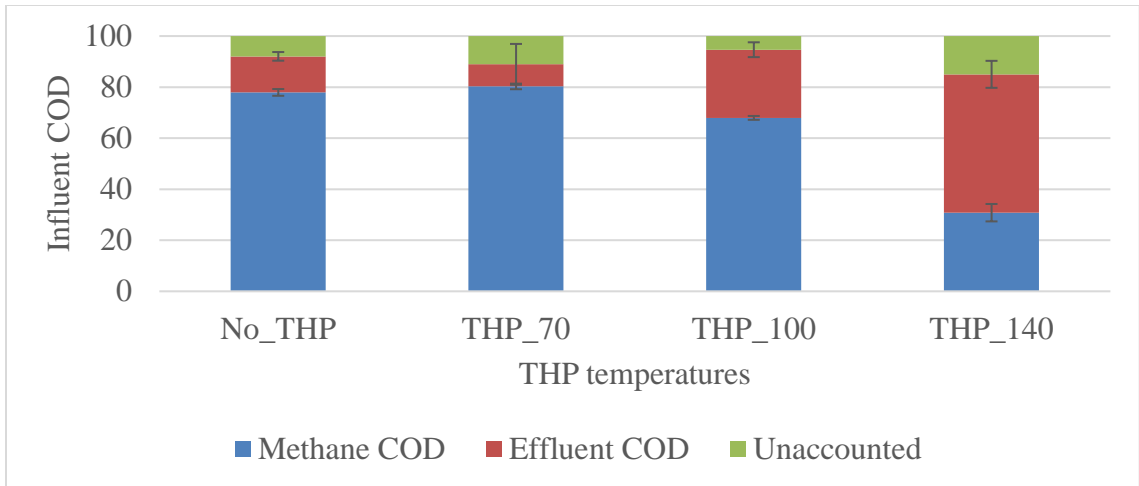


Figure 23: COD balance from potato starch

Table 8 compares the AD reactor performance on HRT due to THP at different temperatures. HRT is a crucial parameter to determine the volume of the reactor required. Although the effect of THP at 140 °C brought down the HRT, it also reduced the reactor performance, both in terms of VFAs and methane production.

Table 8: Effect of THP on HRT of reactors

THP Temp.	Time required for steady-state operation (days)		Effect on HRT	
	VFAs	Methane	VFAs	Methane
No_THP	15	33	-	-
THP_70	-	28	-	15.15 % lower
THP_100	9	31	40.0 % lower	6.06 % lower
THP_140	7	31	53.34 % lower	6.06 % lower

4.5 Co-digestion of potato starch and PHL

The characteristics of the substrates used for this study are summarized in Table 9. The potato starch and PHL differed in their aggregate composition. The aggregate parameter analysis showed that PHL had a very high tCOD value of approximately 94 g/L, the majority of which was present in a soluble form equivalent to 83 mg/L. The ratio between BOD₅ to tCOD is an indicator of the biodegradability of a waste stream (Speece, 1996). The BOD₅/tCOD value for PHL was found to be approximately 0.50. This is a low value of BOD₅/tCOD in comparison to its counterpart, i.e. potato starch which had the BOD₅/COD ratio of 0.75. The low BOD₅/COD value of PHL can be attributed to the presence of slow biodegrading organic such as lignin in the PHL waste stream (Kotze et al., 1969; Speece, 1996).

Table 9: Substrate analysis for co-digestion of PHL and potato starch

Parameters	PHL	Potato Starch	Potato Starch and PHL
TS (mg/L)	114635	8468	9973
TSS (mg/L)	2120	7671	4357
VSS (mg/L)	1723	6922	3564
pH	3.76	7.56	6.12
BOD₅ (mg/L)	47495	6627	6094
tCOD (mg/L)	94011	8856	8724
fCOD (mg/L)	88741	203	5681
sCOD (mg/L)	83372	128	5369
BOD₅/tCOD	0.50	0.75	0.69

The pH of the PHL was close to 3.76, acidic than that of potato starch which was close to 7.56. This low pH of PHL was mainly due to the presence of acetic acid. The TSS and VSS values for potato starch were approximately 25-27% higher than the TSS and VSS values of PHL. PHL was diluted using distilled water and simultaneously mixed with equal volumes of the starch solution to give tCOD (50 % potato starch and 50 % PHL) value of approximately 8750 mg/L.

The reactors were operated at an OLR of 3.5 kg-COD/m³·day and HRT of 2.5 days for a duration of 75 days. Figure 24 shows the initial increase in methane production using 100% PHL was attributed to its soluble component, i.e. acetic acid, a precursor of methane production. As methanogens prefer pH to be close to neutral conditions, alkalinity (sodium bicarbonate) needed to be added.

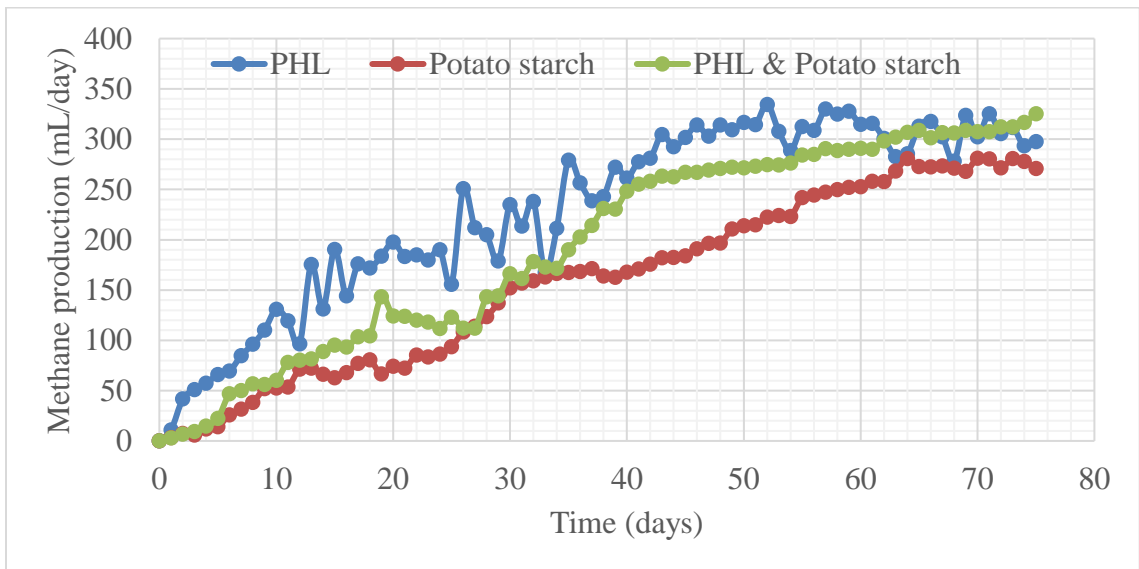


Figure 24: Methane production during the co-digestion of PHL and potato starch

The methane production from 100 % potato starch showed a slow and steady linear increase in methane production. The rate of increase in methane production for 100 %

potato starch as a substrate was lower than 100 % PHL and was attributed to particulate nature and insolubility of potato starch. Table 10 shows the effect of THP on methane production. The substrate (50 % potato starch and 50 % PHL) showed improvement in methane production without any inhibition.

Table 10: Effect of co-digestion of potato starch and PHL on methane production

Substrate	PHL		Potato starch		PHL and Potato starch	
Gas composition	CH ₄	CO ₂	CH ₄	CO ₂	CH ₄	CO ₂
%	62.53	28.05	58.32	27.85	63.04	27.69
Theoretical CH₄ production (mL/day)	360		360		360	
Experimental CH₄ production (mL/day)	299		270		305	
CH₄ Conversion Efficiency (%)	83 %		75 %		84 %	

The alkalinity requirement for these reactors was calculated in terms of the amount of sodium bicarbonate (NaHCO₃) fed to the system. The optimum alkalinity inside the reactor should be 2500 to 4000 mg-CaCO₃/L (McCarty, 1964). The alkalinity was controlled by adjusting the pH value, which is one of the important parameters to be

controlled during the AD process. The relatively low pH values of PHL required that the alkalinity of the substrate solution be adjusted in order to maintain the pH in a reasonable range for methane production. For stable reactor operation, 6.1 g/L (around 4.1 g-CaCO₃/L) and 9.7 g/L (around 6.5 g-CaCO₃/L) of NaHCO₃ was required for 100% potato starch and 100 % PHL reactor systems respectively.

On the contrary, the co-digestion of PHL and potato starch required 7.2 g/L of NaHCO₃ (around 4.7 g-CaCO₃/L) for stable reactor operation. This alternative way of digestion reduced NaHCO₃ requirements by approximately 25% and simultaneously provided higher methane yield than PHL and raw starch digested alone. Souza et al. (1992) found that an alkalinity/COD ratio of 0.5 in the influent decreased the pH to 6.6, which was considered as the lower limit value recommended for methanogenesis. In this study, these ratios were 0.41, 0.65 and 0.47 for reactors fed with the raw potato starch, PHL, and the mixture of potato starch and PHL respectively. Since 7.2 g/L of NaHCO₃ concentration provided an optimum buffering capacity to convert the influent COD to methane and was provided an optimum alkalinity concentration in influent to maintain the pH above 6.6. This value corresponds to an alkalinity/COD ratio of 0.4 and 0.5 proposed by González et al. (1998) and Souza et al. (1992) respectively.

Figure 25 shows the methane yield (mL-CH₄/mg-COD_{applied}) and biogas yield (mL-biogas/mg-COD_{applied}) with COD removal efficiency. The reactors fed with equal volumes of PHL and potato starch had the highest methane yield of 0.336 mL-CH₄/mg-COD_{applied} with the highest COD removal efficiency of 91.3%. The reactors fed with 100% PHL and 100% potato starch provided with 0.321 and 0.298 mL-CH₄/mg-COD_{applied} respectively. The reactors in the above case performed well but were 11.3%

and 4.46% lower than its co-digestion counterpart. The COD removal efficiency of 87.3% was achievable for PHL (100%), in comparison to 82.8% for potato starch (100%).

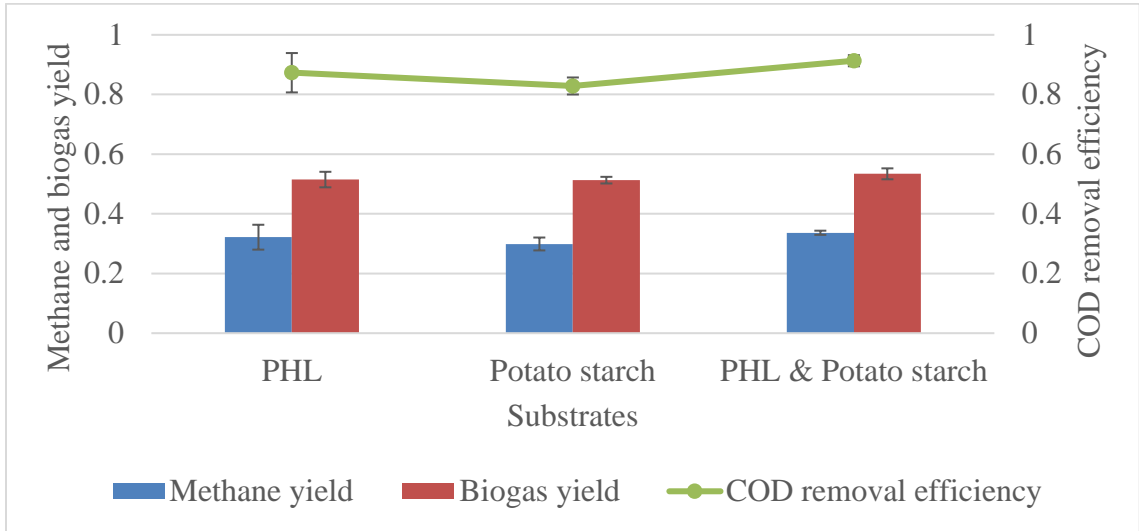


Figure 25: Methane and biogas yield from co-digestion of PHL and potato starch

Figure 26 shows the COD balance of reactors for the co-digestion of PHL and potato starch. The COD balance has been calculated in order to determine the methane gas from COD (CH_4 as COD) and to compare it with the actual methane gas produced from the reactors. There was no considerable difference found on the percentage of influent COD going towards methane for all the reactors. The methane conversion efficiency was 81.8% for PHL, 75.67% for potato starch, and 85.17% for PHL and potato starch. No effects of slowly biodegrading substrates such as lignin and furfural were noticed. The COD balance for reactors fed with potato starch (No_THP) during methane production in Section 4.4 was comparable to results obtained during methane production from starch (100%).

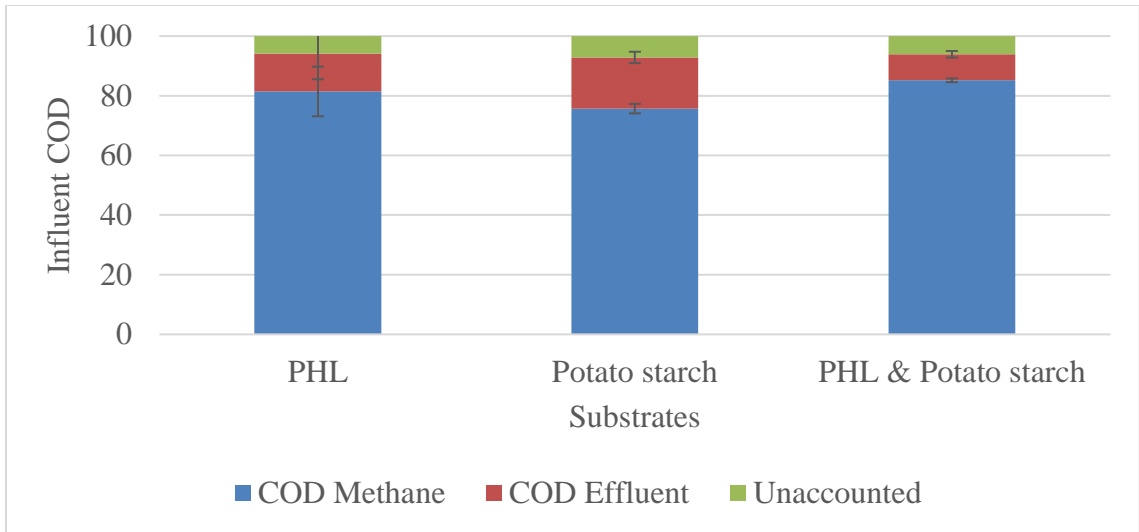


Figure 26: COD balance for co-digestion of PHL and potato starch

4.4 Anaerobic treatability study of food waste

As discussed before, food waste can be generated from several sources and only vegetative food waste from grocery stores was considered in this study; which was conducted to determine the anaerobic digestibility of food waste, another abundantly available waste source. The goal of this study was to compare the results with methane production from starch (raw and thermally pretreated) and its co-digestion with PHL.

This was done to determine the type of waste best suited for methane production.

The average COD and solids analysis of food waste samples are shown in Table 11. The tCOD for 3, 4, and 5 g/L of food waste was approximately 3088, 4058 and 5115 mg/L.

The TS in all cases ranged between 65-70% of tCOD. The VS/TS was relatively high ranging from 0.82-0.90, favorable for the AD process. All the substrate analysis was carried out after blending the solid vegetative food waste and passing the slurry through

1.6 mm sieve. The nutrients were supplied along with the substrate as discussed in Section 3.7.8.

Table 11: Substrate analysis for food waste

Parameters	3 g/L	4 g/L	5 g/L
TS (mg/L)	2302	2747	3340
TSS (mg/L)	2289	2608	3092
VSS (mg/L)	2071	2164	2634
pH	4.99	5.31	5.26
BOD₅ (mg/L)	2154	2713	3091
tCOD (mg/L)	3088	4058	5115
sCOD (mg/L)	1090	1288	2099
BOD₅/tCOD	0.69	0.66	0.60

The reactors were operated at an OLR of 1.2, 1.6, 2.0 kg-COD/m³-day at an HRT of 5 days for 40 days. This study was conducted to assess the performance and treatability of food waste via the mesophilic AD process to produce methane. This test involved exposing wastewater constituents to anaerobic bacteria and recording the amount of methane or total biogas produced (Owen et al., 1979). Figure 27 shows the initial increase in methane production using three different concentrations of food waste. The methane production was relatively low during the first 15 days of digestion, and then increased until day 25 and remained almost constant until the end of the experiment. The

methane production was highly variable especially for reactors fed with 5 g/L (OLR of 2.0 kg-COD/m³·day) of food waste. This was attributed to the particulate nature of food waste, even after blending.

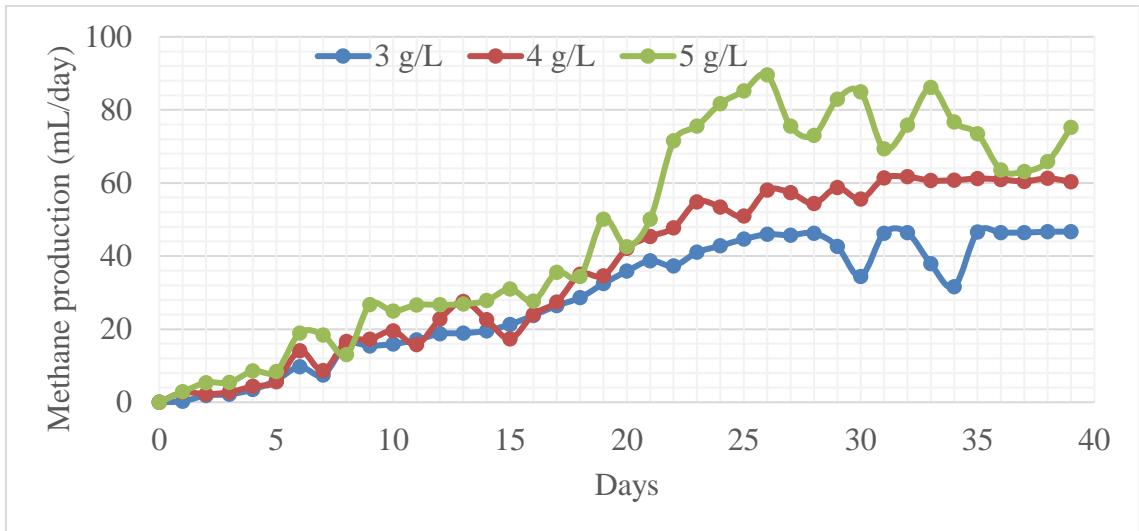


Figure 27: Methane production from food waste

The steady-state methane production from the reactors operated at an OLR of 1.2, 1.6, 2.0 kg-COD/m³·day was approximately 46, 63 and 75 mL-CH₄/mg-COD_{applied}, respectively as shown in Table 11. The biogas composition during digestion of food waste at three reactor loadings is shown in Table 12 as well. However, the biogas produced from the digesters of higher loading (2.0 kg-COD/m³·day) had higher methane content (3% higher) compared with the digesters that had lower loading of 1.2 and 1.6 kg-COD/m³·day. The average CH₄ and CO₂ contents were measured to be in the range of 44-45% and 24-34% respectively for all loadings. The biogas composition obtained in this study was less than obtained by Wang et al. (2005) who studied the anaerobic batch digestion at 35 °C of food waste using laboratory and pilot-scale hybrid solid-liquid

anaerobic digesters. Their results showed that the methane contents of the biogas produced were 71% and 72%, respectively.

Table 12: Methane production from food waste

Food waste	1.2 kg-COD/m³·day		1.6 kg-COD/m³·day		2.0 kg-COD/m³·day	
Gas composition	CH ₄	CO ₂	CH ₄	CO ₂	CH ₄	CO ₂
%	44.63	30.65	44.24	34.28	45.78	24.72
Theoretical CH₄ production (mL/day)	59.25		79		98.75	
Experimental CH₄ production (mL/day)	43		58		74	
CH₄ Conversion Efficiency (%)	72 %		73 %		75 %	

The methane yield (mL-CH₄/mg-COD_{applied}) and biogas yield (mL-biogas/mg-COD_{applied}) along with COD removal efficiency are shown in Figure 28. There was no significant difference in the methane and biogas yields for reactors fed with 4 and 5 g/L of food waste. It should be mentioned that the BOD₅/COD of raw starch was 0.75, higher than the ratio for the food waste, which was in the range of 0.6-0.7. Even though the raw starch was more biodegradable than the food waste, reactors fed with food waste had

approximately the same COD removal efficiency of 77% at OLR of 1.6 and 2.0 kg-COD/m³·day with approximately the same methane and biogas yield. HRT of 5 days was applied to the reactors in this study, in comparison to the previous two studies (methane production from starch and its co-digestion study with PHL) where the HRT applied to the reactors was 2.5 days.

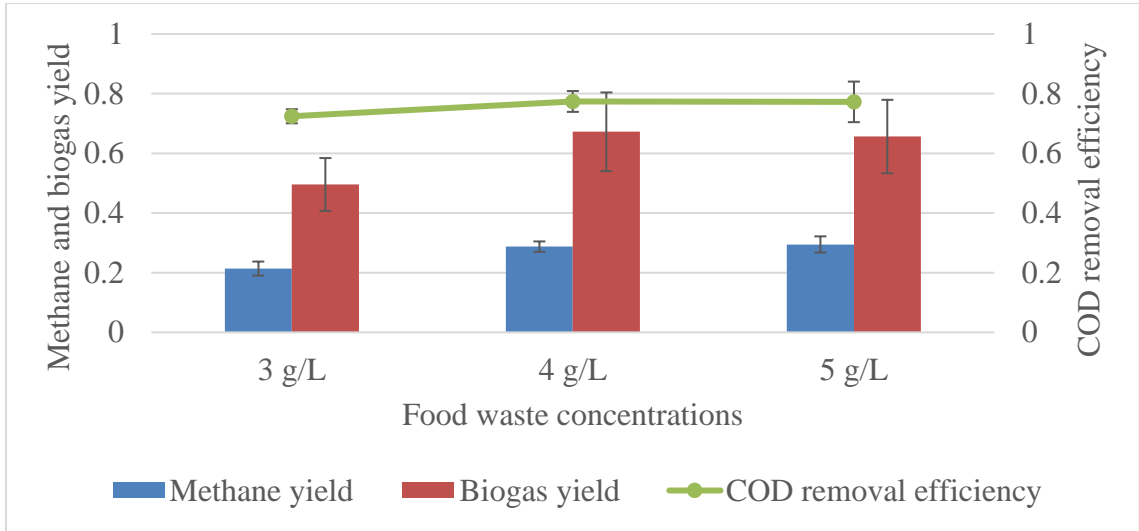


Figure 28: Methane and biogas yield from food waste

Approximately 55, 72, and 75% of the influent COD were contributed towards methane production for reactor loading of 1.2, 1.6 and 2.0 kg-COD/m³·day respectively as shown in Figure 29. As previously stated, that the methane production was highly variable, but still the reactors at increased loadings performed better. Based on this study, it could be inferred that the OLR of 1.6 kg-COD/m³·day was as good as OLR of 2.0 kg-COD/m³·day based on reactor performance which was measured as biogas and methane yield, and COD removal efficiency and COD balance. The methane production from potato starch closely resembled the methane production from food waste. This can be justified by

comparing methane yields, COD conversion and COD removal efficiency obtained from raw potato starch and food waste. It can be found that CH₄ conversion efficiency obtained from starch was 78.98%, in comparison to 75% obtained for food waste at 2 kg-COD/m³·day. Methane yield of 0.308 mL-CH₄/mg-COD was obtained in comparison to 0.294 mL-CH₄/mg-COD for food waste. COD removal efficiencies for raw starch and food waste were approximately 86% and 77%, respectively. Hence, VFAs production using food waste was not conducted.

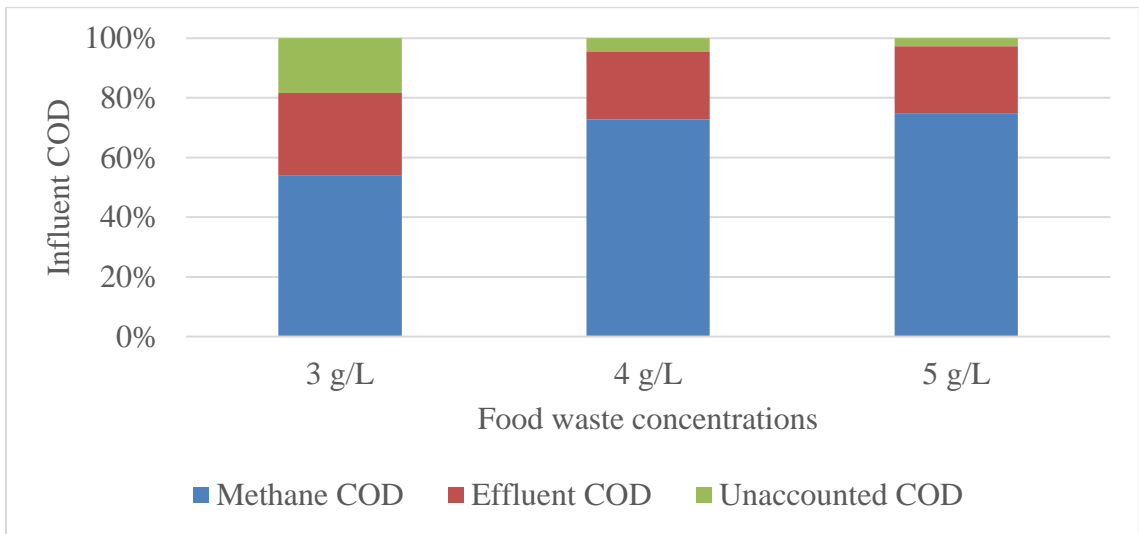


Figure 29: COD balance for food waste

4.3 VFAs production using anaerobic trickling filter (ATF)

Anaerobic downflow attached growth reactors are generally used for easily degradable wastewater with an inherent advantage of short HRT (Metcalf and Eddy, 2003). ATF was operated based on attached growth technology in which the biomass grows and attaches to porous packing material, and waste stabilization takes place in a layer of biofilms

attached to the surface of the reactor support material. In this study, Kaldnes K1 media was used. The inert growth media helps in retaining the biomass in the reactor. Since the volumetric flow rate was 31.68 L/day, concentration in terms of COD of 1000-2650 mg/L was used for OLRs of 3.6, 5.5, and 9.5 kg-COD/m³·day. ATF before startup was fed with 10 L of heat-shocked sludge in order to inhibit methanogenesis. The reactor is shown in Figure 30.



Figure 30: ATF after adding heat-shocked mixed culture

The first 25 days were dedicated to the reactor startup, and the substrate used was 100% sucrose (COD basis). The influent volumetric flow rate of 31.68 L/day and OLR of 3.6 kg-COD/m³·day were applied to the reactor with HRT of 0.28 days (6.72 hours). The recycle ratio was kept constant throughout the study and was around 0.7 ± 0.05 . The reactor was operated for a total duration of around 67 days. VFAs profile for ATF is shown in Figure 31.

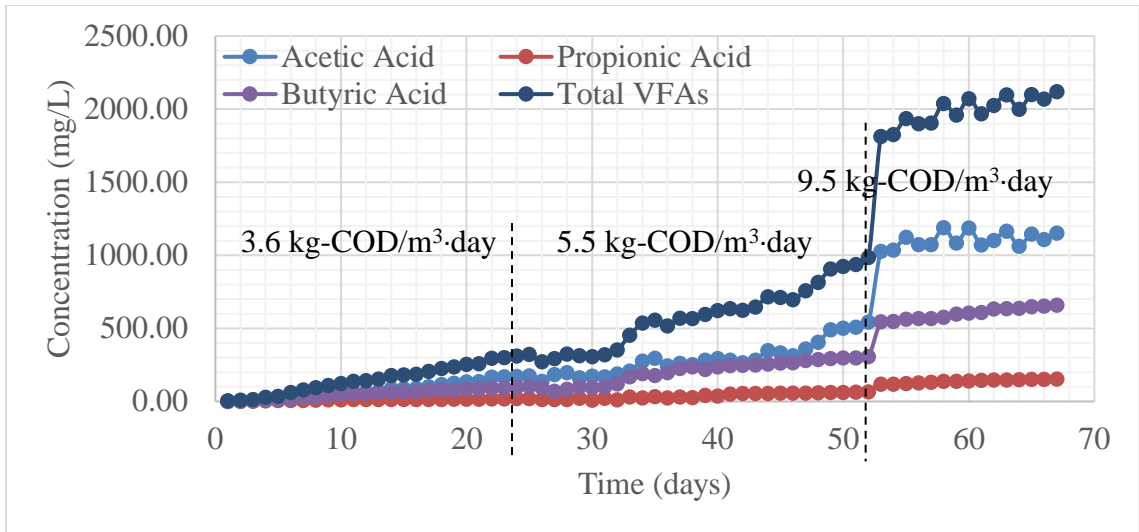


Figure 31: VFAs profile at OLR of 9.5 kg-COD/m³·day at HRT of 0.28 days

After 25 days of operation, VFAs concentration seemed to have stabilized, and the substrate was changed to sucrose and starch (50% each on COD basis) to provide a COD of 1500 mg/L increasing the OLR to 5.5 kg-COD/m³·day. The starch was introduced to the reactor to examine the effect of particulate substrate. With increasing influent waste concentration, there was a decrease in VFAs concentration by approximately 15%. This was attributed to the particulate nature of starch, leading to accumulation as all other operating parameters remained the same. However, the ATF recovered quickly, and after 7 days of operation, VFAs concentration started to increase. After day 52, the substrate was changed back to 100% sucrose providing a COD of 1500 mg/L. It was required as starch started accumulating within the system as shown in Figure 32. This was indicative of the fact that the degradation of starch requires a longer time in comparison to a simple substrate such as sucrose. The volumetric flow rate of 31.68 L/day should have been reduced to increase the HRT to provide more time for the degradation of starch. This was

not done because for a trickling filter arrangement, where the wastewater pours down the media through the void spaces, it may create dead zone inside the reactors which was already causing starch accumulation. To counteract the clogging/accumulation of particulate substrates, the fluidized bed reactor has been developed. In these reactors, the biomass grows attached to solid media that remains in suspension by the upward flowing motion of the fluid (Grady et al., 2011).



Figure 32: Starch accumulation

After day 52, OLR was increased to $9.5 \text{ kg-COD/m}^3\text{-day}$ and was operated with 100% sucrose from until day 67, as acetic acid concentration reached a steady state. This was done by increasing sucrose COD to 2650 mg/L . VFAs concentration increased further reaching the maximum value of $750\text{-}800 \text{ mg/L}$ with a yield of $0.75 \text{ mg-VFAs/mg-COD}_{\text{applied}}$. VFAs profile obtained from ATF (continuous mode) remained the same as the VFAs profile obtained from the batch mode of operation using respirometric studies.

Acetic acid was the dominant product followed by butyric acid and together combined to cover approximately 62-66% of the product spectrum. Propionic acid was one of the main VFAs detected in the effluent. Throughout the study, there was not any measurable quantity of biogas production, and bubbles appeared on media as shown in Figure 33.



Figure 33: Biogas bubbles inside ATF

Although ATF was able to perform better even after starch accumulation, there were foaming events at the OLR of $9.5 \text{ kg-COD/m}^3\cdot\text{day}$, as shown in Figure 34. Chen et al. (2014) reported that the process becomes unstable at higher OLRs and shorter HRTs. To counteract foaming, the reactor loading should have been increased in steps; instead of one step increase from 5.5 to $9.5 \text{ kg-COD/m}^3\cdot\text{day}$. The maximum VFAs yield of $0.30 \text{ mg-VFAs/mg-COD}_{\text{applied}}$ was obtained at HRT of 2 days and OLR of $9 \text{ kg-COD/m}^3\cdot\text{day}$ using from respirometric studies (batch mode of operation) in Section 4.2. ATF performed 3.7 times better than the batch mode in terms of HRT applied to the reactors simultaneously producing higher VFAs yield of $0.78 \text{ mg-VFAs/mg-COD}$. The VFAs yield is provided in Figure 35.



Figure 34: Foaming event inside ATF

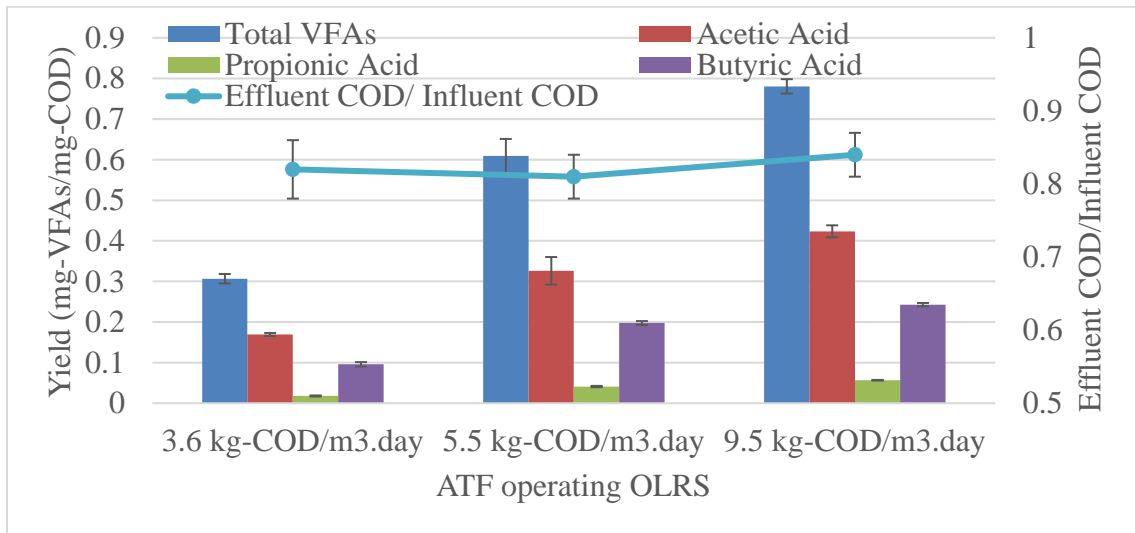


Figure 35: VFAs profile using ATF

5. Conclusion and Recommendations

5.1 Conclusions

The first research objective of the study was to determine the effect of THP on potato starch solubilization. This objective was successfully completed as the starch solubilization was evaluated at different THP temperatures and a linear correlation exists between solubilization and THP temperature with R^2 values ranging from 0.74-0.90. The fCOD and sCOD increased with a simultaneous reduction in TSS and VSS.

The second objective of the study was to evaluate the impact of solubilization of potato starch, due to THP, on VFAs production. This objective was successfully completed as there appeared to be little to no difference overall, with respect to VFA production using No_THP and THP_100 starch, and VFAs production from THP_140 starch fell significantly. Acetic acid and butyric acid were the dominant products for all operating conditions. The HRT of 10 days was found to be best suited for increasing the production of VFAs.

The third objective of the study was to determine the impact of solubilization of potato starch on methane production. A direct correlation (R^2 ranging from 0.82-0.96) exists between the increase in fCOD and sCOD with the reduction in TSS and VSS. Lower temperature THP i.e. THP_70 was found to be better suited for substrates containing high carbohydrate content, resulting in the enhanced methane yield. For THP_140 starch, the solubilization was highest, but the methane yields went down because of Maillard and caramelization reactions.

The fourth objective was to conduct the co-digestion of PHL and potato starch. The objective was successfully met as PHL was found to be a promising substrate for co-digestion with potato starch. The alkalinity requirements reduced by 25% when co-digesting 50 % PHL and 50 % potato starch (COD basis) and the highest methane yield of 0.32 mL-CH₄/mg-COD was obtained.

The next phase of the study was to determine the anaerobic treatability of food waste. The food waste was found to be a good substrate for the AD process. The reactors were operated at HRT of 5 days, but there were no significant differences in methane yield and COD removal efficiency compared to reactors operated at HRT of 2.5 days for potato starch. The methane accounted for 45% of the biogas produced, with methane yield of 0.28 mL-CH₄/mg-COD_{applied}.

The final objective was to evaluate the production of VFAs using ATF. ATF performed successfully treating low strength (1000-2650 mg/L) synthetic wastewater (sucrose and potato starch) with a maximum OLR of 9.5 kg-COD/m³·day and HRT of 0.28 days. ATF treating 3.5 and 5.5 kg-COD/m³·day was the most stable at the HRT tested. It was found that OLR should be increased in steps, instead of one step increase from 5.5 to 9.5 kg-COD/m³·day, as at higher loading rates, reactor operation was unstable.

5.2 Recommendations for future work

The results obtained during this thesis suggest that further research should focus on:

1. Comparative energy and cost-benefit analysis of the range of experimental conditions concluded in the study. This evaluation will allow determination of the

feasibility of the proposed process and the benefit with respect to the conventional AD process without any pretreatment.

2. An evaluation of other parameters apart from solubilization, i.e. to assess the performance of THP such as the particle size distribution.
3. More in-depth insight into the compounds formed during THP_140, which lowers AD reactor performance.
4. An evaluation of different ratios for co-digestion of potato starch and PHL, apart from 50% PHL and 50% potato starch. This may allow improving reactor performance further, such as improved reactor loading.
5. Better characterization of food waste is required to simulate the real-world scenario.
6. Considering the performance of ATF with sucrose and potato starch, co-digestion of food waste should be studied with PHL.

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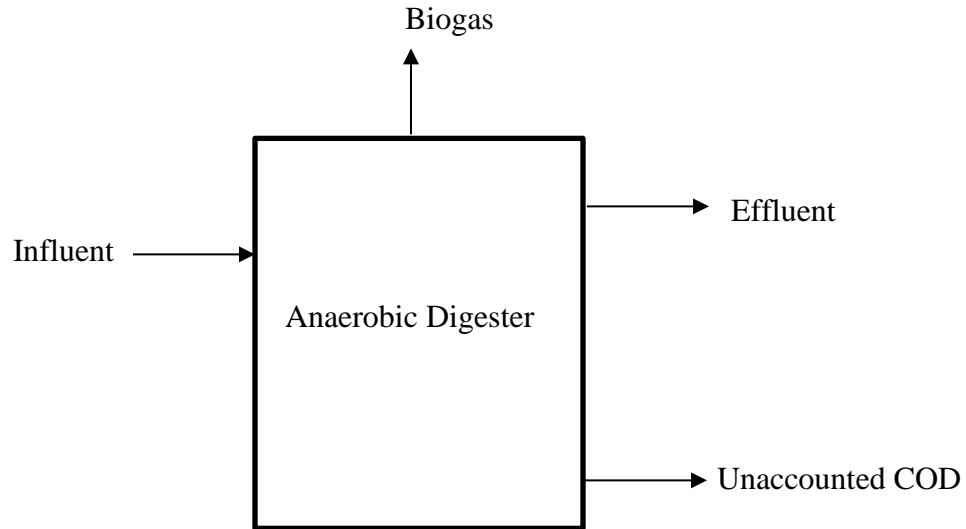
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Appendix A: COD Balance

Sample Calculations: COD Balance in the reactor

Sources of COD input and output from the reactor are identified below:



Sample calculations for bench-scale studies:

$$\begin{aligned}\text{Influent COD} &= C_{\text{in}} \times M_{\text{in}} \\ &= 15000 \text{ mg-COD/L} \times 20 \text{ mL/day} \\ &= 300 \text{ mg-COD/day}\end{aligned}$$

$$\begin{aligned}\text{Effluent COD} &= C_{\text{out}} \times M_{\text{out}} \\ &= 2000 \text{ mg-COD/L} \times 20 \text{ mL/day} \\ &= 40 \text{ mg-COD/day}\end{aligned}$$

$$\begin{aligned}\text{Biogas COD} &= (\text{Biogas volume} \times \text{CH}_4 \text{ Percentage}) \div \text{CH}_4 \text{ Equivalent @ } 35^\circ\text{C} \\ &= (150 \text{ mL/day} \times 0.60) \div 0.395 \text{ mL-CH}_4/\text{mg-COD} \\ &= 227.84 \text{ mg-COD/day}\end{aligned}$$

$$\begin{aligned}\text{Unaccounted COD} &= \text{Influent COD} - \text{Effluent COD} - \text{Biogas COD} \\ &= 300 - 40 - 227.84 \\ &= 32.16 \text{ mg-COD/day}\end{aligned}$$

Percentage relative to influent COD

$$\text{Biogas COD percentage} = (227.84/300) = 75.94\%$$

$$\text{Effluent COD} = (40/300) = 13.34\%$$

$$\text{Unaccounted COD} = (32.16/300) = 10.72\%$$

Appendix B: COD Data before and after THP

Table 13: COD data for potato starch at 10 g/L

Temp (°C)	Before			After		
	tCOD	fCOD	sCOD	tCOD	fCOD	sCOD
50	10662.4	258.944	192.9387	10662.4	5965.867	4950.4
	10408.53	205.632	190.4	11170.13	6854.4	4823.467
	10789.33	208.1707	154.8587	10408.53	7108.267	4315.733
	10281.6	192.9387	180.2453	10408.53	6473.6	4696.533
70	10789.33	208.1707	144.704	10408.53	7742.933	5965.867
	9900.8	190.4	116.7787	10916.27	7235.2	6473.6
	10535.47	220.864	157.3973	10281.6	7362.133	5585.067
	10027.73	266.56	134.5493	10789.33	7235.2	6092.8
80	10662.4	264.0213	172.6293	10789.33	8631.467	7616
	9773.867	190.4	177.7067	9900.8	9266.133	7235.2
	10281.6	233.5573	129.472	11424	9012.267	7742.933
	9900.8	246.2507	228.48	10027.73	8758.4	6854.4
100	11424	233.5573	192.9387	10408.53	10027.73	8885.333
	10281.6	195.4773	114.24	11550.93	10154.67	8377.6
	9646.933	281.792	192.9387	10789.33	10027.73	7996.8
	10408.53	167.552	119.3173	10789.33	10408.53	7996.8
120	10408.53	236.096	172.6293	10281.6	10535.47	9900.8
	11170.13	200.5547	175.168	9646.933	11297.07	9266.133
	10281.6	200.5547	185.3227	10027.73	10281.6	9900.8
	10027.73	182.784	139.6267	10662.4	10916.27	9900.8
140	10281.6	218.3253	142.1653	10789.33	9900.8	10027.73
	10916.27	264.0213	192.9387	10535.47	9773.867	10027.73
	10408.53	279.2533	144.704	10535.47	10535.47	10027.73
	9900.8	213.248	175.168	10916.27	9773.867	10281.6

Table 14: COD data for potato starch at 15 g/L

Temp (°C)	Before			After		
	tCOD	fCOD	sCOD	tCOD	fCOD	sCOD
50	15485.87	261.4827	114.24	14978.13	7996.8	4188.8
	15232	220.864	192.9387	15739.73	7235.2	4188.8
	15739.73	246.2507	126.9333	15739.73	7616	4188.8
70	15232	233.5573	198.016	16501.33	7869.867	6981.333
	15485.87	248.7893	190.4	14216.53	8631.467	6473.6
	14724.27	279.2533	119.3173	15232	7362.133	6854.4
80	16247.47	198.016	152.32	16247.47	8885.333	7869.867
	15232	187.8613	149.7813	14470.4	9393.067	7235.2
	15232	192.9387	144.704	15232	8885.333	7616
100	15739.73	213.248	180.2453	14470.4	10662.4	9266.133
	15485.87	185.3227	177.7067	14724.27	10281.6	9393.067
	13454.93	210.7093	132.0107	14724.27	10916.27	10281.6
120	14978.13	233.5573	187.8613	15739.73	13962.67	11170.13
	15739.73	192.9387	137.088	15232	12947.2	10916.27
	14724.27	152.32	154.8587	15232	13201.07	10281.6
140	14724.27	246.2507	157.3973	15739.73	14978.13	14470.4
	14216.53	243.712	144.704	15993.6	14470.4	14216.53
	16247.47	213.248	142.1653	15739.73	14724.27	13962.67

Table 15: COD data for potato starch at 25 g/L

Temp (°C)	Before			After		
	tCOD	fCOD	sCOD	tCOD	fCOD	sCOD
50	24371.2	159.936	142.1653	25386.67	7108.267	4442.7
	26148.27	187.8613	142.1653	25894.4	7489.067	4442.7
	25386.67	185.3227	121.856	26656	7616	4442.7
70	25386.67	205.632	124.3947	25132.8	8504.533	5712
	25132.8	220.864	144.704	26656	7996.8	5712
	26909.87	187.8613	129.472	26909.87	7996.8	5965.7
80	25132.8	185.3227	121.856	24371.2	9139.2	7489.067
	24371.2	190.4	137.088	23863.47	9012.267	7489.067
	25894.4	192.9387	144.704	24625.07	9139.2	7742.933
100	24371.2	167.552	132.0107	24878.93	11170.13	10027.73
	24117.33	147.2427	132.0107	26656	11043.2	9520
	25386.67	159.936	134.5493	25386.67	10281.6	9266.133
120	26656	167.552	137.088	25132.8	14470.4	12693.33
	25640.53	180.2453	144.704	25894.4	13454.93	12693.33
	24625.07	195.4773	144.704	25132.8	13962.67	12058.67
140	24117.33	187.8613	144.704	25640.53	16247	14216.53
	25132.8	182.784	147.2427	26656	16755	14470.4
	25640.53	187.8613	129.472	25386.67	17263	13708.8

Appendix C: Solids data before and after THP

Table 16: Solids data for potato starch at 10 g/L

Temp. (°C)	Before			After		
	TS	TSS	VSS	TS	TSS	VSS
50	10213	9568.333	7921.173	10045.33	7966.667	7483.333
	9731.333	8853.333	7572.407	11066	8050	7450
	10069.67	9110	8059.11	9939.333	8866.667	7333.333
	9823	8868.333	7723.713	10238	8100	7400
70	10327.33	9220	7987.79	9774.667	6650	5616.667
	9492	8506.667	7308.84	10353.33	6666.667	5366.667
	10043.33	8803.333	7833.25	9734	6366.667	5766.667
	9306.333	7968.333	7290.837	10043.33	6466.667	5433.333
80	10224.67	9135	7904.853	10148	4733.333	3483.333
	9018	8055	6949.52	9750	4566.667	3266.667
	10048	8875	7832.86	10530.67	4366.667	3150
	9308	8405	7396.527	9394.667	4750	3300
100	11244.33	10081.67	8957.943	9905.333	2616.667	2116.667
	9580	8640	7625.273	10614	3100	2100
	9271.667	8493.333	7451.943	10470	2933.333	2266.667
	9914.333	9151.667	7800.107	10093.33	2783.333	1833.333
120	10010.33	8905	7939.913	9948.667	1433.333	783.3333
	10648.67	9466.667	8229.85	9284.667	1216.667	1100
	9755.333	8726.667	7783.737	9582	1533.333	650
	9722.333	9040	7647.83	10022	1300	916.6667
140	9616.667	8645	7276.26	10103.33	1033.333	1066.667
	10445.33	9568.333	8321.83	9820	1733.333	1200
	10029.33	9338.333	8018.46	10052.67	1583.333	933.3333
	9679.333	8845	7383.053	10262	1466.667	900

Table 17: Solids data for potato starch at 15 g/L

Temp. (°C)	Before			After		
	TS	TSS	VSS	TS	TSS	VSS
50	14831.33	12768.33	10466.67	15901.33	12383.33	8550
	14800.67	12923.33	10843.33	17029.33	12333.33	8283.333
	15040	13188.33	10821.67	16637.33	11633.33	8400
70	14700.67	12453.33	10903.33	17722.67	10316.67	7900
	14630	12738.33	10751.67	15216	9516.667	6733.333
	14188	12296.67	10346.67	15604	9266.667	6283.333
80	15529.33	13285	11385	17043.33	7783.333	5550
	14503.33	12501.67	10513.33	15098	7300	6083.333
	14440.67	12781.67	10956.67	16025.33	6716.667	5266.667
100	14684	12661.67	10453.33	14799.33	5933.333	4300
	14920.67	12823.33	11070	15726	6383.333	4166.667
	12886	11418.33	9738.333	15412.67	5350	4283.333
120	13809.33	12475	10790	16872	3283.333	2500
	14936.67	12805	11061.67	15630.67	3766.667	1866.667
	14104	12553.33	10770	16250.67	3100	2200
140	14112	12428.33	10538.33	16392.67	2800	1283.333
	13104.67	11533.33	10180	16803.33	2050	916.6667
	15669.33	13565	11606.67	16375.33	2333.333	1083.333

Table 18: Solids data for potato starch at 25 g/L

Temp. (°C)	Before			After		
	TS	TSS	VSS	TS	TSS	VSS
50	23821.33	21348.33	18508.33	24117.33	19000	17600
	25505.33	22940	19393.33	25369.33	18900	18500
	24786.67	22046.67	18676.67	25833.33	19333.33	17833.33
70	24546.67	21678.33	18980	23959.33	16800	13200
	24442	20851.67	19075	25318	14866.67	13600
	26440	23445	20278.33	25188	15766.67	12733.33
80	25077.33	22823.33	19226.67	23216	13266.67	10666.67
	23776	20930	18615	23148	12966.67	10133.33
	25151.33	22878.33	19868.33	23503.33	11433.33	10000
100	23790.67	21818.33	18481.67	23878.67	10933.33	7966.667
	23464	21385	17755	25582.67	10966.67	8033.333
	24614.67	21903.33	19366.67	24213.33	10833.33	7966.667
120	25618.67	23211.67	19553.33	23795.33	9500	5033.333
	24914.67	22525	19110	24946.67	9533.333	4933.333
	23998.67	21888.33	18483.33	24129.33	9366.667	4800
140	23460.67	21593.33	17988.33	24259.33	8433.333	4400
	24347.33	21420	18846.67	25427.33	8166.667	4166.667
	24949.33	22786.67	19451.67	24107.33	8333.333	3966.667

Appendix D: VFAs data at different operating conditions

Table 19: VFAs profile for reactors fed with starch (No_THP)

OLR	6 kg-COD/m ³ ·day			9 kg-COD/m ³ ·day		
HRT (d)	0.5	1	2	0.5	1	2
AA (mg/L)	284.6	838.9	2028.9	383.5	1164.6	2886.4
PA (mg/L)	59.4	177.3	348.4	226.7	428.74	771.4
BA (mg/L)	149.8	374.8	707.7	188.2	586.1	1081.1
VFAs (mg/L)	534.8	1484.9	3304.7	868.3	2381.7	5218.0
VFAs yield	0.171	0.247	0.271	0.185	0.260	0.289

VFAs yield = (mg-VFAs/mg-COD)

Table 20: VFAs profile for reactors fed with starch (THP_100)

OLR	6 kg-COD/m ³ ·day			9 kg-COD/m ³ ·day		
HRT (d)	0.5	1	2	0.5	1	2
AA (mg/L)	332.6	931.5	2116.8	590.9	1204.7	2871.7
PA (mg/L)	111.4	197.2	450.2	195.8	302.48	598.6
BA (mg/L)	121.9	355.1	730.3	212.4	524.6	1182.1
VFAs (mg/L)	678.1	1736.8	3818.9	1217.0	2450.3	5399.5
VFAs yield	0.216	0.288	0.308	0.247	0.273	0.297

Table 21: VFAs concentration at HRT of 10 days

HRT	10 days
AA (mg/L)	2174.9
PA (mg/L)	1087.4
BA (mg/L)	1928.2
VFAs (mg/L)	6486.7

Table 22: VFAs profile for reactors fed with starch (THP_140)

OLR	6 kg-COD/m ³ ·day			9 kg-COD/m ³ ·day		
HRT (d)	0.5	1	2	0.5	1	2
AA (mg/L)	184.1	369.3	782.2	230.2	445.4	970.8
PA (mg/L)	119.5	169.7	272.3	137.3	325.2	567.6
BA (mg/L)	0	239.7	465.1	0	322.9	722.3
VFAs (mg/L)	331.9	869.1	1717.2	478.4	1303.9	2668.7
VFAs yield	0.097	0.121	0.150	0.097	0.143	0.138

Appendix E: Methane production from potato starch

Table 23: Effect of THP on starch solution

	No_THP	THP_70	THP_100	THP_140
tCOD (mg/L)	10313	10598	10884	10694
fCOD (mg/L)	221	7393	10154	9996
sCOD (mg/L)	138	6029	8314	10091
TS (mg/L)	9792	9976	10270	10059
TSS (mg/L)	8624	6537	2858	1454
VSS (mg/L)	7605	5545	2079	1025

Appendix F: Anaerobic treatability study of food waste

Table 24: Methane production from food waste at 1.2 kg-COD/m³·day

Days	Influent COD (mg/L)	Effluent COD (mg/L)	CH₄ (%)	CO₂ (%)	Biogas Volume (mL)	mL-CH₄/day
1	3978.45	3520.71	9.64	60.35	2.87	0.276668
2	4107.88	3652.62	3.08	66.91	60.06	1.849848
3	3962.45	3503.71	0.58	69.41	376.72	2.184976
4	4148.54	3525.97	9.9	60.09	35.02	3.46698
5	4124.28	3505.47	7.03	62.96	85.38	6.002214
6	4059.68	3178.75	9.02	60.97	107.53	9.699206
7	4153.43	3192.46	8.74	68.25	84.91	7.421134
8	4054.02	2865.97	24.23	45.76	65.2	15.79796
9	3913.15	2707.23	34.48	35.51	44.8	15.44704
10	4043.20	2863.43	24.41	45.58	65.34	15.949494
11	4075.37	2859.81	31.77	38.22	53.66	17.047782
12	4094.46	2768.87	28.52	41.47	65.65	18.72338
13	4194.10	2837.1	27.74	42.25	68.15	18.90481
14	4150.67	2841.77	39.16	30.83	49.94	19.556504
15	4140.13	2690.66	31.29	38.7	68.08	21.302232
16	4023.21	2461.98	28.26	41.73	83.98	23.732748
17	4053.31	2334.4	20.78	49.21	127.15	26.42177
18	3955.43	2133.64	26.26	43.73	109.01	28.626026
19	3920.48	1972.8	24.28	45.71	133.75	32.4745
20	4188.42	1980.55	25.35	44.64	141.46	35.86011
21	4153.68	1786.9	25.31	44.68	153.02	38.729362
22	4032.62	1454.05	37.87	32.12	98.54	37.317098
23	3943.73	1304.88	31.35	38.64	130.91	41.040285
24	4065.37	1285.17	42.27	27.72	101.25	42.798375
25	3972.21	1114.01	49.1	20.89	90.91	44.63681
26	4165.26	1205.35	32.66	37.33	140.69	45.949354
27	4044.47	1122.98	46.85	23.14	97.58	45.71623
28	4131.26	1093.1	42.41	27.58	109.06	46.252346
29	4077.59	1185.7	34.04	35.95	125.39	42.682756
30	4037.97	1114.57	33.37	36.62	103.16	34.424492
31	3997.39	1003.81	48.71	31.28	95	46.2745
32	3971.99	955.33	51.45	28.54	90.16	46.38732
33	4179.24	1132.58	33.81	46.18	112.01	37.870581
34	4107.19	1135.81	30.26	49.73	104.41	31.594466

35	4026.10	1068.29	53.41	26.58	87.28	46.616248
36	4162.14	1147.95	53.52	26.47	86.77	46.439304
37	3913.73	933.37	60.11	19.88	77.25	46.434975
38	4199.52	1182.54	58.81	21.18	79.36	46.671616
39	4135.12	1071.3	56.68	23.31	82.34	46.670312

Table 25: Methane production from food waste at 1.6 kg-COD/m³·day

Days	Influent COD (mg/L)	Effluent COD (mg/L)	CH₄ (%)	CO₂ (%)	Biogas Volume (mL)	mL-CH₄/day
1	4049.69	3495.49	11.93	47.99	22.5	2.68425
2	4020.73	3467.72	11.67	46.5	18.85	2.199795
3	4309.57	3682.58	11.67	46.71	23.66	2.761122
4	4009.83	3242.9	11.54	42.28	37.88	4.371352
5	4312.34	3373.98	11.72	76.36	47.86	5.609192
6	4389.03	3067.62	12.11	48.92	116.14	14.06455
7	4327.63	3409.02	11.87	82.38	72.88	8.650856
8	4027.03	2697.25	12.35	78.2	134.56	16.61816
9	4033.09	2650.04	12.25	32.29	140.59	17.22228
10	4028.35	2496.16	11.93	55.17	163.43	19.4972
11	4139.9	2667.1	24.63	65.58	63.96	15.75335
12	4269	2397.6	21.03	81.05	108.19	22.75236
13	4199.59	2317.13	11.95	48.15	230.57	27.55312
14	4238.03	2516.84	18.75	30.25	120.6	22.6125
15	3865.51	2125.34	26.54	49.22	65.44	17.36778
16	4000.21	2051.55	27.5	40.35	87.39	24.03225
17	3841.14	1647.23	31	35.97	88.54	27.4474
18	3929.16	1348.83	35.39	52.44	98.92	35.00779
19	4118.45	1654.36	33.12	36.92	104.51	34.61371
20	4015.7	1310.31	30.59	22.61	137.81	42.15608
21	4105.11	1149.31	36.89	21.97	123.01	45.37839
22	4365.51	1306.25	31.65	69.15	150.89	47.75669
23	3950.19	790.91	27.31	54.77	200.75	54.82483
24	4272.2	1017.76	27.73	57.37	192.75	53.44958
25	3893.82	1052.42	37.84	25.57	134.61	50.93642
26	4053.11	1098.51	35.19	28.99	165	58.0635
27	3933.4	857.86	42.3	48.28	135.55	57.33765
28	4098.22	1056.83	52.81	31.5	103.03	54.41014
29	3907.88	898.33	48.47	33.02	121.18	58.73595

30	3913.96	873.14	45.71	52.65	121.6	55.58336
31	4177.52	1017.29	63.07	36.52	97.27	61.34819
32	4144.13	900.27	46.38	36.53	133.01	61.69004
33	4356.02	1160.49	44.69	34.46	135.78	60.68008
34	4282.74	1151.74	44.31	39.34	137.12	60.75787
35	4044.67	978.52	46.73	21.89	131.03	61.23032
36	4032.67	883.16	44.01	31.99	138.55	60.97586
37	3859.36	659.03	48.75	8.13	123.99	60.44513
38	3961.6	708.15	43.66	25.84	140.3	61.25498
39	3991.91	778.17	46.33	25.17	130.31	60.37262
40	3816.19	609.46	51.05	25.19	101.72	51.92806

Table 26: Methane production from food waste at 2.0 kg-COD/m³·day

Days	Influent COD (mg/L)	Effluent COD (mg/L)	CH₄ (%)	CO₂ (%)	Biogas Volume (mL)	mL-CH₄/day
1	5031.11	4563.69	14.98	54.98	18.99	2.844702
2	5187.25	4520.77	15.76	55.76	34.01	5.359976
3	4852.16	4180.82	15.97	55.97	33.96	5.423412
4	4991.42	4273.85	10.52	35.52	81.41	8.564332
5	5044.78	4331.82	24.73	59.73	34.1	8.43293
6	4828.38	3282.98	20.63	45.63	91.8	18.93834
7	4870.3	3760.61	20.25	45.25	90.86	18.39915
8	5298.83	4519.65	28.72	53.72	45.29	13.00729
9	5094.9	3562.09	20.93	45.93	127.4	26.66482
10	5184.21	3397.57	33.73	58.73	73.96	24.94671
11	5269.59	3656.44	10.81	35.81	246.43	26.63908
12	5198.58	3443.3	29.11	54.11	91.6	26.66476
13	5075.41	3429.15	23.83	38.83	112.65	26.8445
14	5186.6	3105.06	33.73	48.73	82.44	27.80701
15	5206.11	2853.83	41.7	56.7	74.42	31.03314
16	5006.16	3383.12	46.14	61.14	60.02	27.69323
17	5256.54	2864.1	42.76	57.76	83.17	35.56349
18	5285.02	2768.16	41.3	56.3	83.35	34.42355
19	4869.31	1526.87	36.05	41.05	139.01	50.11311
20	5245.99	2154.12	45.93	50.93	92.61	42.53577
21	5216.45	2037.36	38.4	23.4	130.5	50.112
22	5141.61	1004.7	49.29	50.29	145.08	71.50993
23	5035	1017.48	40.56	45.56	186.23	75.53489

24	4889.95	191.99	33.89	28.89	241.13	81.71896
25	5072.87	712.44	33.4	18.4	255.05	85.1867
26	4874.62	236.04	41.73	6.73	214.56	89.53589
27	4982.24	727.35	41.04	16.04	184.21	75.59978
28	4978.93	1163.71	54	39	135.28	73.0512
29	5297.35	1056.9	40.68	5.68	203.79	82.90177
30	5084.28	638.96	41.85	6.85	202.89	84.90947
31	4970.74	1225.8	45.87	0.87	151.22	69.36461
32	5116.39	1232.63	44.52	19.52	170.32	75.82646
33	5113.93	715.69	36.61	21.61	235.31	86.14699
34	5044.64	876.5	41.36	27.63	185.36	76.6649
35	5085.73	1274.24	41.7	16.7	176.16	73.45872
36	4838.8	1395.33	45	30	141.21	63.5445
37	4945.17	1708.1	41.56	36.56	151.88	63.12133
38	5230.23	1755.8	52.61	37.61	125.12	65.82563
39	4967.39	876.07	54.06	39.06	139.06	75.17584
40	4973.84	994.83	55.34	40.34	140.44	77.7195

Appendix G: Co-digestion of PHL and starch

Table 27: PHL characterization

pH	Solids (mg/L)			BOD (mg/L)	COD (mg/L)		
	TS	TSS	VSS		tCOD	fCOD	sCOD
3.99	118198.3	2139.52	1705.74	46678.54	94593.63	87370.74	83205.3
3.63	115261.7	2161.12	1788.02		95014.58	88701.9	83616.43
3.98	111199.6	2258.07	1903.21	47813.1	93840.68	87779.56	83096.63
3.97	113547.1	2037.36	1685.37		92578.24	90279.15	82803.12
3.8	113436.3	2019.48	1603.53	50413.27	95688.1	88485.28	84862.69
3.56	118699.7	2232.92	1739.46		93809.5	87476.35	82847.35
3.85	113239.1	2141.76	1827.91	45221.3	96205.53	90402.97	82715.91
3.96	115785.3	2085.84	1793.75		92319.63	87728.09	83927.71
3.69	111893.5	2116.2	1655.41	44721.9	92971.67	88312.3	84785.29
3.8	115705	2238.28	1791.82		96651.84	90287.75	82876
3.71	113635.3	2003.03	1836.1		92550.2	89341.1	83398.3
3.83	116320.2	2270.47	1601.78		92292.42	87353.41	82307.08
3.58	111874.7	2093.93	1687.29	49579.65	93929.49	90063.31	82709.62
3.92	115789.5	2028.62	1842.59		96959.26	90063.51	83692.33
3.62	114095.5	2046.15	1628.78	50044.88	96247.92	87841.22	83827.79
3.55	112730.2	2091.42	1729.02		93681.81	87421.62	82780.85
3.89	114919.2	2135.04	1610.74	46339.73	92549.93	89094.03	84493.29
3.74	115092.6	2107.52	1666.44		92004.99	89252.31	83418.6
3.58	116641.8	2077.4	1644.11	46649.31	92320.31	88832.99	82718.87

Table 28: Potato starch characterization

pH	Solids (mg/L)			BOD (mg/L)	COD (mg/L)		
	TS	TSS	VSS		tCOD	fCOD	sCOD
7.45	8586.92	7737.47	6937.56	6744.75	8825.29	191.62	132.93
7.67	8541.6	7528.36	6819.99		8864.35	218.86	127.2
7.49	8436.28	7615.65	6836.16	6777.31	8842.79	213.24	119.61
7.77	8490.27	7794.88	7099.02		8983.63	195.06	130.67
7.5	8541.23	7554.01	6938.56	6506.43	8748.19	197.32	119.45
7.46	8328.48	7794.76	6900.19		8857.71	189.26	136.07
7.75	8525.1	7733.59	7015.14	6519.41	8882.58	203.69	114.52
7.72	8539.06	7749.65	6822.91		8932.54	216.1	125.83
7.42	8420.11	7737.52	6981.39	6609.49	8985.7	209.28	133.52
7.37	8551.15	7695.55	6810.62		8993.72	206.47	137.01
7.49	8466.2	7640.84	6841.04	6592.46	8998.34	211.18	128.87
7.32	8347.91	7676.89	6831.11		8713.95	195.42	128.82
7.68	8363.43	7551.77	7080.96	6510.43	8862.61	202.91	127.06
7.56	8596.08	7741.79	6902.93		8912.58	196.47	117.23
7.5	8405.84	7633.56	6857.3	6593.31	8820.34	218.1	136.21
7.45	8373.31	7763.08	6850.77		8753.68	206.02	136.26
7.64	8390.96	7527.13	6904.75	6795.88	8706.52	197.29	125.86
7.79	8567	7648.54	7059.15		8749.44	189.92	127.14
7.58	8432.44	7638.24	7029.06		8843.17	214.09	137.18

Table 29: Potato starch and PHL characterization (50% each COD basis)

pH	Solids (mg/L)			BOD (mg/L)	COD (mg/L)		
	TS	TSS	VSS		tCOD	fCOD	sCOD
6.18	10077.27	4406.6	3592.75	6077.14	8752.26	6261.17	5717.78
6.07	10066.62	4404.52	3568.54		8555.41	5024.05	5994.27
6.26	9875.57	4291.8	3576.23	5986.29	8585.27	5316.56	5408.75
6.19	9961.66	4296.86	3640.74		8779.81	6083.39	5311.41
6.05	9882.97	4461.76	3678.98	5956.64	8791.89	5948.86	4857.45
6.27	10055.24	4411.66	3418		8840.99	5155.13	5659.45
6.21	9842.03	4282.02	3704.81	6191.51	8814.53	5513.65	5235.69
6.25	10034.79	4364.89	3549.71		8711.91	4946.45	5968.07
5.98	10059.35	4340.07	3519.53	6032.64	8715.65	6460.44	5008.76
6.05	10082.07	4474.42	3628.57		8591.2	4895.32	6000.29
6.01	9968.13	4218.61	3591.07	6170.83	8567.76	6280.64	4707.08
6.24	9828.95	4308.34	3462.63		8662.96	5456.73	4921.03
6	9892.91	4471.59	3548.77	6195.87	8747.22	5216.07	5481.98
5.9	9952.6	4231.39	3491.53		8807.16	5923.1	4632.33
6.28	10061.2	4308.29	3548.59	6120.29	8746.47	5132.1	5981.87
6.17	10037.05	4424.09	3447.82		8792.76	6430.11	5353.06
6.16	10002.17	4427.66	3638.8	6116.94	8659.2	6295.26	5948.11
6.18	10008.87	4300.08	3493.38		8830.57	6238.4	4820.35
6.01	9801.64	4366.39	3626.57		8812.39	5371.09	5013.14

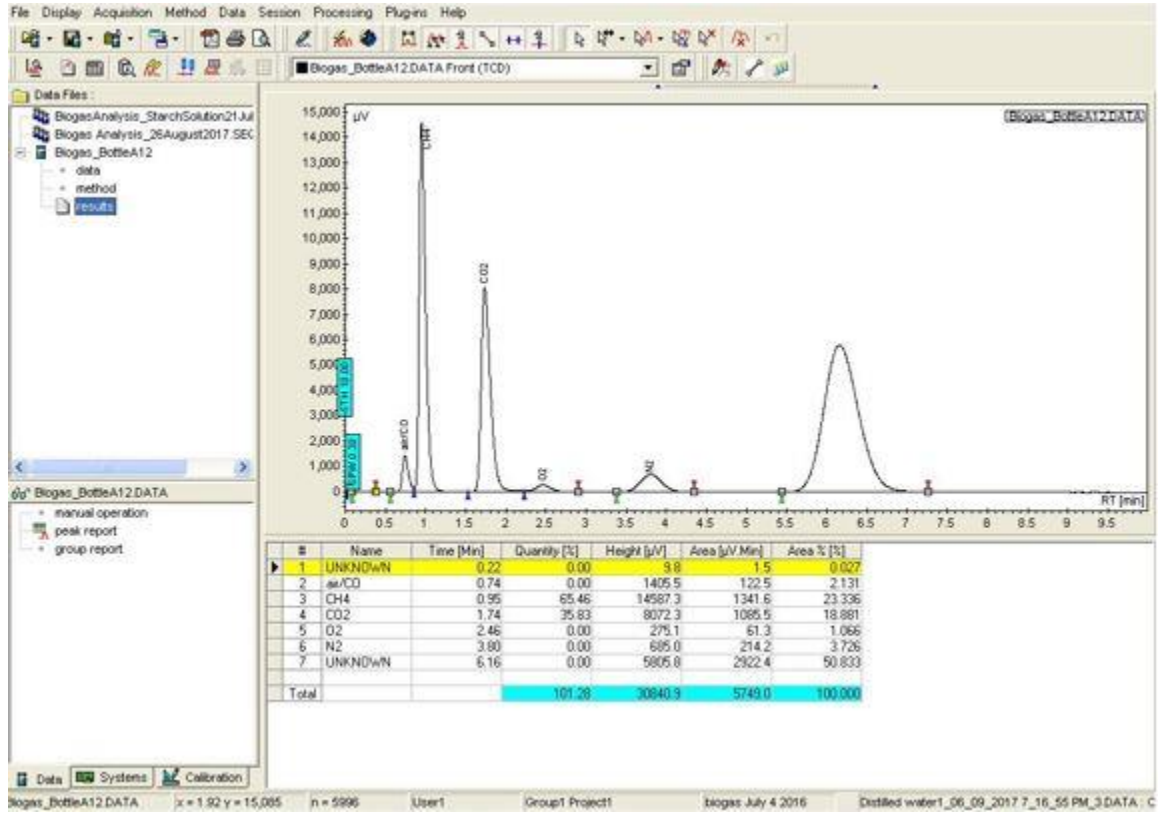
Table 30: Biogas data for co-digestion of PHL and starch

Day	Influent COD	Effluent COD	Biogas Volume (mL)	CH₄ (%)	CO₂ (%)	mL-CH₄/day
0	0	0	0	0	0	0
1	8992.09	8364.08	29.14	10.32	20.03	3.007248
2	9297.59	8563.24	63.37	10.79	23.71	6.837623
3	9286.67	8488.6	85.17	10.99	23.1	9.360183
4	9349.43	8412.14	107.6	13.61	22.19	14.64436
5	8805.13	7686.91	157.52	14.17	22.84	22.32058
6	8852.09	7097.78	204.2	22.91	24.14	46.78222
7	8946.98	7123.2	216.68	23.08	22.72	50.00974
8	9015.88	7030.79	167.62	33.76	22.59	56.58851
9	9055.83	7067.58	153.15	36.7	23.22	56.20605
10	9083.86	6994.68	125.19	48.02	29.37	60.11624
11	9210.95	6681.97	198.58	39.21	27.62	77.86322
12	9239.45	6658.81	186.46	42.98	22.86	80.14051
13	8916.23	6300.24	151.09	53.85	26.95	81.36197
14	9199.25	6386.36	160.94	55.12	26.18	88.71013
15	9077.67	6112.78	181.97	52.3	20.3	95.17031
16	9382.66	6469.23	149.52	62.34	28.01	93.21077
17	9272.32	6102.39	202.71	50.98	23.79	103.3416
18	9152.89	5952.42	182.03	57.39	27.28	104.467
19	9034.16	4840.78	222.01	64.51	26.97	143.2187
20	9195.03	5484.62	336.09	36.94	28.9	124.1516
21	9046.51	5349.24	317.86	38.94	34.12	123.7747
22	9059.37	5470.28	262.76	45.66	32.91	119.9762
23	8916.34	5357.8	241.97	48.8	20.63	118.0814
24	9160.58	5768.82	174.61	64.07	22.93	111.8726
25	9397.94	5734.53	229.76	53.45	20.91	122.8067
26	8864.68	5458.27	152.6	73.51	31.01	112.1763
27	9390.38	5984.98	149.52	74.99	24.24	112.125
28	9016.75	4840.17	222.84	64.21	27.99	143.0856
29	8812.01	4604.25	205.99	70.01	25.56	144.2136
30	9312.03	4544.1	315.73	52.63	27.05	166.1687
31	9007.83	4373.89	222.21	72.55	28.31	161.2134
32	9112.34	4037.86	320.12	55.71	34.83	178.3389
33	8810.6	3879.81	244.63	70.6	23.09	172.7088
34	8829.1	3922.53	230.11	74.54	21.39	171.524
35	9058.89	3679.9	266.32	71.34	34.9	189.9927

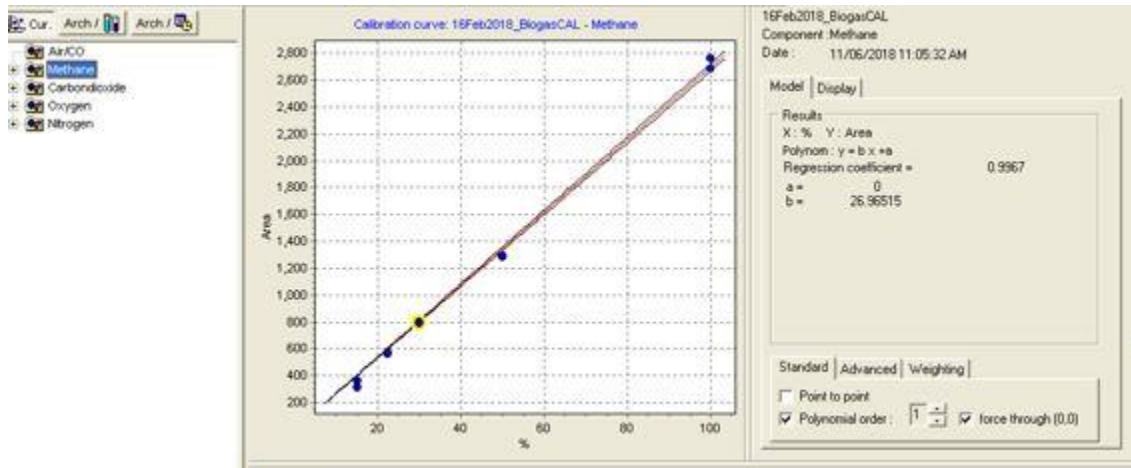
36	9213.7	3530.91	367.44	55.17	22.95	202.7166
37	8922.64	2941.89	314.81	68.04	28.13	214.1967
38	8989.14	2573.45	461.49	50.05	22.07	230.9757
39	9275.57	2884.62	451.55	51.02	32.39	230.3808
40	9170.89	2329.56	406.77	61.01	28.21	248.1704
41	9207.66	2189.55	399.16	63.91	24.74	255.1032
42	9334.9	2242.4	414.01	62.32	27.8	258.011
43	8909.23	1683.79	430.96	61.04	22.87	263.058
44	9307.82	2111.72	409.81	64.02	29.56	262.3604
45	9310.47	2002.04	415.6	64.2	28.45	266.8152
46	9389.11	2074.24	413.69	64.5	33.72	266.8301
47	9027.51	1646	437.77	61.48	25.65	269.141
48	9206.33	1797	443.55	61.03	31.52	270.6986
49	8902.8	1450.17	446.53	60.91	23.33	271.9814
50	8939.26	1502.51	425.78	63.71	23.29	271.2644
51	9340.02	1860.39	427.82	63.84	24.51	273.1203
52	9251.58	1734.38	431.42	63.63	33.57	274.5125
53	9342.21	1847.64	427.56	64.14	21.22	274.237
54	9356.13	1801.97	425.27	64.96	20.03	276.2554
55	9082.53	1327.54	463.26	61.36	34.16	284.2563
56	8903.36	1123.75	462.38	61.59	33.57	284.7798
57	9247.62	1325.18	484.04	60.03	30.27	290.5692
58	9329.32	1454.73	459.62	62.78	30.89	288.5494
59	8891.88	994	466.87	62.09	34.57	289.8796
60	9116.5	1202.84	475.66	61.12	34.13	290.7234
61	8858.47	954.85	464.71	62.41	31.68	290.0255
62	8906.63	810.16	486.57	61.25	28.42	298.0241
63	9107.64	902.87	486.8	62.06	25.36	302.1081
64	9300.37	967.11	484.48	63.31	23.49	306.7243
65	9273.66	896.3	475.23	64.97	20.52	308.7569
66	9142.96	955.62	463.98	64.96	34.4	301.4014
67	8985.54	670.42	500.58	61.22	23.52	306.4551
68	9015.23	714.83	487.95	62.72	29.98	306.0422
69	9224.01	863.77	499.91	61.71	29.71	308.4945
70	8908.6	566.73	479.5	64.11	28.9	307.4075
71	9274.22	941.19	475.96	64.59	29.33	307.4226
72	8876.87	417.89	498.7	62.6	31	312.1862
73	9180.48	716.24	506.72	61.62	34.43	312.2409

Appendix H: Sample chromatograms and GC calibration

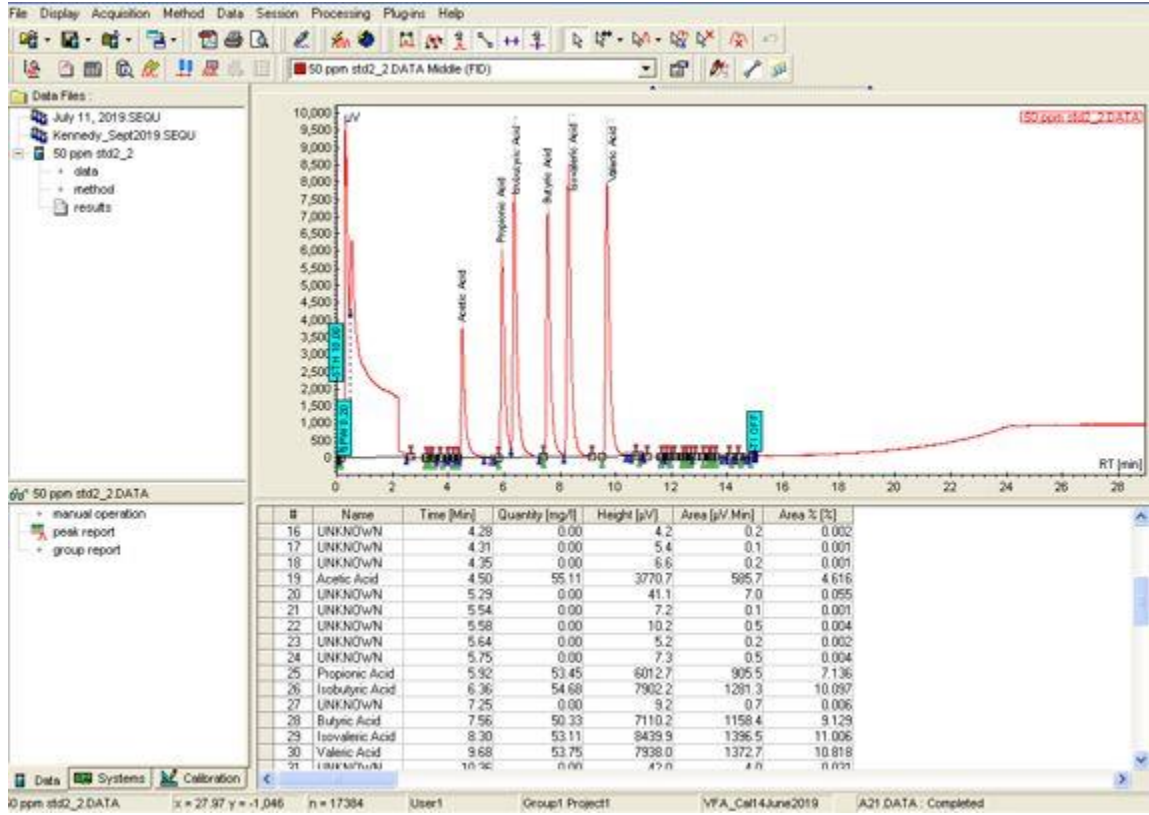
Biogas chromatogram



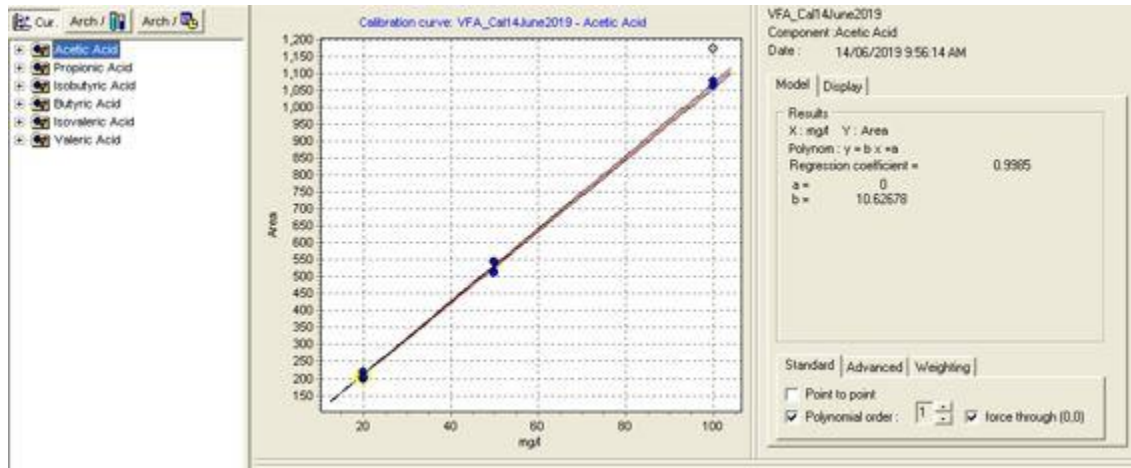
Biogas (methane) calibration



VFAs chromatogram



VFAs (acetic acid) calibration



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Bhargav, M., Singh, K.. Volatile fatty acids production from thermally pre-treated starch using mesophilic anaerobic digestion. ASCE Journal of Environmental Engineering. Under peer review.

Conference Presentations:

Bhargav, M. and Singh, K. (2018). Volatile Fatty Acid production from potato waste using Thermal Hydrolysis Process during mesophilic anaerobic digestion. Conference proceedings from the annual conference of the Canadian Society of Civil Engineering, Fredericton, New Brunswick, Canada.

Bhargav, M. and Singh, K. (2019). Volatile fatty acids production from thermally hydrolyzed potato starch using different modes of reactor operation (Poster presentation). Proceedings of the 16th International Water Association (IWA) World Congress on Anaerobic Digestion, Delft, The Netherlands.