INVESTIGATION OF BIO-HYDROGEN AND BIO-METHANE PRODUCTION FROM POTATO WASTE

Mina Aminnejad
University of Windsor

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INVESTIGATION OF BIO-HYDROGEN AND BIO-METHANE PRODUCTION FROM POTATO WASTE

By

Mina Aminnejad

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

Windsor, Ontario, Canada

2017

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August 30, 2017
DECLARATION OF ORIGINALITY

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ABSTRACT

An evaluation of single-stage and two-stage anaerobic digestion processes for biomethane and biohydrogen production using potato waste was performed to assess the viability of biohydrogen production from potato waste and the impact of separating the acidogenic and methanogenic stages on anaerobic digestion with hydrogen production in the first stage. Potato waste has the potential to improve hydrogen production with a maximum yield of 0.51 L H_2 / g COD_{consumed} with anaerobic digester sludge (ADS). A comparison of the initial substrate-to-biomass S_o/X_o of 0.5 and 1 g COD_{substrate} / g VSS_{seed} demonstrates that the optimum experimental range of S_o/X_o for hydrogen production is 0.5 g COD_{substrate} / g VSS_{seed} using anaerobic digester sludge ADS.

The optimum experimental range of S_o/X_o for methane production is 0.5 g COD_{substrate} / g VSS_{seed} using ADS as a seed and supernatant as a feed. However, when using mixed substrate, there is not a significant difference between different S_o/X_o.

Potato waste has the potential to improve methane production with a yield of 0.39 m^3 CH_4 / kg TCOD_{removed} when using supernatant as a feed as tests with mixed feed only revealed a maximum potential of 0.35 m^3 CH_4 / kg TCOD_{removed}.

In this research, the use of two-stage digestion for potato waste led to an increase in the TVFAs to TCOD ratio due to the acidification process during hydrogen production in the first stage. The methane yield in the anaerobic digestion stage increased from 0.29 m^3 CH_4 / kg TCOD_{removed} in the single-stage process to 0.39 m^3 CH_4 / kg TCOD_{removed} in the two-stage and single-stage processes, respectively.
DEDICATION

To my beloved husband, Dariush, for his love, endless support and knowledge: this project would not have run as well without his guidance and help. I will always appreciate all he has done to help me develop my technology skills.

To my father, mother, and sister for their love and support. Their words of encouragement and push for tenacity rang in my ears throughout my research.

To my supervisor, Dr. Biswas, for the patient guidance, encouragement and advice he has provided throughout my time as his student. I have been extremely fortunate and proud to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly.
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Finally, the author would like to express her deep gratitude and appreciation to her father, mother, sister and husband for their continuous support and encouragement throughout the course of this work. Without my husband's support, help and patience, this work would not have been possible.
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<tr>
<td>ADM1</td>
<td>Anaerobic digestion model no. 1</td>
</tr>
<tr>
<td>ADS</td>
<td>Anaerobic digester sludge</td>
</tr>
<tr>
<td>AMPTS II</td>
<td>Automatic Methane Potential Test System</td>
</tr>
<tr>
<td>ANN</td>
<td>Artificial neural network</td>
</tr>
<tr>
<td>APB</td>
<td>Anaerobic packed-bed</td>
</tr>
<tr>
<td>BOD</td>
<td>Biological oxygen demand</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>CSTR</td>
<td>Continuous stirred tank reactor</td>
</tr>
<tr>
<td>F/M</td>
<td>Food to microorganisms ratio</td>
</tr>
<tr>
<td>GHG</td>
<td>Greenhouse gas</td>
</tr>
<tr>
<td>H</td>
<td>Cumulative hydrogen value</td>
</tr>
<tr>
<td>Hmax</td>
<td>Maximum cumulative hydrogen value</td>
</tr>
<tr>
<td>HP</td>
<td>Hydrogen production</td>
</tr>
<tr>
<td>HPR</td>
<td>Hydrogen production rate</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>HY</td>
<td>Hydrogen yield</td>
</tr>
<tr>
<td>K</td>
<td>Maximum rate of substrate utilization/first-order kinetic constant</td>
</tr>
<tr>
<td>LCFA</td>
<td>Long chain fatty acids</td>
</tr>
<tr>
<td>OLR</td>
<td>Organic loading rate</td>
</tr>
<tr>
<td>OFMSW</td>
<td>Organic fraction of municipal solid waste</td>
</tr>
<tr>
<td>P</td>
<td>Ultimate/Cumulative hydrogen production</td>
</tr>
<tr>
<td>Pmax</td>
<td>Maximum cumulative hydrogen production</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>pH</td>
<td>Initial pH</td>
</tr>
<tr>
<td>$R^2$</td>
<td>Coefficient of determination</td>
</tr>
<tr>
<td>$R_m$</td>
<td>Rate of hydrogen production</td>
</tr>
<tr>
<td>$S_o/X_o$</td>
<td>Substrate to biomass ratio</td>
</tr>
<tr>
<td>SBR</td>
<td>Sequencing batch reactor</td>
</tr>
<tr>
<td>SCOD</td>
<td>Soluble chemical oxygen demand</td>
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<td>SRT</td>
<td>Solid retention time</td>
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<tr>
<td>$T$</td>
<td>Temperature</td>
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<tr>
<td>$T_{\text{carbi}}$</td>
<td>Initial total carbohydrates</td>
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<tr>
<td>$T_{\text{carbf}}$</td>
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<tr>
<td>TCOD</td>
<td>Total chemical oxygen demand</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
</tr>
<tr>
<td>TSS</td>
<td>Total suspended solids</td>
</tr>
<tr>
<td>TVFAs</td>
<td>Total volatile fatty acids</td>
</tr>
<tr>
<td>UASB</td>
<td>Up-flow anaerobic sludge blanket</td>
</tr>
<tr>
<td>$V_s$</td>
<td>Volume of sludge</td>
</tr>
<tr>
<td>$V_p$</td>
<td>Volume of potato waste</td>
</tr>
<tr>
<td>VFAs</td>
<td>Volatile fatty acids</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solids</td>
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<tr>
<td>VSS</td>
<td>Volatile suspended solids</td>
</tr>
<tr>
<td>$X_o$</td>
<td>Initial biomass concentration</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Lag phase duration</td>
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CHAPTER 1
INTRODUCTION

1.1. Introduction
Large amounts of fruit and vegetable wastes are produced by the food industry, and this creates a
major challenge to the managers of the landfills because of its volume and their high biodegradability [Bouallagui, 2004].

According to the UN Food and Agricultural Organization (FAO) Statistical Yearbook 2012 and FAO Global Food Losses and Food Waste 2011 report, one-third of the food formed for human consumption is lost or wasted, mostly before it ever reaches human. That is almost 1.3 billion tonnes per year. According to the Cut Waste, Grow Profit 2014 report, Canadians waste $31 billion of food produced yearly; this is roughly 40% of food formed per year in Canada. In North America, more than 30% of fruits and vegetables are refused by stores because they are not aesthetically pleasing enough for consumers.

The cumulative cost of related wastes, such as energy, water, land, labour, capital investment, infrastructure, machinery, transport, has been assessed by the United Nations’ Food and Agricultural Organization at 2.5 times larger than the “face value” of wasted food, which means that the inclusive cost of food waste in Canada likely exceeds 100 billion [Gooch et al., 2014].

Organic waste in landfills generates methane gas, which is 25 times more harmful to the environment than carbon dioxide [Environmental Commissioner of Ontario, 2012]. Waste was highlighted in the Environmental Commissioner of Ontario’s Annual Report as a nascent subject that can be escaping broader public attention and has the potential for important environmental effects.

Source Separated Organics (SSO) is the system through which waste producers separate compostable materials from other wastes at the source for separate collection. SSO plans have been started in a wide range of venues, including single-family residential units, commercial businesses, major events locations, food processing facilities, schools, hospitals, and airports. The organic portion of the waste stream is progressively observed as a resource. The ensuing products-
renewable energy and compost—not only benefit the environment by increasing the nutrient composition of soil, but also by decreasing a number of harmful trends: greenhouse gas emissions, dependency on foreign energy imports, the amount of waste passing to landfills, the amount of wet, runny waste going to other methods of disposal, the leachate related with storm water management at landfills, the greenhouse gas emissions from unbounded landfill processes, and erosion and storm water control through biofiltration [Schwab, 2000].

Organic materials composed together in SSO agendas are transported to composting facilities where the waste is changed into nutrient-rich soil amendments recognized as compost. Organic feedstock can also be transported to anaerobic digestion services that produce biogas, a source of renewable energy. The subsequent biogas (methane) can then be employed for cogeneration (electricity and heat preferably on or close to the site of production) and can be applied in gas combustion engines or turbines. When used in synthetic natural gas, methane can be fed into the natural gas network or more refined to hydrogen for use in stationary cogeneration fuel cells.

Canada has the second highest methane emissions from waste disposal on land among all the countries in the United Nations Framework Convention on Climate Change Parties [UNFCC, 2003]. Landfill gas is essentially comprised of primarily of methane and carbon dioxide (CO₂) - two effective greenhouse gases- as well as slight amounts of hydrogen, oxygen, nitrogen, hydrogen sulphide and trace amounts of non-organic compounds and volatile organic compounds [Gardner et al., 1993; Schumacher, 1983]. Methane is naturally produced during a 30 to 50 year period as waste undertakes anaerobic decomposition. It is the fundamental concern for greenhouse gas emissions from landfills as it has 23 times the global warming possibility of CO₂. The methodical retrieval and utilization of landfill gas produced during anaerobic decomposition of municipal solid wastes both decreases GHG emissions and makes an alternative renewable source of energy to replace fossil fuel use [Pembina Institute, 2003; Smith et al., 2001]. If the methane was recovered from one tonne of waste it could yield approximately 1000 kilowatt hours (kWh), as one cubic meter of methane gas has an energy rate of four to five kWh [Pembina Institute, 2003]. Methane recovery of landfill gas signifies one of the most cost-effective resources to lessen GHG emissions due to both fuel sales and credits from GHG decrease [Rovers and Associates, 1999]. In addition, the capture and use of landfill gas supplies the additional benefits as it reduces odours, allows damage to vegetation to be monitored, lessens owner liability, offers a potential basis of
Anaerobic biogas digesters are airtight reactors in which organic waste is broken down and transformed into biogas by an anaerobic digestion. Biogas is captured and is then converted into heat or other forms of energy. The residual sludge consists of various nutrients and can be employed in agriculture (optionally after an aerobic post-composting). This technology has been progressed over the past centuries, primarily in industrialized countries, leading to several strategies that each have different levels of complexity. To solve the problem of municipal waste disposal and rising fuel prices, low-tech set-ups—particularly those adapted in developing countries—have been established today.

Using anaerobic digestion has several advantages:

- It produces biogas and fertilizer (complete retention of the fertilizer nutrients (N, P, K)
- It decreases greenhouse gas emissions through methane recovery
- It facilitates the united treatment of a wide variety of organic waste and wastewaters
- It lessens the number of solids that need to be handled
- It offers an effective pathogen removal that relies on temperature
- It facilities process constancy: high-loads can be treated and anaerobic sludge can be conserved for prolonged periods without any feeding

1.2. Anaerobic digestion
Anaerobic digestion is getting more attention, both as a solution to environmental problems and also as an energy supply for today’s energy-demanding life style [Asam et al., 2011]. With 244 plants in Europe and a volume of 8 million tonnes of organics treatment capabilities, anaerobic digestion is already carrying out of about 25% of the biological treatment in Europe [Baere, 2000].

In anaerobic digestion, organic materials are degraded by bacteria, in the absence of oxygen, transformed into a methane and carbon dioxide combination. The remaining matters or slurry from the digester has ammonium and other nutrients that could be employed as an organic fertilizer [Nas, 1977]. Microorganism from two biological groups, the bacteria and the archaea, carry out this process under anaerobic conditions [Dugba, 1999]
There are examples where anaerobic digestion systems have been used in the agricultural industry. For instance, when dealing with livestock, it is used to reduce the overall environmental impact in the production of manures and for energy generated through the production of biogas [Rapport et al., 2008]. Most of these anaerobic digestion structures are single-stage systems. In a single-stage (one-stage), all biological reactions happen in a single reactor. Studies shows that two-stage anaerobic digestion could offer greater advantages over the single-stage digestion because it is faster and more stable [Baere, 2000]. In practice, however, it is contended that the two-stage digestion has not been able to validate its asserted advantages in the market, and the added benefits in increasing the rate of hydrolysis and methanization have not been affirmed [Pohland, 1977].

1.2.1. The Anaerobic digestion process

Anaerobic digestion is often believed to be a multifaceted process; the digestion itself is based on a reduction process containing several biochemical reactions occur under anoxic conditions [Aslanzade, 2014]. Methane formation in anaerobic digestion includes four different steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

Hydrolysis

Hydrolysis is the first stage in anaerobic digestion process and consists of the enzyme-mediated changes in insoluble organic materials—including lipids, polysaccharides, proteins, fats, and nucleic acid—into soluble organic materials, such as compounds appropriate for the use as source of energy and cell carbon. This include monosaccharides, amino acids, and other modest organic compounds. This step is fulfilled due to strict anaerobes, such as bacterizes, clostridia, and facultative bacteria like streptococci [Christy, 2014]. This first step is vital because large organic molecules are basically too large to be directly absorbed and applied by microorganisms as a substrate/food source.

Acidogenesis

The second stage is acidogenesis, during which the monomers shaped in the hydrolytic step are taken up by wide variety of facultative and obligatory anaerobic bacteria and are degraded into short-chain organic acids, including butyric acids, propanoic acids, acetic acids, alcohols, hydrogen, and carbon dioxide. The concentration of hydrogen shapes as an intermediate result in this stage effects the type of final product formed during the fermentation process.
Acetogenesis

The products formed in the acidogenic stage are used as substrates for the other microorganisms that are active in the third phase: acetogenesis. In this, also referred to as the acidogenic phase, anaerobic oxidation is performed [Aslanzade, 2014]. Products that cannot be directly changed into methane by methanogenic bacteria are formed into methanogenic substrates, while volatile fatty acids and alcohols (VFA) are oxidized into methanogenic substrates, such as acetate, hydrogen and carbon dioxide. VFA with carbon chains longer than one unit are oxidized into acetate and hydrogen [Elseadi, 2008]

Methanogenesis

In the methanogenic stage, methanogenic bacteria produce methane and carbon dioxide from intermediate products in strict anaerobic conditions [Aslanzade, 2014]. Methanogenesis is an important step in the whole anaerobic digestion process as it is the slowest biochemical reaction of the process [Elseadi, 2008].

1.2.2. Substrate for anaerobic digestion process
A wide range of biomasses can be applied as substrate (feedstock) for the making of biogas from anaerobic digestion process. The substrate should have the all nutritional requirements of the microorganisms in their energy sources and numerous components vital for making new cells. The substrate should also contain different components needed for the activity of microbial enzymes systems, such as trace elements and vitamins [Aslanzade, 2014]. Substrate composition is critical in the anaerobic digestion process.

1.3. Problem statement
Microbial cultures can have an effect on biomethane production from soluble substrates like glucose [Ling et al., 2009]. Many studies applied common anaerobic digester sludge to assess biomethane production from different wastes. For example, [Chen et al., 2006] and [Yu et al., 2002] applied it to process food wastes. [Parawira et al., 2004] conducted anaerobic batch biodegradation of potato waste at different concentrations and found that a maximum methane yield of 0.32 L CH₄/g VS degraded was obtained at Sₒ/Xₒ of 1.5. In another study, [Parawira et al., 2005] investigated the anaerobic digestibility of potato waste in a laboratory-scale UASB reactor
and an APB reactor treating potato. The methane yield was 0.23 L CH\textsubscript{4}/g COD\textsubscript{degraded} in the UASB reactor and 0.16 L CH\textsubscript{4}/g COD\textsubscript{degraded} in the APB reactor.

Linke, [2006] examined the anaerobic treatment of solid wastes from potato processing in CSTR. The biogas and methane yields obtained were 58% and 50%, respectively. In long term lab-scale experiments it could be demonstrated that thermophilic anaerobic digestion is applicable for treatment of solid wastes from potato processing [Linke, 2006]. Zhu et al., [2007] investigated a two-stage anaerobic digestion process from potato waste for co-production of hydrogen and methane. The hydrogen stage was done in continuous mode and the methane stage was operated in both continuous and semi-continuous modes. A maximum gas production rate of 270 mL/h and an average of 119 mL/h were produced from the hydrogen stage during the operation of over 110 days.

Aiming to maximize the acidification process, the acidogenic and methanogenic stages in a two-stage anaerobic digestion process were studied individually in several studies [Vinas et al., 1993; Pavan et al., 2000; Demirel and Yenigun, 2002]. Some studies investigated the effect of hydrogen production in the first stage on the methane production in the second stage. Chu et al., [2008] investigated a two-stage process comprised of thermophilic hydrogen production and mesophilic methane production for the treatment of OFMSW. They maintained a stable performance for simultaneous hydrogen and methane production for 150 days with hydrogen and methane yields of 0.25 m\textsuperscript{3}/kg VS\textsubscript{added} and 0.464 m\textsuperscript{3}/kg VS\textsubscript{added}, respectively. Furthermore, Han and Shin, [2004] tested food waste in a leaching-bed reactor for hydrogen production and an UASB reactor for methane production in mesophilic conditions; they reached hydrogen and methane yields of 0.31 m\textsuperscript{3}/kg VS\textsubscript{added} and 0.21 m\textsuperscript{3}/kg VS\textsubscript{added}.

Many studies investigated various factors in the production of biohydrogen and biomethane from different types of substrate with different reactors. However, these studies did not focus on comparing two types of substrate-mixed and supernatant from final biohydrogen stage in the second stage of a two-stage anaerobic digestion process in the production of biomethane from potato waste under mesophilic condition and in the batch system.
1.4. Research objectives
The current study investigates the use of potato waste in hydrogen and methane production with four central objectives:

1. To assess the viability of biomethane production from potato waste in batch studies, with substrate to biomass \( S_0/X_0 \) of 0.5 and 1 and the maximum biomethane production potential. The potato waste which was used in the current study is uncooked and comes from a food processing industry.
2. To conduct a comparative evaluation of single-stage and two-stage anaerobic digestion processes using potato waste.
3. To conduct a comparative evaluation of two substrates-supernatant and mixed liquid from biohydrogen stage-in two-stage anaerobic digestion processes using potato waste.
4. Assess the viability of biohydrogen production from potato waste in batch studies and determine if a substrate to biomass \( S_0/X_0 \) ratio of 0.5 and 1 facilitates maximum hydrogen production potential.

1.5. Research contributions
Methane production potentials of different waste streams have been studied in the literature using common anaerobic digester sludge [Wang and Wan, 2009]. In addition, a two-stage anaerobic digestion process was proven to be more robust, easily achieving steady state condition, than the single-stage digestion; higher methane production rates and yields were observed in the second stage [Demirel and Yenigun, 2002].

The primary contribution of the current study is that it confirms the potential advantages of two-stage anaerobic digestion over single-stage for potato waste treatment: increased acidification leads to improved biogas production and enhanced biosolids destruction efficiency.

1.6. Thesis organization
This thesis includes five chapters and conforms to the “integrated-article” format as outlined in the Thesis Regulation Guide by the School of Graduate Studies of University of Windsor.

I. Chapter 1 provides a literature review that includes background of hydrogen and methane production, different reactors used for this purpose, a wide variety of substrates that have the potential to achieve biogas.
II. Chapter 2 presents a two-stage anaerobic digestion process.

III. Chapter 3 outlines an assessment of the viability of biohydrogen production from potato waste in batch studies and a determination of the optimal substrate to biomass \((S_o/X_o)\) ratio and the maximum hydrogen production potential.

IV. Chapter 4 presents a comparative assessment of single-stage and two-stage anaerobic digestion of potato wastes.

V. Chapter 5 summarizes the main conclusions of this investigation and provides future research recommendations based on the findings of this study.
CHAPTER 2
LITERATURE REVIEW

2.1. Introduction
Environmental friendly energy carriers and sources are a highlighted topic in the energy and
environmental zone. Currently, the global energy demand is primarily fulfilled with fossil fuels,
which are depleting, and the world is facing rigorous pollution quandaries from the by-products of
fossil fuels uses [Ghimire et al., 2015].

Researchers have broadly acknowledged the fact that the expanding $CO_2$ level is exacerbated by
the utilization of fossil fuels, which increasing the impact that greenhouse gases have on global
warming. Thus, various methods are being developed to harness energy from clean renewable
sources, and multiple energy sources are being explored.

According to the Cut Waste, Grow Profit 2014 report, Canadians waste $31 billion worth of food
yearly, roughly 40% of the food produced for consumption in Canada. In North America, more
than 30% of fruits and vegetables are refused by stores because they are deemed to not be
aesthetically pleasing enough for consumers.

The cumulative cost of related wastes -which includes energy, water, land, labour, capital
investment, infrastructure, machinery, and transport- has been assessed by the United Nations’
Food and Agricultural Organization to be 2.5 fold larger than the “face value” of wasted food,
meaning the cost of food waste in Canada may exceed $100 billion [Gooch et al., 2014].

Organic material in landfills generate methane gas, which is 25 times more harmful to the
environment than carbon dioxide [Environmental Commissioner of Ontario, 2012]. Waste was
highlighted in the Environmental Commissioner of Ontario’s Annual Report (2011/2012) as a
nascent subject that is escaping broader public attention and has the potential for important
environmental effects.

SSO is the system through which waste producers separate compostable materials from other
wastes at the source for separate collection. SSO plans have been started in a widespread range of
venues, including single-family residential units, commercial businesses, major events locations,
food processing facilities, schools, hospitals, and airports. The organic portion of the waste stream is progressively observed as a resource.

Renewable energy and compost offers a number of benefits with respect to the environment [Schwab, 2000]:

- They decrease greenhouse gas emissions;
- They decrease dependency on foreign energy imports;
- They increase the nutrient composition of soil;
- They decrease the amount of waste in landfills;
- They diminish the amount of wet, runny waste going to other methods of disposal;
- The decrease the leachate relate to stormwater management at landfills;
- They decrease the greenhouse gas emissions from unbounded landfill processes;
- They slow the progress of erosion and stormwater control via biofiltration.

Organic materials composed together in SSO agendas are transported to composting facilities where the waste is changed into nutrient-rich soil amendments recognized as compost. Organic feedstock can also be transported to anaerobic digestion services that produce biogas, a source of renewable energy. The subsequent biogas (methane) can then be employed for cogeneration (electricity and heat preferably on or close to the site of production) and can be applied in gas combustion engines or turbines. When used in synthetic natural gas, it can be fed into the natural gas network or more refined to hydrogen for use in stationary cogeneration fuel cells [Pembina Institute, 2003].

Canada has the second highest methane emissions from waste disposal on land among the many countries in the United Nations Framework Convention on Climate Change Parties [UNFCC, 2003]. Landfill gas is essentially including half methane and half carbon dioxide (CO₂), two effective greenhouse gases, as well as slight amounts of hydrogen, oxygen, nitrogen, hydrogen sulphide and trace amounts of non-organic compounds and volatile organic compounds [Gardner et al., 1993; Schumacher, 1983]. Methane is naturally produced from landfill during a 30 to 50 year period as waste undertakes anaerobic decomposition. If the methane was recovered from one tonne of waste it could yield approximately 1000 kilowatt hours (kWh), as one cubic meter of methane gas has an energy rate of four to five kWh [Pembina Institute, 2003]. Methane recovery
of landfill gas signifies one of the most cost-effective resources to lessen GHG emissions due to both fuel sales and credits from GHG decrease [Rovers and Associates, 1999]. In addition, the capture and use of landfill gas supplies the additional benefits of restraining odours, monitoring damage to vegetation, lessening owner liability, dropping risk from explosions, fires and asphyxiation, and smog while giving a potential basis of revenue and yield [Smith et al., 2001].

Hydrogen is also a carbon-free clean fuels and is the primary by-product of water combustion [Andriani et al., 2013]. It can likewise be useful in dealing with global warming and expanding contamination and pollution issues. Moreover, it is favored over methane derived from its more extensive industrial applications. For example, H₂ is utilized in the synthesis of ammonia and hydrogenation of edible oil, petroleum, coal, and shale oil [Niesner et al., 2013].

2.2. Potential sources of organic biomass for biogas production

2.2.1. Food waste
Food waste is an energy source found primarily in landfills, where it rots, thereby releasing greenhouse gases into the atmosphere. Treating and recycling food waste is difficult since it consists of large amounts of sodium salt, and moisture, and is joined with other waste during collection.

The load of food waste in industry is gradually increasing; therefore, an appropriate food waste management strategy needs to be conceived to maintain its eco-friendly and sustainable disposal. Consequently, there is an urgent need to investigate more effective recycling options. Anaerobic digestion has been successfully applied in European and Asian countries to stabilize food wastes, and to produce advantageous end-products [Zhang et al., 2007].

2.2.2. Potato waste
Potato waste derives in many forms, including whole potatoes, peels, frying oil, and spoiled product. It is all discarded into huge bins and travels by digesters, pumps, and other equipment which break it down and change the organics into gas. Potato waste is too weak in quality to apply effectively as animal feed, which is also disposed as a slurry [Parawira et al., 2004].

Potato peels and other “zero value” wastes from potato processing are full of starch that can be liquefied and fermented to yield fuel-grade ethanol. A study in Canada’s potato-growing province
of New Brunswick suggests that 44,000 tons of processing waste could gain 4-5 million liters of ethanol [International potato center, 2011].

Lignocellulose refers to plant dry matter (biomass), so called lignocellulosic biomass. The lignocellulosic biomass can be broadly classified into virgin biomass, waste biomass, and energy crops. Potatoes are one the most important sources of lignocellulose [International potato center, 2011].

**Composition of Lignocellulosic materials**

The composition of biomass is based on feedstock nature. [Mosier et al., 2005]. Cellulose is most plentiful, representing 30–70% of lignocellulosic biomass; hemicelluloses and lignin represent 15–30% and 10–25% of the biomass, respectively [Monlau et al., 2011].

**Cellulose**

Cellulose is comprised of D-glucose subunits and is linked by β-(1→4) glyosidic bonds [Fengel, 1992; Fengel and Wegener, 1984]. The cellulose in a plant is comprised of parts that have a prearranged crystalline structure and parts with weakly organized, amorphous structures [Liang and Marchessault, 1959]. The cellulose strains are bundled together and form cellulose fibrils, also known as cellulose bundles. These cellulose fibrils are primarily independent and weakly bound through hydrogen binding [Atalla and Vanderhart, 1984]. Cellulose, insoluble in water and most organic solvents, is chiral and biodegradable. It can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature. Many properties of cellulose depend on its chain length, crystallinity, or degree of polymerization [Monlau et al., 2011].

**Hemicelluloses**

Hemicelluloses can be any of the heteropolymers (matrix polysaccharides) present in nearly all plant cell walls lengthwise, with cellulose [Aman, 1993]. While cellulose is crystalline, sturdy, and resistant to hydrolysis, hemicelluloses have a chance, amorphous structure with a slight forte. Hemicelluloses have less molecular weight than cellulose and has branches with small side chains that contain different sugar monomers and can include xylose, mannose, galactose, rhamnose, and arabinose, which are polymers that can be simply hydrolyzed [Ebringerová and Heinze, 2000; Fengel and Wegener, 1984; Kacurakova et al., 1999] by dilute acid, a base or by many
hemicellulase enzymes. Xylose is the sugar monomer that is mostly present and is the dominant chemical present, though uronic and ferulic acids also tend to be present [Monlau et al., 2011].

**Lignin**

After cellulose and hemicelluloses, lignin is the third most abundant polymer in the environment and is present in cell walls. It is an amorphous heteropolymer consisting of three different phenylpropane alcohols: p-coumaryl (H), coniferyl (G), and sinapyl (S). The nature and the amount of lignin monomers (H, G, S) differ according to species, maturity, and the space localization in the cell [Yoshizawa et al., 1993]. For instance, an increase in lignin content from 3-7% was detected through the maturing of grass [Nizami et al., 2009].

**2.2.3. Agricultural residues**

Agricultural residues, which primarily contain lignocellulosic wastes, are an economically applicable and renewable source of second generation carbon neutral biofuels. These make up plant biomass waste, which is usually comprised of cellulose, hemicellulose, and lignin shaped by photosynthesis. Agricultural residues are formed when the economically valued products of crops are harvested and the residues, such as straw, stover, peelings, cobs stalks, and bagasse, are left over. The 2010 global annual production of agricultural residues was about 5.1 billion dry tones. The waste made by the agricultural, forestry, and aquaculture industry is growing with the raising population; thus, the waste from this part will increase [Ghimire et al., 2015].

**2.2.4. Livestock waste (manure)**

Livestock waste includes solid animal manure waste; fodder waste, which normally covers a lignocellulosic fraction; and wastewater, which contains urine. A considerable amount of livestock manure derives from cattle feedlots and poultry and swine structures. These livestock recognized as pollution causes. Because they threaten the atmospheric and water environment. The present practices of livestock waste management include its application in agricultural fields as well as biological stabilization or treatment, such as composting and AD. Manure management practices can decrease direct and indirect greenhouse gas emissions by making energy in the form of biogas from the manure prior to its land application.

However, for manure substrates need to be done physical and chemical treatment. It will cause prevention in the methanogenic activity. Another, problematic case that might happen throughout
the use of this feedstock is the inhibition of the biohydrogen production by ammonia as its extreme nitrogen content might cause bioreactor failure [Ghimire et al., 2015].

2.2.5. Industrial waste
Enormous amounts of carbohydrate-rich, non-toxic waste in the form of solid waste and wastewater are produced by agro-industries waste, which includes palm oil mill and olive mill wastewater; food industries, such as breweries; and tapioca and dairy industries. It can be potential feed for dark fermentative biohydrogen production. Ren et al., [2009] confirmed that waste molasses is a brilliant feed in a pilot scale system worked under mesophilic conditions (35°C) where positive outcomes were gained in terms of H₂ production [Ghimire et al., 2015].

2.2.6. Organic fraction of municipal waste
The OFMSW usually creates food waste, 85-95% of which is volatile solids, and 75–85% of which is moisture content production. This means it is comprised of a high percentage of biodegradable carbohydrates, which makes it an ideal substrate for DF. Food waste current in municipal waste is primarily responsible for methane emissions and leachate production from landfills. AD has been proposed as the most appropriate treatment choice for OFMSW or food waste with energy recovery and other environmental credentials. Thus, food waste has been used widely in DF trials. Ghimire et al., [2015] reviewed studies on DF processes that apply OFMW or food waste for dark fermentative biohydrogen production. They found that a considerable amount of waste biosolids or sludge are made from municipal wastewater treatment plants, which are normally comprised of carbohydrates or polysaccharides and proteins. Several researchers have used the available carbohydrates present in these biosolids in fermentative hydrogen production. However, the sludge requires pre-treatment, such as ultrasonication, acidification, sterilization, freezing–thawing, or alkaline pre-treatment, to enable the fermentative process. In addition, Kim et al. confirm the value of sewage sludge as co-substrate in the DF of food waste [Ghimire et al., 2015].

2.3. Anaerobic digestion
The anaerobic digestion of solid waste is a procedure similar to the one applied in biogas production. In the absence of oxygen, anaerobic bacteria are used to break down the organic matter of biomass, and throughout the transformation, a blend of methane and carbon dioxide gases are produced. The characteristic ratio of gas mixture is 60–70% methane and 30-40% carbon dioxide. The gas has a heating value 650–750 Btu/ft³. Owing to growing cost of energy, the anaerobic
digestion of biomass is an effective option for the creation of fuel and bio fertilizer for organic cultivation. Anaerobic digestions in landfills are possible sources of methane production from solid waste. The anaerobic digestion of the biodegradable fraction of the municipal solid waste produces methane and carbon dioxide in uneven volumes [Molino et al., 2012].

2.3.1 Principles
Anaerobic digestion is a normal biological process when bacteria break down organic material in environments with no oxygen. A controlled enclosed version of the anaerobic breakdown of organic waste is a kind of landfill process, which produce methane as an end product. Numerous research groups have shown that the AD process can be split into three main stages: hydrolysis, acidogenesis, and methanogenesis [Molino et al., 2012].

Anaerobic fermentation decreases the total mass of waste, makes solid or liquid fertilizer, and yields energy. In the first step of hydrolysis, or liquefaction, fermentative bacteria change the insoluble complex organic matter, such as cellulose, into soluble molecules, such as sugars, amino acids, and fatty acids. The complex polymeric matter is hydrolyzed to monomers. For example, hydrolytic enzymes buried by microbes transform cellulose into sugars, or alcohols and proteins into peptides or amino acids. The hydrolytic action is importance waste has high organic content that might develop rate limiting. Some industrial operations overcome this limitation by using chemical reagents to increase the hydrolysis process. The use of chemicals to increase the first stage has been found to shorten digestion time and increase methane yields [Molino et al., 2012].

2.3.2 Four important steps in this process
In the first stage, four transformations occur: lipids are converted into fatty acids, polysaccharides are converted into monosaccharides, protein are converted into amino acids, and nucleic acids are converted into purines and pyrimidines.

In the second stage, acetogenic bacteria, also identified as acid formers, change the products of the first stage to simple organic acids, carbon dioxide, and hydrogen [Molino et al., 2012].

The main acids formed are acetic acid (CH₃COOH), propionic acid (CH₃CH₂COOH), butyric acid (CH₃CH₂CH₂COOH), and ethanol (C₂H₅OH). The products shaped during acetogenesis are caused by various microbes. The acetogenesis reaction is shown below:

\[ C₆H₁₂O₆ \rightarrow 2C₂H₅OH + 2CO₂ \quad (2.1) \]
In the third and final phase, the methane is formed by bacteria named methane formers, also known as methanogens, is caused by two phenomena: the cleavage of acetic acid molecules that generate carbon dioxide and methane, and the reduction of carbon dioxide with hydrogen.

Methane production is higher when carbon dioxide is reduced, but when digesters have inadequate hydrogen concentration, it causes an acetate reaction, which is the main producer of methane. The methanogenic bacteria include *methanobacterium, methanobacillus, methanococcus*, and *methanosarcina*. Methanogens can be broken up into two groups: acetate and H\textsubscript{2}/CO\textsubscript{2} consumers [Molino et al., 2012].

The methanogenesis reactions can be stated as follows:

The biogas products from the anaerobic digestion are comprised of methane, carbon dioxide, hydrogen, hydrogen sulfide, ammonia, siloxanes, and other materials that may prevent the anaerobic digestion process or affect corrosion issues in the pipelines or distribution systems of waste treatment facilities. Several research groups have shortened methods for biogas purification, specifically for hydrogen sulfide, ammonia, and siloxane removal. In conclusion of the purification process the biogas still comprises hydrogen, carbon dioxide, and trace amounts of sulphidric acid and ammonia (<100 ppm) that should be detached from the stream to yield biomethane [Molino et al., 2012].

**2.4. Anaerobic bioreactors used for biomethanation**

**2.4.1 Batch systems**

In batch systems, digesters are completed with or without addition of seed materials and let to go through all degradation stages consecutively. The symbol of batch systems is the pure separation between the first phase, where acidification proceeds much quicker than methanogenesis, and the second phase, where acids are converted into biogas.

Converti et al., [2008], tried the anaerobic batch digestion under both mesophilic and thermophilic conditions. The results demonstrated that, under mesophilic and thermophilic situations, the blend of vegetable wastes could be digest. The anaerobic batch digestion of mixed vegetable waste was done effectively at 5% total solid concentration. Digestion of the waste after 47 days lead to 0.16 m\textsuperscript{3}biogass/kg TS\textsubscript{applied} with a maximum gas production on day 26. Two other studies
demonstrated that the anaerobic treatment at 8% TS in a batch digester was achieved by VFA accumulation and immutable declining pH problems [Bouallagui et al. 2003, and Marouani et al., 2003]. In the past, batch systems have not succeeded in captivating a considerable market share [Naik et al., 2009]. However, the specific structures of batch processes, like simple design and process control, robustness towards coarse and heavy contaminants, and lower investment costs cause them more appealing for developing countries. Using the SBR technology in anaerobic treatment is worthy of consideration because of its operational flexibility, which is characterized by three factors: a high degree of process flexibility in relation to cycle time and sequence, a lack of separate clarifiers, and the retention of a higher concentration of slow-growing anaerobic bacteria within the reactor. Research into the ASBR process has demonstrated that it can achieve reasonably high solid content waste degradation and suspended solid removal (90–93%) using the ASBR were informed [Naik et al., 2009].

2.4.2. Continuous one-stage systems

Of the waste treatment facilities in Europe that conduct anaerobic digestion of the organic portion of municipal solid wastes and bio wastes, approximately 89% depend on continuous one-stage systems [Mata-Alvarez et al., 2000]. Nevertheless, a significant volume of studies have explored waste treatment in two phases, specifically an acid creating phase followed by a methanogenic stage. The reason for this is that two-stage structure has more potential and offers more possibilities to investigate the intermediate stages of the digestion process. Alternately, industrialists desire one-stage structures because of their simpler designs and lesser investment costs. Different tests on vegetable wastes anaerobic digestion were done using a wide variety one-stage systems. Mata-Alvarez et al., [2000], observed the mesophilic one-stage system can adequately stir a reactor during the treatment of the organic portion of the wastes coming from a large food market. The maximum OLR that was attempted was under 3 kg TVS/(m³ day). The OLR of 6 kg TVS/(m³ day) was discovered to be a limit condition for similar waste digestion. Furthermore, as cited by Mata-Alvarez et al., [2000], this waste apparently was more biodegradable, which allowed greater and quicker VFA production: this underscored the validity of this OLR limit. Overloading digesters by more than 4 kg TVS/m³ day was also investigated by Lane, who found a reduction in pH and gas yield and a surge in the CO₂ content of gas formed when using a CSTR [Naik et al., 2009].
A semi-continuously mixed tubular digester was tried. The best outcomes were gained by using an HRT for 20 days with an OLR of 2.8 kg TVS/ (m$^3$day). The pH may drop in the hydrolysis shortly to 6.1, but then it leftovers most of the time at 7.2. When the HRT dropped to 10 days, the pH decreased to 5 and inhibition was detected. The most important factor of the tubular reactor was its capability to distinguish acidogenesis and methanogenesis longitudinally down the reactor, which allowed the reactor to act as a system of two phases [Naik et al., 2009].

In one-step anaerobic digestion of solid wastes, problems might occur if the substrate is simply degradable because there is no option for the accumulation/retention of biomass within the reactor in solid waste digestion; hence, the slower increasing methanogens are overfed at higher loading rates.

In a one-stage system, merging acidogens and methanogens are in one vessel and hydrogen produced by acidogenic metabolism, which is integrated by the methanogens to diminish carbon dioxide to methane and water. The feeding rate of the substrate is improved when acidogenic actions-including acetate, carbon dioxide, and hydrogen production-are present and while the methanogenic population cannot rise to an equivalent extent. At a loading rate were the hydrogen consuming reactions become soaked, the accumulation of hydrogen partially inhibits its extra creation, and more organic electron sink will be shaped accordingly. This creates imbalances and a cessation of methane production [Naik et al., 2009].

**2.4.3. Continuous two-stage systems**

Both clusters of acidogenic and methanogenic organisms are dissimilar with respect to their nutritional supplies, physiology, pH optima, growth, nutrient uptake kinetics, and their capability to survive environmental stress factors. When merging acidogens and methanogens in one reactor during conventional digestion procedures, uniform conditions are forced on both clusters. However, two-phase anaerobic digestion indicates a process configuration that uses isolated reactors for acidification and methanogenesis linked in a series, which allows the optimization of both processes [Naik et al., 2009].

The two-phase anaerobic digestion of a combination of fruit and vegetable wastes has been considered by different researchers [Rajesh Wari et al., 1999]. The two-step technology used by Rajesh Wari et al., [1999] facilitated the conversion of more than 94% of the vegetable market.
waste into biogas. The raw waste was acidified in a solid bed reactor. After achieving the acidification phase, the leachate gained was further treated in an UASB reactor for biogas production. The hydrolysis–acidification step was passed out in ASBR and methane fermentation was performed in a fixed film reactor that used the up-flow method. The global degradation yield endured more than 87% and the biogas production yield was about 0.29 L/g of the initial TCOD. Using a two-stage system related to a thermophilic liquefaction CSTR reactor and a mesophilic anaerobic filter, over 95% volatile solids were transformed into methane at a volumetric loading rate of 5.65 g VS/L d. The methane production yield was about 420 L/kg VS_{added} [Naik et al., 2009].

2.5. Hydrogen production
Hydrogen production can be categorized into chemical-physical and biological methods [Cai et al., 2004]. The chemical-physical systems are energy-intensive and costly [Mizuno et al., 2000], while making biological hydrogen offers some promising environmental solutions and uses less energy.

2.5.1. Biohydrogen production processes
Bio-hydrogen can yield several processes:

- Direct Biophotolysis
- Indirect Biophotolysis
- Photofermentation
- Dark Fermentation

In the following units, the typical explanation of these systems is provided with their key benefits and drawbacks [Ghirardi et al., 2000]

2.5.1.1. Direct Biophotolysis
Specific green algae can yield hydrogen gas by applying solar energy to switch water [Ghirardi et al., 2000], which is instantly available and fed into oxygen and hydrogen through the following reaction:

\[ 2 \text{H}_2 \text{O} + \text{light energy} \rightarrow 2 \text{H}_2 + \text{O}_2 \]  

(2.2)

The primary benefit of this process is its carbon-free character, where water is divided by solar energy producing hydrogen and oxygen [Resnick, 2004]. Using solar energy itself is a negative
side for this method [Das and Veziroglu, 2001] and the important issue with direct biophotolysis is the requirement to separate hydrogen and oxygen, which makes the process impractical. Simultaneous hydrogen and oxygen production with this process has attained little concentrations of hydrogen owing to the need for an inert gas [Hallenbeck and Benemann, 2002]. By using this procedure, 0.07 mmol/L-h [Levin et al., 2004] of hydrogen was produced. It was confirmed by applying the Biochemistry Process (Figure 2.1):

2.5.1.2. Indirect Biophotolysis
In an indirect biophotolysis process, a specific category of autotrophic microalgae, identified as cyanobacteria, synthesizes hydrogen by breaking down water in a two-step process [Resnick, 2004]:

\[
6\text{H}_2\text{O} + 6\text{CO}_2 + \text{light energy} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \quad (2.3)
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} + 6\text{H}_2\text{O} \rightarrow 12\text{H}_2 + 6\text{CO}_2 \quad (2.4)
\]

In the first phase, cyanobacteria changes water and carbon dioxide into glucose and oxygen via a multifaceted process of photosynthesis. In the second phase, glucose is converted into hydrogen and carbon dioxide. The benefit of the indirect biophotolysis in comparison to the direct biophotolysis process is that cyanobacteria can consume nitrogen from the atmosphere to satisfy its nutritional requirements. One of the drawbacks for this method the fact that the gas mixture that is formed contains not only oxygen and hydrogen, but carbon dioxide as well [Das and Veziroglu, 2001]. When utilizing this process, Kotay and Das, [2008] found that the maximum hydrogen production in their experiment was 0.36 mmol/L-h, which is five times that reported for direct biophotolysis [Kotay and Das, 2008]. In addition, a solar efficiency of 10% has been confirmed by using indirect biophotolysis in open ponds [Benemann, 1998].

2.5.1.3. Photofermentation
A class of purple non-sulfur bacteria can yield hydrogen in the absence of nitrogen [Levin et al., 2004] by leading the flow of electrons to the reduction of hydrogen as an alternative of fixing nitrogen when growing on poor nitrogen basis [Brentner et al., 2010]. They produce hydrogen and carbon dioxide by converting water by using the following chemical equation:
Potato Waste: cellulose, hemicellulose, lignin, fat, reducing and non-reducing sugar, protein, pectin

Amino acids, sugars, alcohols, long chain fatty acids

Hydrolysis

Acidogenesis

Intermediary product:

Inhibition

\[ H_2O + CH_4 + CO_2 \]

H2

CO2

Acetate

Figure 2.1. The Biochemistry Process [Bouallagui et al., 2005]
Studies have tried using numerous microalgae in hydrogen production by utilizing photo fermentation, such as *Rhodopseudomonas* capsulata [Jouanneau et al., 1984, Levin et al., 2004], *Rhodobacterspheroids* [Resnick, 2004], and *Rhodospirillum rubrum* [Resnick, 2004]. A wide variety of types of wastes, such as whey and distillery effluents, can be applied as a source of glucose in photo fermentation. The significant drawbacks are the existence of carbon dioxide in the gas combination and the water pollution produced by the fermented broth that must be wasted after fermentation [Das and Veziroglu, 2001]. A maximum hydrogen making rate of 0.16 mmol/L-h using *Rhodobacter spheroids* was confirmed by Kotay and Das, [2008], and a feed conversion efficiency of up to 91% when applying *Rhodopseudomonas palustris* was confirmed by Brentner et al., [2010].

### 2.5.1.4. Anaerobic Dark Fermentation

Dark fermentation proposals offer a vast possibility of hydrogen production by relating different anaerobic bacteria species, such as *Clostridium* [Lin et al., 2007], *Enterobacter* [Yokoi et al., 2001], or *Bacillus* [Kalia et al., 1994] when activated at varied reaction temperatures. It can be separated into mesophilic (25-40°C), thermophilic (40-65°C), extreme thermophilic (65-80°C), or hyper thermophilic (>80°C) [Levin et al., 2004]. Dark fermentative hydrogen production rests on the type of carbohydrates source, such as glucose, hexose, starch, or cellulose [Guo et al., 2010], and on the procedure situations like the pH [Ginkel and Sung, 2001]. Furthermore, the final products can be different and include acetate, butyrate, propionate, lactic acid, and ethanol [Guo et al., 2010].

In addition to the wide range of final products made by the various microbial metabolisms, acetate and butyrate are the only final products that have theoretical yields of four and two moles of hydrogen per each mole of glucose [Batstone et al., 2002]:

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2.8)$$

$$C_6H_{12}O_6 \longrightarrow \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{CO}_2 + 2\text{H}_2 \quad (2.9)$$
Nevertheless, the growth of acetate in the medium does not essentially indicate higher biohydrogen production as numerous microbial species can produce acetate in a hydrogen-consuming path by converting hydrogen and carbon dioxide [Guo et al., 2010]:

\[
2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2\text{H}_2\text{O} \quad (2.10)
\]

The by-products of the fermentation procedure consist of propionate, ethanol, and lactic acid. Propionate is a metabolite of a hydrogen-consuming pathway (Equation 2.8), while ethanol and lactic acid are incorporated in a zero-hydrogen balance pathway (Equations 2.9 - 2.10) [Batstone et al., 2002]:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2 \rightarrow 2\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \quad (2.11)
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2 \quad (2.12)
\]

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CHOHCOOH} + 2\text{CO}_2 \quad (2.13)
\]

Nandi and Sengupta [1998] categorized the main hydrogen-producing and hydrogen-consuming bacteria in anaerobes (Clostridia, Methylo trophs, Methanogenic bacteria, Rumen Bacteria, Archaea) and facultative anaerobes (Escherichia coli, Enterobacter). In a mixed culture, both facultative and anaerobic hydrogen-producing and hydrogen-consuming microorganisms can stay.

Operating situations vastly affect the bacterial metabolism and thus hydrogen yields. Small hydrogen yields have been attained in fermentation processes, which were improved for biomass instead of hydrogen production [Hallenbeck and Benemann, 2002]. To increasing the hydrogen yield, feed metabolism should be focused on the producing of VFAs instead of alcohols or lactic acid.

2.6. Conversion of organic biomass to biomethane

2.6.1. Biomethanation

Biomethanation (biogas formation) indicates one of the important types of bio-energy and can be formed from organic solid wastes and organic wastewaters [Plugge et al., 2010]. Anaerobic methanogenic action of organic solid waste and wastewaters can decrease dependence on fossil fuels. Anaerobic processing offers an effective waste treatment that requires less energy when creating bio-energy in the form of methane [Plugge et al., 2010].
Anaerobic wastewater treatment procedures are beneficial compared to aerobic processes. Anaerobic wastewater treatment has some benefits compared to common aerobic treatment procedure [Lier, 2008]:

- It leads to a decrease of almost 90% of the sludge made.
- It requires 90% less space when operating expanded sludge bed systems.
- It offers considerable COD loading rates, achieving to 20–35 kg COD $m^{-3}$ reactor capacity. $day^{-1}$, causes less reactor capacity.
- Its lack of fossil fuel usage during treatment removes almost 1 kWh/kg COD.
- It facilitates the formation of 13.5 MJ CH$_4$ energy/kg COD removed.
- It allows rapid start-up (< 1 week) by using seed granular anaerobic sludge.
- Its usage of chemicals is almost zero.
- It has considerable COD treatment efficiency

Methanogenesis is the last step in the anaerobic digestion procedure, and the feed employed by the methanogenic organisms consist of hydrogen, formic acid, carbon monoxide, methanol, methylamine, and acetate [George et al., 2003]. The representative reactions, including these compounds, are outlined in Equations 2.10 to 2.15.

Costa et al., [2013] outline the four main steps of the biochemical process of biomethanation.

Step 1: Hydrolysis

In the first step, carbohydrates are converted into soluble sugars, primarily by cellulases, amylases, and xylanases. Proteins are then degraded via peptides and amino acids and by lipases, and lipids are converted into LCFA and glycerol.

Step 2: Acidogenesis

Primary feed for acidogenesis consist of soluble saccharides, amino acids, and glycerol. The results in the formation of mostly acetate and hydrogen, among other products such as propionate, butyrate, carbon dioxide, and other organic materials, like lactate and alcohols.

Step 3: Acetogenesis
Hydrogen producing acetogens can be grown and lead to the formation of more fermentation products (short chain fatty acids and alcohols), LCFA, and acetate. Fatty acids oxidation is linked to the reduction of hydrogen ions either or bicarbonate. It is an effective external electron acceptor that can be used to convert to hydrogen and formats.

Step 4: Methanogenesis

Methanogenesis is achieved by methanogenic archaea, which metabolizes the final products of the prior reactions and converts them to methane. This procedure primarily facilitates two pathways:

1. Carbon dioxide reduction (hydrogenotrophic methanogenesis).
2. Acetate dissimilation (acetoclastic methanogenesis).

\[ \text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad (2.14) \]
\[ 4\text{HCOOH} \rightarrow 4\text{CH}_4 + 3\text{CO}_2 + 2\text{H}_2\text{O} \quad (2.15) \]
\[ 4\text{CO} + 2\text{H}_2\text{O} \rightarrow 4\text{CH}_4 + 3\text{CO}_2 \quad (2.16) \]
\[ 4\text{CH}_3\text{OH} \rightarrow 3\text{CH}_4 + \text{CO}_2 + 2\text{H}_2\text{O} \quad (2.17) \]
\[ 4(\text{CH}_3)_3\text{N} + 6\text{H}_2\text{O} \rightarrow 9\text{CH}_4 + 3\text{CO}_2 + 4\text{NH}_3 \quad (2.18) \]
\[ \text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2 \quad (2.19) \]

George et al., [2003] found the COD equivalent of methane through stoichiometry and showed that the theoretical total of CH\(_4\) can be formed by anaerobic situations is 0.35 L CH\(_4\) per g COD. His studies also explored the biomethanation of pretreated lignin and black liquor, which were contrasted with the theoretical COD yield [George et al., 2003].

2.6.2. Coupling biohydrogen and biomethane two-stage processes

Only approximately 10–20% of the energy potential of an organic feed is achieved by dark fermentation process [Cooney et al., 2007]. The end-product of dark fermentation includes VFA (mostly acetic and butyric acids) and other materials, which will not be converted into H\(_2\) because of thermodynamic restrictions [Hawkes et al., 2007]. There are numerous ways to employ such residues in a second stage, which include converting the by-products to H\(_2\) using photosynthetic bacteria or converting VFA to CH\(_4\) throughout an anaerobic procedure [Ren et al., 2009]. In the
second stage, acetate and butyrate obtained from soluble metabolites of the dark fermentation can be transformed into hydrogen by photosynthetic bacteria—recognized for their main leaning to transform organic acids to hydrogen in the presence of light—and through the action of the nitrogenase enzyme [Claassen et al., 2004]. The mixture of dark and photo fermentation can attain a theoretical maximum hydrogen yield of 12 mol H₂/mol hexose. This type of two-stage bioprocess has been studied by means of a lignocellulosic feed, like potato steam peel and cassava starch [Claassen et al., 2004; Su et al., 2009]. Through a mixture of dark and photo fermentation, the practical maximum hydrogen yield from cassava starch improved to 18 mmoles H₂/g starch from the original 10.7 mmoles H₂/g starch in dark fermentation only [Su et al., 2009]. Nevertheless, one of the primary disadvantages of this method is its costs since photoheterotrophic bacteria uses light as their main energy source and organic materials as the carbon source [Claassen et al., 2004].

Another encouraging path is the use of a two-stage H₂-CH₄ process, which has offered important improvements in hydrolysis and offers more energy yields compared to a one-stage methanogenic process [Hawkes et al., 2007]. In the first stage, the operational situations—the presence of acid pH and short retention time—are set to prefer the fermentation of the feed to hydrogen by developing the growth of acidogenic bacteria. In the second stage, conditions are reformed to suit methanogenesis. This includes the presence of neutral pH and more retention time. This type of method offers several benefits because the first stage efficiently solubilizes the joint hydrogen-methane mixture (20–30% H₂, 80–70% CH₄), which has been proven to burn cleaner than methane alone [Bauer and Forest, 2001; Ueno et al., 2007]. Some studies have been carried out using a two-stage H₂ and CH₄ process. For example, Pakarinen et al. [2009] studied mesophilic CH₄ formation from grass silage in a one-stage process to mix thermophilic H₂ and mesophilic CH₄ formation in a two-stage process. In addition to the hydrogen formation of 5.6 ml H₂/g VS, an 8% growth in CH₄ yields were attained from grass sillage in the two-stage process, where the one-stage process 467 ml CH₄/g VS vs. 431 ml CH₄/g VS [Pakarinen et al., 2009]. However, from the energy aspect, a growth of 7% in MJ/kg VS was detected with the two-stage process, in which only 0.4% formed from hydrogen production. This top leveled methane yield in the two-stage process was ascribed to the thermophilic H₂ formation stage, which improved the hydrolysis of
the solid substrates and caused in enlarged solubilization and increase the formation of VFA [Pakarinen et al., 2009].

A two-stage process was done for maize. Hydrogen formation of 158 ml H₂/g DM for maize was gained, and the methane production reached to 426 ml CH₄/g DM [Rechtenbach and Stegmann, 2009; Xie et al., 2007].

Lastly, this type of process was tried by Antonopoulou et al., [2006], who employed sweet sorghum (hydrolysate and solid portion). The two-stage H₂–CH₄ method was employed to the hydrolyzed portion that was bright in easily fermentable sugars, whereas the one-stage CH₄ process was used exclusively for the solid portion. The formation of 10.4 ml H₂/g DM and 29 ml CH₄/g DM were attained for the hydrolyzate and 78 ml CH₄/g DM for the solid part. A two-stage process can expand methane formation, though the upsurge in the CH₄ yield should be measured in the light of the investment needed in the higher mixed two-stage process [Pakarinen et al., 2009].

2.7. Optimization of biomethane production

2.7.1. Comparative methane yields from different energy crops

A variety of energy crops have been considered for the past 30 years with respect to their methane possibility after anaerobic digestion. Frigon et al., [2010] has completed general review of methane production from fruit and vegetables, grass, woody biomass, terrestrial weed, marine biomass, and freshwater biomass. Two large groups of energy crops obtain most of the consideration: starch crops for their high methane yield, and lignocellulosic products as the second generation of biofuel products. The first category contains the sugar and starch crops, which are quite effective at using of solar energy and can yield either fermentable sugars (sugarcane, sugarbeet), or starch (corn, potatoes). The sugar and starch products are the significant energy crops now employed on a commercial part for the formation of biomethane [Frigon et al., 2010].

While these products produce high amount of methane, they have other usage as food and/or feed as well, which may regularly compete with biofuel production. Cellulosic or lignocellulosic crops are characterized by a wide variety of grasses covering small percentage of lignin, such as hay, clover, reed canary grass, while other energy products, such as Miscanthus or switchgrass, consist of top levels of lignin (12–20%).
2.7.2. Methane yield from starch crops and potato waste
Several studies have assessed the methane potential gained from starch crops such as sugar beets, corn, and potatoes, some of which are offered here to prove the high-top biofuel yield reachable from anaerobic digestion. It must be noted that preparing these crops prior to anaerobic digestion do not need more than a size decreasing [Frigon et al., 2010].

Even though it is not itemized as such by the experimenters, the researchers consider the ensiling of crops to constitute a pretreatment in itself. Sugar and starch crops reveal the best methane yield per hectare, at 5 300–12 390, 6604 and 5400 m$^3$CH$_4$/ha for corn, triticale and sugar beets, respectively. The higher yield gained from sugar and starch crops, however, should be evaluated against the of quality land required, their impact on the cost of food and feed crops, and the more thorough care involved to these kinds of cultures (nutrients, pesticide) [Frigon et al., 2010].

<table>
<thead>
<tr>
<th>Crops</th>
<th>Operating Conditions</th>
<th>Yield (m$^3$CH$<em>4$/kg VS$</em>{added}$)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato</td>
<td>BMP</td>
<td>0.31-0.33</td>
<td>Parawira W, 2004</td>
</tr>
<tr>
<td>Potato</td>
<td>CSTR, OLR 2.5 Kg TS/L.d, HRT 20d</td>
<td>0.43</td>
<td>Stewart DJ, 2015</td>
</tr>
<tr>
<td>Corn</td>
<td>BMP</td>
<td>0.25-0.40</td>
<td>Li &amp; Chen, 2007</td>
</tr>
<tr>
<td>Corn</td>
<td>CSTR, OLR 2.5 - 4.0 Kg TS/L.d, HRT 10-20d</td>
<td>0.18-0.41</td>
<td>Badger DM, 1997</td>
</tr>
<tr>
<td>Wheat</td>
<td>BMP</td>
<td>0.14-0.34</td>
<td>Zauner E, 1986</td>
</tr>
<tr>
<td>Oats</td>
<td>BMP</td>
<td>0.254</td>
<td>Amon T, 2007</td>
</tr>
<tr>
<td>Sugarbeets</td>
<td>BMP</td>
<td>0.25-0.45</td>
<td>Badger DM</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>BMP</td>
<td>0.23-0.30</td>
<td>Xu Q, 2002</td>
</tr>
<tr>
<td>Rye</td>
<td>BMP</td>
<td>0.14-0.36</td>
<td>Petersson A, 2007</td>
</tr>
</tbody>
</table>
3.1. Introduction
Hydrogen production from natural substrates is quickly developing as a substitute to fossil fuels, as it has three times the energy content of hydrocarbon fuels [Rifkin, 2002], and its only byproduct is water, which has no CO, CO₂, hydrocarbons, or other particles when burnt [Liu, 2008]. Hydrogen can be formed with numerous methods: electrolysis, photolysis, bio-photolysis, photo-fermentation, or dark fermentation. Fermentative technology is well recognized, and the co-products, such as organic acids, in this technology are valuable [Liu, 2008].

Dark fermentation is the most commonly used method for biological hydrogen production, particularly when integrated with waste treatment [Mizuno et al., 2000]. Potato waste is a strong candidate for biological hydrogen production. It is characterized by high COD of up to 120 g/L, VS of 77 g/L, VSS of 54 g/L, and VFAs of 5 g/L (Table 3.3).

$S_o/X_o$ is an important parameter that affects hydrogen production with hydrogen yield growing linearly at $S_o/X_o$ of 4 to 6.6 g COD/g VSS.d [Hafez et al., 2010a]. For organic wastes, the term of $S_o/X_o$ is complicated as the VSS impacts both the food and microorganisms’ controls. Furthermore, as depicted in Table 3.1, there are only a handful of studies on biohydrogen production from particulate wastes [Pan et al., 2008; Chen et al., 2006a; Yu et al., 2002; Lay et al., 2010].

There is a proof that $S_o/X_o$ directly impacts the formation of microorganisms [Speece et al., 1973]. The effect of microbial cultures on biohydrogen production from soluble substrates is detailed in the literature listed in Table 3.1.

As shown in Table 3.1, the extensive work by Pan et al., [2008] demonstrates that when they increased the rate of $S_o/X_o$ from 1 to 6 g VS_{substrate}/ g VS_{seed}, hydrogen production potential grew, though the hydrogen production declines after a $S_o/X_o$ of 7.

Table 3. 1. Hydrogen production potentials and yields in batch experiments
For instance, biohydrogen production from glucose ranges from 1.08 mol H\(_2\)/mol glucose [Oh et al., 2003] to 3.09 mol H\(_2\)/mol glucose [Wang and Wan, 2008]. The hydrogen yields from glucose consuming *Clostridium* species ranges from 1.17 mol H\(_2\)/mol glucose [Oh et al., 2003] to 2.8 mol H\(_2\)/mol glucose [Taguchi et al., 2000].

Most studies on biohydrogen production have been done in batches due to concerns of enduring constancy of continuous-flow systems. These systems were linked with contamination because of the methanogens in the feeds. In such cases, batch experiments are prejudiced because they are proceeded on pre-treated seed biomass, as opposed to the enhanced or acclimatized cultures in sustained continuous-flow systems. Pretreatment of anaerobic digester sludge is required primarily to restrain the hydrogen consuming microorganisms and enhance the hydrogen producing bacteria. This may be achieved by a variety of approaches, such as heat, acid, base, aeration, or ultrasonication pretreatment [Elbeshbishy et al., 2010]. Acclimatization of anaerobic digester sludge is the greatest typical microbial culture for valuation of biohydrogen production potential from numerous substrates. The hydrogen producers increase in a hydrogen bioreactor, where methanogens are exhausted and hydrogen producers represent the main active community in the
sludge in continuous-flow systems [Hafez et al., 2010a; Ozkan et al., 2010]. Literature search shows that no previous work has been conducted on hydrogen production in batch experiments using acclimatized anaerobic digester sludge from a continuous-flow biohydrogen system.

The primary objectives of this study are: to assess the feasibility of biohydrogen production from potato waste, and complete a comparative evaluation of $S_o/X_o$ and its maximum hydrogen production potential.

3.2. Materials and methods

3.2.1. Seed sludge
ADS was collected from the secondary anaerobic digester at Stradford’s wastewater treatment plants (Ontario, Canada). The TSS and VSS concentrations of the ADS were 28.9 and 16 g/L, respectively. Heat pretreatment for the ADS was conducted by heating the sludge at 70°C for 30 minutes [Hafez et al., 2010 a]. Table 3.2 lists the various characteristics of the secondary sludge measured in triplicates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Waste water (AV. ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>40 ± 0.5</td>
</tr>
<tr>
<td>VS</td>
<td>16.8 ± 0.6</td>
</tr>
<tr>
<td>TSS</td>
<td>28.9 ± 1.2</td>
</tr>
<tr>
<td>VSS</td>
<td>16 ± 0.5</td>
</tr>
<tr>
<td>TCOD</td>
<td>28 ± 0.5</td>
</tr>
<tr>
<td>SCOD (mg/L)</td>
<td>560 ± 5</td>
</tr>
<tr>
<td>TVFA’s (mg/L)</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>Total Carbohydrates (mg/L)</td>
<td>5500 ± 5</td>
</tr>
<tr>
<td>Soluble Carbohydrates (mg/L)</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>pH</td>
<td>8.3</td>
</tr>
<tr>
<td>Alkalinity (mg/L) as NaHCO₃</td>
<td>7500</td>
</tr>
</tbody>
</table>

3.2.2. Potato waste (substrate)
Uncooked potato waste was collected from a food process company and used as the substrate to evaluate the hydrogen production rates. Table 3.3 lists the various characteristics of the potato waste measured in triplicates.
Table 3.3. Potato waste characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Potato Waste (AV± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS</td>
<td>90 ± 1.8</td>
</tr>
<tr>
<td>VS</td>
<td>77 ± 2.1</td>
</tr>
<tr>
<td>TSS</td>
<td>85 ± 2.4</td>
</tr>
<tr>
<td>VSS</td>
<td>54 ±2.5</td>
</tr>
<tr>
<td>TCOD</td>
<td>120 ± 2</td>
</tr>
<tr>
<td>SCOD</td>
<td>68 ± 1.4</td>
</tr>
<tr>
<td>TVFA’s</td>
<td>5 ± 0.2</td>
</tr>
<tr>
<td>Total Carbohydrates</td>
<td>60 ± 1.9</td>
</tr>
<tr>
<td>Soluble Carbohydrates</td>
<td>31 ± 0.9</td>
</tr>
<tr>
<td>pH</td>
<td>12.3</td>
</tr>
<tr>
<td>Alkalinity (mg/L) as NaHCO₃</td>
<td>27000</td>
</tr>
</tbody>
</table>

3.2.3. Batch experiments

Batch anaerobic studies were conducted in bottles with a liquid volume of 420 mL and head space volume of 80 mL. Experiments were done in triplicates for \( S_0/X_0 \) of 0.5 and 1 g COD\(_\text{substrate} \)/g VSS\(_\text{seed} \). Volumes of potato waste and sludge used in batches were calculated by using the following equation:

\[
S_0/X_0 = \frac{V_p(L) * \text{Potato waste TCOD (g/L)}}{V_s(L) * \text{Sludge VSS (g/L)}}
\]

Where \( V_p \) is the volume of potato waste and \( V_s \) is the volume of sludge, and Table 3.4 shows the volumes used in bottles for each \( S_0/X_0 \). A 2 g/L buffer solution (NaHCO\(_3\)) was also added for pH control. The initial pH value for the mixed solution in each bottle was adjusted using HCl and measured to be 5.47±0.2 for all runs.
Table 3.4. Batches design for biohydrogen production

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>Substrate</th>
<th>$S_0/X_0$</th>
<th>Sludge Volume (mL)</th>
<th>Substrate Volume (mL)</th>
<th>Glucose (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Deionized water</td>
<td>0.5</td>
<td>370</td>
<td>50</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Deionized water</td>
<td>0.5</td>
<td>370</td>
<td>50</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>&amp; glucose</td>
<td>1</td>
<td>330</td>
<td>90</td>
<td>5.1</td>
</tr>
<tr>
<td>Potato waste</td>
<td>Potato waste</td>
<td>0.5</td>
<td>370</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>sample</td>
<td></td>
<td>1</td>
<td>330</td>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

Twenty milliliter samples of the mixtures were collected initially. The head space was flushed with oxygen-free nitrogen gas for a period of 30 seconds and capped tightly with rubber stoppers. The bottles were then placed in their own place in AMPTS II operating at 180 rpm and maintained at a temperature of 37°C. Six blank bottles were prepared using ADS without potato waste, and six control bottles were prepared using ADS and Glucose. Final samples were taken at the end of the batch experiment, and the final pH for the mixed solution in each bottle were measured to be 5.05±0.2 for runs.

The AMPTS II measures ultra-low biogas flows produced from the anaerobic digestion of any biological degradable substrate at laboratory scale.

3.2.4. Analytical methods

The biogas production was measured by AMPTS II. The AMPTS II can report gas production in cumulative gas in ml and rate of producing gas. TVFAs, as well as TCOD and SCOD were measured using HACH methods [Hafez et al., 2010b]. In addition, TSS and VSS concentrations were measured using standard methods [APHA, 1995], while soluble parameters were analyzed after filtering the samples by 0.45 μm filter paper. Table 3.5 lists the sample characteristics in initial and final steps. All were done in triplicates.
Table 3. 5. Samples characteristics for biohydrogen batches

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>$S_o/X_o$ gCOD/gVSS</th>
<th>Initial (mg/L)</th>
<th>Final (mg/L)</th>
<th>Cumulative $H_2$ (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
<td>PH</td>
<td>TCOD</td>
<td>SCOD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5</td>
<td>19030</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5</td>
<td>19050</td>
<td>189</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5</td>
<td>19043</td>
<td>186</td>
</tr>
<tr>
<td>Control 0.5</td>
<td></td>
<td>5.5</td>
<td>31905</td>
<td>8665</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5</td>
<td>32011</td>
<td>8546</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5</td>
<td>34625</td>
<td>12930</td>
</tr>
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<td></td>
<td></td>
<td>5.5</td>
<td>34425</td>
<td>13100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5</td>
<td>34532</td>
<td>12980</td>
</tr>
<tr>
<td>Potato waste 0.5</td>
<td></td>
<td>5.5</td>
<td>33825</td>
<td>5265</td>
</tr>
<tr>
<td>Sample 1</td>
<td></td>
<td>5.5</td>
<td>33805</td>
<td>4965</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>37400</td>
<td>8799</td>
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<td></td>
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<td>5.5</td>
<td>37175</td>
<td>8990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.5</td>
<td>37600</td>
<td>8802</td>
</tr>
</tbody>
</table>

34
3.3. Results and discussion

3.3.1. Hydrogen production
Figures 3.1 and 3.2 show the cumulative hydrogen production at two \( S_o/X_o \). In batches as the \( S_o/X_o \) increased from 0.5 to 1 g COD/g VSS, hydrogen production increased from 500 mL at \( S_o/X_o \) of 0.5 g COD/g VSS to a maximum of 540 mL at \( S_o/X_o \) of 1 g COD/g VSS.

Figure 3. 1. \( \text{H}_2 \) cumulative for batches with \( S_o/X_o = 0.5 \) g COD/g VSS

Figure 3. 2. \( \text{H}_2 \) cumulative for batches with \( S_o/X_o = 1 \) g COD/g VSS
3.3.2. Hydrogen Yields

Table 3.6 shows the hydrogen yield based on the total carbohydrates converted in batch studies. A low hydrogen yield of 94 mL H$_2$/g of T-carb was obtained with an $S_o/X_o$ of 0.5 g COD/g VSS. This may be due to an insufficient amount of feed. After this hydrogen yield reached an average of 143 mL H$_2$/g T-carb converted with an $S_o/X_o$ of 1 g COD/g VSS. It is likely that the maximum $S_o/X_o$ range is 1 g COD/g VSS. Potato waste contains a significant amount of carbohydrates (Table 3.3), which are the most preferable substrate for producing H$_2$. It is clear that in absence of sludge acclimatization, the low percentage of hydrogen producers in the ADS, the carbohydrates in potato waste was partially converted to hydrogen. The maximum conversion efficiency of 70% removal with an $S_o/X_o$ of 0.5 g COD/g VSS was achieved.

Table 3.6. Carbohydrate balance in potato waste batches

<table>
<thead>
<tr>
<th>$S_o/X_o$ gCOD/gVSS</th>
<th>T-Carb$_{in}$ mg/L</th>
<th>T-Carb$_{out}$ mg/L</th>
<th>ΔCarb mg/L</th>
<th>Carb Removal %</th>
<th>mL H$_2$/gT-Carb.converted</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6584</td>
<td>1955</td>
<td>4629</td>
<td>70</td>
<td>94</td>
</tr>
<tr>
<td>1</td>
<td>10214</td>
<td>6632</td>
<td>3582</td>
<td>35</td>
<td>143</td>
</tr>
</tbody>
</table>

3.3.3. Gompertz model

Table 3.7 shows the kinetic data from the Gompertz model [Lay et al., 1999]. The coefficient of determination, $R^2$, was 0.999 for all Gompertz data. It is obvious that the lag phase in the $S_o/X_o$ of 1 g COD/g VSS batches with an average of 1 hour is much lower than that in the $S_o/X_o$ of 0.5 g COD/g VSS batches, where the average was 1.8 hours. This also can be related to the increase in the percentage of hydrogen producers in an $S_o/X_o$ of 1 g COD/g VSS relative to an $S_o/X_o$ of 0.5 g COD/g VSS. The maximum hydrogen production rate in batches using an $S_o/X_o$ of 0.5 g COD/g VSS was 62.8 mL/hr, which is 1.5 times of the 40.3 mL/hr in batches using an $S_o/X_o$ of 1 g COD/g VSS.
Table 3.7. Gompertz data for batches

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>$S_0/X_0$ (g COD/g VSS)</th>
<th>P (mL)</th>
<th>$R_m$ (mL/hr)</th>
<th>$\lambda$ (hr)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
<td>42.6</td>
<td>0.8</td>
<td>5.8</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42.2</td>
<td>0.8</td>
<td>5.8</td>
<td>0.999</td>
</tr>
<tr>
<td>Control</td>
<td>0.5</td>
<td>500.4</td>
<td>45.4</td>
<td>3.2</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>512.2</td>
<td>46.3</td>
<td>3.2</td>
<td>0.999</td>
</tr>
<tr>
<td>Potato waste sample</td>
<td>0.5</td>
<td>489.2</td>
<td>62.8</td>
<td>1.8</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>481.9</td>
<td>40.3</td>
<td>1.0</td>
<td>0.999</td>
</tr>
</tbody>
</table>

P: Ultimate hydrogen production, $R_m$: Rate of hydrogen production, $\lambda$: Lag phase duration, $R^2$: Coefficient of determination
Table 3. 8. Final results for the biohydrogen batches

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>$S_i/X_o$</th>
<th>Net ΔCOD</th>
<th>Net H₂</th>
<th>COD balance (%)</th>
<th>Actual yield</th>
<th>Theoretical yield</th>
<th>Actual yield</th>
<th>LH₂/g COD&lt;sub&gt;added&lt;/sub&gt;</th>
<th>LH₂/g COD&lt;sub&gt;consumed&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/L</td>
<td>mg</td>
<td>mL</td>
<td>mg COD</td>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blank</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>140</td>
<td>56</td>
<td>28</td>
<td>18</td>
<td>100%</td>
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<tr>
<td>Blank</td>
<td>141</td>
<td>55</td>
<td>27</td>
<td>17</td>
<td>99%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>142</td>
<td>57</td>
<td>29</td>
<td>19</td>
<td>97%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
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<td>54</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>137</td>
<td>55</td>
<td>29</td>
<td>19</td>
<td>96%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>136</td>
<td>53</td>
<td>27</td>
<td>17</td>
<td>97%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2780</td>
<td>1112</td>
<td>515</td>
<td>324</td>
<td>93%</td>
<td>0.17</td>
<td>0.20</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Control 0.5</td>
<td>2655</td>
<td>1062</td>
<td>472</td>
<td>297</td>
<td>94%</td>
<td>0.16</td>
<td>0.19</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Control 0.5</td>
<td>2723</td>
<td>1098</td>
<td>505</td>
<td>311</td>
<td>95%</td>
<td>0.15</td>
<td>0.18</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>2710</td>
<td>1084</td>
<td>563</td>
<td>354</td>
<td>94%</td>
<td>0.09</td>
<td>0.11</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>2700</td>
<td>1080</td>
<td>554</td>
<td>348</td>
<td>94%</td>
<td>0.10</td>
<td>0.10</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>2698</td>
<td>1083</td>
<td>560</td>
<td>351</td>
<td>95%</td>
<td>0.10</td>
<td>0.12</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td><strong>Potato Waste Sample</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato Waste Sample</td>
<td>2660</td>
<td>1064</td>
<td>467</td>
<td>294</td>
<td>94%</td>
<td>0.16</td>
<td>0.19</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Potato Waste Sample 0.5</td>
<td>2405</td>
<td>962</td>
<td>473</td>
<td>297</td>
<td>95%</td>
<td>0.16</td>
<td>0.17</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Potato Waste Sample 0.5</td>
<td>2365</td>
<td>946</td>
<td>362</td>
<td>228</td>
<td>94%</td>
<td>0.12</td>
<td>0.17</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Potato Waste Sample 1</td>
<td>2475</td>
<td>990</td>
<td>509</td>
<td>320</td>
<td>95%</td>
<td>0.09</td>
<td>0.10</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Potato Waste Sample 1</td>
<td>3050</td>
<td>1220</td>
<td>513</td>
<td>323</td>
<td>94%</td>
<td>0.10</td>
<td>0.12</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Potato Waste Sample 1</td>
<td>2555</td>
<td>1022</td>
<td>517</td>
<td>325</td>
<td>95%</td>
<td>0.10</td>
<td>0.10</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>
3.3.4. COD balance
The COD mass balance data is depicted in Table 3.8. The COD balance at 96±2% shows conforming data. The average COD removal was 95±1% for both of ratios. As shown in Table 3.8, there is no significant differences in COD reduction between the two ratios.

3.4. Conclusions
This investigation shows the importance of $S_0/X_o$ in hydrogen production studies in batch experiments.

Potato waste has potential for hydrogen production with a maximum yield of 0.51 $\text{LH}_2/\text{g COD}_{\text{consumed}}$ with ADS. In comparing an $S_0/X_o$ of 0.5 and 1 $\text{g COD}_{\text{substrate}}/\text{g VSS}_{\text{seed}}$, the optimum experimental range of $S_0/X_o$ for hydrogen production is 0.5 $\text{g COD}_{\text{substrate}}/\text{g VSS}_{\text{seed}}$. 
CHAPTER 4

COMPARATIVE ASSESSMENT OF SINGLE-STAGE AND TWO-STAGE ANAEROBIC DIGESTION FOR THE TREATMENT OF POTATO WASTE

4.1. Introduction

Potato waste is characterized by a high TCOD of up to 120 g/L, 77 g/L of VS, and 60 g/L of total carbohydrates. Therefore, it is an effective substrate candidate for anaerobic digestion.

In a single-stage anaerobic digestion, Parawira et al., [2004] observed promising results from the mesophilic digestion of potato waste (95% VS; 19%TS). For example, a batch system with a methane yield of 0.32 m$^3$CH$_4$/kg TCOD$_{removed}$ can provide up to 60% of the daily energy requirement of a bioethanol plant. One pilot scale UASB reactor achieved 76% TCOD removal with 0.33 L CH$_4$/g VS$_{degraded}$. It was also tested in a CSTR where the methane yield was 50-58% of biogas [Linke, 2006]. In the anaerobic digestion process, the separation between the acidogenic and methanogenic phase provides superior stability to the overall process; this separation also provides an opportunity for better process control [Demirel and Yenigun, 2002]. The goal of a two-stage anaerobic digestion system is not only to stabilize/degrade extra waste, but also to obtain more energy from the system [Thompson, 2008]. In a two-stage anaerobic digestion method, the final product of the acidification phase applies thin stillage that is suitable for anaerobic treatment. In this process, the TVFAs could reach 29.5 g COD/L [Pavan et al., 2000; Nasr et al., 2011].

Vinas et al., [1993] succeeded in getting a methane yield of 0.31 L/g COD in a two-stage method with a 13% growth over the single-stage method applying a cellulosic material as the feed. In addition, the difficulty and extra expense of construction and operating commercial two-stage systems did not help the rate of its development [Rapport et al., 2008]. The theoretical higher biogas yields have also been questioned since the acidogenic stage separation avoids the hydrogen to methane pathway [Reith et al., 2003].

The primary aims of this study are to relate and assess the methane yield from potato waste in single-stage and two-stage anaerobic digestion processes; to investigate the impact of the acidogenic stage in hydrogen production and the methane production in batch studies under
mesophilic condition; and to determine if there is a significant difference in energy yields between single-stage and two-stage anaerobic digestion process.

4.2. Materials and methods

4.2.1. Seed sludge
Secondary digested sludge (ADS) was collected from the secondary digester at Stratford’s wastewater treatment plant (Ontario, Canada) and was applied as seed sludge for the single-stage anaerobic digestion and the second stage of the two-stage anaerobic digestion for methane production. The TSS and VSS were 28.9 and 16 g/L, respectively (Table 3.2).

4.2.2. Feed (substrate)
Potato waste collected from a food processing company was used as the substrate to evaluate its hydrogen and methane production potentials. For the single-stage methane production and the first stage hydrogen production, potato waste was used as the substrate with TCOD, TVFAs, TSS, and VSS of 120, 5, 85, and 54 g/L, respectively (Table 3.3).

Hydrogen batch tests were done at an $S_o/X_o$ of 0.5 and 1 g COD/g VSS based on the TCOD of the potato waste and seed sludge VSS concentration. After the hydrogen production stage, the bottles of the two different $S_o/X_o$ were centrifuged for 20 minutes at 4000 rpm, and the supernatant was then used as substrate for the second stage methane production. The TCOD of the supernatants from an $S_o/X_o$ of 0.5 and 1 g COD/g VSS were 7.2 and 8.3 g/L, respectively.

4.2.3. Batch experiments
Hydrogen and methane batch anaerobic experiments were conducted in AMPTS bottles with a liquid volume of 420 mL and head space volume of 80 mL. Table 4.1 and 4.2 display the volumes of substrates and seeds used in bottles and primary pH for each stage. For hydrogen production as a first stage, the experiments were conducted in triplicates for an $S_o/X_o$ of 0.5 and 1 g TCOD_{substrate}/g VSS_{seed} using ADS as the seed and potato waste as the substrate. For production of methane, the tests were done in triplicates for an initial $S_o/X_o$ of 0.5 and 1 g COD/g VSS using ADS as the seed and the supernatant from the hydrogen production stage as the
substrate. The volumes of potato waste and supernatant as substrates used in batches were calculated using the following Equation:

\[
\frac{S_0}{X_0} = \frac{V_{\text{substrate}}(L) \times \text{substrate TCOD} \left(\frac{g}{L}\right)}{V_{\text{sludge}}(L) \times \text{Sludge VSS} \left(\frac{g}{L}\right)}
\]  

(4.1)

where \(V_{\text{substrate}}\) is the volume of substrate and \(V_{\text{sludge}}\) is the volume of sludge.

A buffer (NaHCO\textsubscript{3}) with concentrations of 2 g/L was added for pH control in both hydrogen and methane batches. The initial pH for the mixed solution in each bottle was subsequently adjusted using HCl or NaOH and measured to be 7.17±0.1 for methane batches.

Table 4.1. Batch design for two-stage biomethane production

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>Substrate</th>
<th>(S_0/X_0)</th>
<th>Sludge Volume (mL)</th>
<th>Substrate Volume (mL)</th>
<th>Acetic acid (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Deionized water</td>
<td></td>
<td>240</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Deionized water</td>
<td></td>
<td>148</td>
<td>268.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Two-Stage Mixed</td>
<td></td>
<td>0.5</td>
<td>303</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>247</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>Centrifuged</td>
<td></td>
<td>0.5</td>
<td>180</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>148</td>
<td>272</td>
<td></td>
</tr>
</tbody>
</table>

In the first step, 20 mL of the mixtures samples were collected. The head space was then flushed with oxygen-free nitrogen gas for a period of 30 seconds and capped tightly with rubber stoppers. The bottles were then placed in an AMPTS II; this operated at 180 rpm and a temperature of 37°C. Blank bottles of seed material and deionized water, without substrate, were prepared using ADS for methane production runs.
Table 4.2. Batch design for single-stage biomethane production

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>Substrate</th>
<th>(S_o/X_o) g COD/g VSS</th>
<th>Sludge Volume (mL)</th>
<th>Substrate Volume (mL)</th>
<th>Acetic acid (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Deionized water</td>
<td>240</td>
<td>180</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Deionized water</td>
<td>1</td>
<td>148</td>
<td>268.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Single-Stage</td>
<td>Potato Waste</td>
<td>0.5</td>
<td>350</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>(Potato waste)</td>
<td></td>
<td>1</td>
<td>300</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

The AMPTS II measures ultra-low biogas flows produced from the anaerobic digestion of any biological degradable substrate at laboratory scale.

4.2.4. Analytical methods

The biogas production was automatically measured by AMPTS II. The TVFAs, TCOD, and SCOD were measured using HACH methods, while the TSS and VSS of the seed were analyzed using standard methods [APHA, 1995]. Soluble parameters were determined after filtering the samples through 0.45 µm filter paper.

4.3. Results and Discussion

4.3.1. Biogas production

The first stage—the acidogenic stage—was carried out with two different substrate to microorganisms ratios: \(S_o/X_o\) of 0.5, and \(S_o/X_o\) of 1 g COD/g VSS. Figure 4.1 shows the hydrogen production rates achieved with ultimate hydrogen production potentials of 501 and 540 mL, respectively. It can be inferred from the Figure that as the \(S_o/X_o\) increased from 0.5 to 1 g COD/g VSS, the hydrogen production rate decreased from 44 mL/hr to 38 mL/hr. This trend did not continue at the same rate and during the following 10 hours the rate of hydrogen production for an \(S_o/X_o\) of 1 was greater than that with ratio of 0.5. It is noted that while methane gas is being produced in the single-stage anaerobic digestion process, there was no hydrogen gas detected. The final pH levels for the mixed solution in each bottle were measured and found to be 7.56±0.01 for methane runs and 5.05±0.15 for the hydrogen runs.
4.3.2. Hydrogen and methane yields

Tables 4.5 and 4.6 show the summary for initial and final data from the batch studies for both single-stage and two-stage anaerobic digestion experiments. Figures 4.2 and 4.3 show the methane yield during the single-stage and two-stage anaerobic digestion. In the two-stage anaerobic digestion, the methane yields based on COD$_{added}$ were 175 mLCH$_4$/gCOD$_{added}$ for the methanogenic batches for centrifuged substrate in an S$_o$/X$_o$ of 0.5 g COD/g VSS, and was 153 mLCH$_4$/gCOD$_{added}$ for the batches of centrifuged substrate in an S$_o$/X$_o$ of 1 g COD/g VSS. The methane yield for mixed substrate in an S$_o$/X$_o$ of 0.5 g COD/g VSS was 140 mLCH$_4$/gCOD$_{added}$ and mixed substrate in an S$_o$/X$_o$ of 1 g COD/g VSS was 113 mLCH$_4$/gCOD$_{added}$. Alternately, a methane yield of only 101 and 71 mL CH$_4$/g COD was achieved in the single-stage experiment with an S$_o$/X$_o$ of 1 and 0.5 g COD/g VSS, respectively. The maximum methane yield of 175 mL/g COD, observed in the two-stage batch, was 75% higher than the yield achieved in the single-stage experiment.
Table 4.3. Samples characteristics for the two-stage batches

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>Substrate</th>
<th>$S_o/X_o$ g COD/g VSS</th>
<th>Initial (mg/L)</th>
<th>Final (mg/L)</th>
<th>Cumulative $\text{CH}_4$ (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PH TCOD SCOD TCARB SCARB</td>
<td>PH TCOD SCOD TCARB SCARB</td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td>Deionized water</td>
<td>7.2 6680 320 3 0.15</td>
<td>7.5 5770 200 2 0.08</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 6673 322 2.9 0.15</td>
<td>7.4 5768 198 2.5 0.08</td>
<td>138</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 6620 325 3.1 0.15</td>
<td>7.5 5728 200 2.8 0.08</td>
<td>137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Deionized water &amp; Acetic acid</td>
<td>7.2 10450 7611 2.4 0.2</td>
<td>8.3 7100 320 1.2 0.16</td>
<td>1101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 10501 7610 2.5 0.2</td>
<td>8.2 6971 310 1.1 0.15</td>
<td>1100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 10500 7608 2.6 0.2</td>
<td>8.3 7052 305 1.3 0.16</td>
<td>1094</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>0.5</td>
<td>7.2 12450 2298 1.2 0.05</td>
<td>7.4 8222 410 1.1 0.04</td>
<td>711</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 12510 2311 1.3 0.04</td>
<td>7.5 8022 408 1.0 0.05</td>
<td>743</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 12404 2300 1.1 0.05</td>
<td>7.5 8504 390 1.2 0.03</td>
<td>657</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 12886 5557 1.5 0.09</td>
<td>7.5 8066 685 1.1 0.04</td>
<td>805</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7.2 12913 5501 1.6 0.09</td>
<td>7.5 8033 694 1.3 0.04</td>
<td>814</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 12789 5600 1.7 0.09</td>
<td>7.5 8109 711 1.2 0.04</td>
<td>799</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two- stage</td>
<td>0.5</td>
<td>7.2 7111 5080 1.1 0.09</td>
<td>7.5 4176 670 0.6 0.03</td>
<td>487</td>
<td></td>
</tr>
<tr>
<td>Centrifuged</td>
<td>7.2 7234 5007 1.2 0.09</td>
<td>7.5 4199 664 0.7 0.03</td>
<td>482</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 7308 5050 1.0 0.09</td>
<td>7.5 4294 655 0.5 0.03</td>
<td>476</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 8344 7885 0.8 0.1</td>
<td>7.5 4432 895 0.3 0.03</td>
<td>635</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 8198 7914 0.7 0.1</td>
<td>7.5 4316 911 0.4 0.03</td>
<td>631</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.2 8512 7812 0.9 0.1</td>
<td>7.5 4474 879 0.4 0.03</td>
<td>625</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.4. Samples characteristics for the single-stage batches

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>$S_0/X_0$</th>
<th>Initial (mg/L)</th>
<th>Final (mg/L)</th>
<th>Cumulative CH$_4$(mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g COD/g VSS</td>
<td>PH</td>
<td>TCOD</td>
<td>SCOD</td>
</tr>
<tr>
<td>Blank</td>
<td>7.2</td>
<td>6880</td>
<td>320</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>6673</td>
<td>322</td>
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<td>6620</td>
<td>325</td>
<td>3.1</td>
</tr>
<tr>
<td>Control</td>
<td>7.2</td>
<td>10450</td>
<td>7611</td>
<td>2.4</td>
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<tr>
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<td>10501</td>
<td>7610</td>
<td>2.5</td>
</tr>
<tr>
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<td>7.2</td>
<td>10500</td>
<td>7608</td>
<td>2.6</td>
</tr>
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<td>Single-stage</td>
<td>7.2</td>
<td>13489</td>
<td>6104</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>13901</td>
<td>6211</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>13800</td>
<td>6301</td>
<td>3.2</td>
</tr>
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<td>0.5</td>
<td>7.2</td>
<td>15399</td>
<td>10502</td>
<td>2.8</td>
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<td>7.2</td>
<td>15490</td>
<td>10311</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>15498</td>
<td>10030</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Figure 4.2 and 4.3 show the maximum methane production rates for the single-stage and two-stage anaerobic digestion processes. The methane production rate in the two-stage anaerobic digestion was higher than that in the single-stage process.

The maximum methane production rate belongs to two-stage runs for two kinds of feeds. For the centrifuged substrate in an $S_0/X_0$ of 0.5 is 80 mL CH$_4$ yield/day and in an $S_0/X_0$ of 1 is 66 mL CH$_4$ yield/day. During the runs with mixed substrate for $S_0/X_0$ of 0.5 and 1, the yield for methane production is 59 and 60 mL CH$_4$ /day, respectively. The results for single-stage method were 40 and 49 mL CH$_4$ /day for $S_0/X_0$ of 0.5 and 1, respectively.
Figure 4.2. CH$_4$ cumulative for single-stage and two-stage batches with S$_o$/X$_o$ = 0.5 g COD/g VSS

Figure 4.3. CH$_4$ cumulative for single-stage and two-stage batches with S$_o$/X$_o$ = 1 g COD/g VSS
In the single-stage anaerobic digestion, the methane yield based on the potato waste COD\textsubscript{consumed} was 0.31 m\textsuperscript{3}CH\textsubscript{4}/kg TCOD\textsubscript{removed} with S\textsubscript{o}/X\textsubscript{o} of 0.5, and also 0.29 m\textsuperscript{3}CH\textsubscript{4}/kg TCOD\textsubscript{removed} with S\textsubscript{o}/X\textsubscript{o} of 1 g COD/g VSS. In the two-stage anaerobic digestion process, the methane yield based on COD\textsubscript{consumed} was 0.39 m\textsuperscript{3}CH\textsubscript{4}/kg TCOD\textsubscript{removed} with centrifuged substrate, when the S\textsubscript{o}/X\textsubscript{o} was 0.5 and the methane yield was 0.36 m\textsuperscript{3}CH\textsubscript{4}/kg TCOD\textsubscript{removed}, with centrifuged substrate, for S\textsubscript{o}/X\textsubscript{o} of 1 g COD/g VSS.
Table 4.5. Final results for the single-stage batches

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>$S_o/X_o$ g COD/g VSS</th>
<th>Net ΔCOD mg/L</th>
<th>Net CH₄ mg</th>
<th>COD balance (%)</th>
<th>Actual yield LCH₄/g CODₜₐₐᵈ</th>
<th>Theoretical m³CH₄/kg TCODₜₐₐᵈRemoved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
<td>904</td>
<td>362</td>
<td>139</td>
<td>350</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>910</td>
<td>364</td>
<td>140</td>
<td>353</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>892</td>
<td>357</td>
<td>137</td>
<td>345</td>
<td>97%</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>6615</td>
<td>2640</td>
<td>1010</td>
<td>2523</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6618</td>
<td>2647</td>
<td>1014</td>
<td>2555</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6630</td>
<td>2652</td>
<td>1008</td>
<td>2538</td>
<td>96%</td>
</tr>
<tr>
<td>single -stage 0.5</td>
<td></td>
<td>2515</td>
<td>1006</td>
<td>295</td>
<td>743</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2462</td>
<td>985</td>
<td>289</td>
<td>728</td>
<td>74%</td>
</tr>
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<td>1084</td>
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<td>796</td>
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<tr>
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<td>1854</td>
<td>97%</td>
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<td></td>
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<td>6010</td>
<td>2404</td>
<td>713</td>
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</tr>
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<td></td>
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<td>5785</td>
<td>2314</td>
<td>728</td>
<td>1834</td>
<td>94%</td>
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</table>
Table 4.6. Final results for the two-stage batches

<table>
<thead>
<tr>
<th>Batch Name</th>
<th>Substrate</th>
<th>So/Xo g COD/g VSS</th>
<th>ΔCOD mg/L</th>
<th>ΔCH₄ mg</th>
<th>COD balance %</th>
<th>Actual yield</th>
<th>Theoretical yield</th>
<th>m³CH₄/kg TCOD&lt;sub&gt;removed&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Deionized water</td>
<td></td>
<td>904</td>
<td>362</td>
<td>350</td>
<td>97%</td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>910</td>
<td>364</td>
<td>353</td>
<td>97%</td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>892</td>
<td>357</td>
<td>345</td>
<td>97%</td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>Control</td>
<td>Deionized water &amp; Acetic acid</td>
<td></td>
<td>6615</td>
<td>2640</td>
<td>2523</td>
<td>96%</td>
<td>0.264</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6618</td>
<td>2647</td>
<td>2555</td>
<td>97%</td>
<td>0.254</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6630</td>
<td>2652</td>
<td>2538</td>
<td>96%</td>
<td>0.288</td>
<td>0.30</td>
</tr>
<tr>
<td>Mixed</td>
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<td>1504</td>
<td>1399</td>
<td>92%</td>
<td>0.147</td>
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<tr>
<td></td>
<td></td>
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<td>0.156</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3375</td>
<td>1350</td>
<td>1210</td>
<td>93%</td>
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<tr>
<td></td>
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<td>2448</td>
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<td>0.177</td>
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<td>Centrifuged</td>
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<td>93%</td>
<td>0.153</td>
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<td>3665</td>
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<td>0.154</td>
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<td>3743</td>
<td>1497</td>
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<td>91%</td>
<td>0.152</td>
<td>0.17</td>
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</tbody>
</table>

50
With mixed substrate, the methane yield was 0.34 m³CH₄/kg TCODremoved when S₀/X₀ was 1. With mixed substrate, for S₀/X₀ of 0.5, the methane yield was 0.34 m³CH₄/kg TCODremoved. It is clear that both mixed and centrifuged substrate have higher yield than single-stage anaerobic digestion process for the same S₀/X₀.

### 4.4. Conclusions

The benefits of two-stage over single-stage anaerobic digestion from this study included higher biomethane production rate and efficiencies, increased net energy production, and total overall enhancement of the process. The positive effect of separating the acidogenic and methanogenic stages of anaerobic digestion was demonstrated through improved performance of the second-stage BMP process. In addition, the feedstock COD removal efficiency was boosted in the second-stage BMP process after acidification when compared to the single-stage BMP process.

The optimum experimental range of S₀/X₀ for methane production was 0.5 g CODsubstrate/g VSSseed when using ADS as a seed and supernatant as a feed. However, with using mixed substrate, no significant difference was observed between the two levels of S₀/X₀.

Potato waste has the potential to improve methane production with a yield of 0.39 m³CH₄/kg TCODremoved when using supernatant as a feed, especially given that tests with mixed feed only revealed a maximum potential of 0.35 m³CH₄/kg TCODremoved.

The current study found that the use of two-stage digestion for potato waste led to an increase in the TVFAs-to-TCOD ratio due to the acidification process during hydrogen production in the first stage. The methane yield in the anaerobic digestion stage increased from 0.29 m³CH₄/kg TCODremoved in the single-stage process to 0.39 m³CH₄/kg TCODremoved in the two-stage process.
5.1. Conclusions
The following findings summarize the main outcomes of this research according to the major objectives as follows:

• **Biohydrogen production:**
  1. In this study, Potato waste shows the potential to higher hydrogen production with a maximum yield of \(0.51 \text{ LH}_2/\text{gCOD}_{\text{consumed}}\) with anaerobic digester sludge (ADS).
  2. In comparing the initial substrate-to-biomass, \(S_0/X_0\), of 0.5 and 1 g COD\(_{\text{substrate}}/g\) VSS\(_{\text{seed}}\), the optimum experimental values of \(S_0/X_0\) for hydrogen production was 0.5 g COD\(_{\text{substrate}}/g\) VSS\(_{\text{seed}}\), when using anaerobic digester sludge, ADS.
  3. Potato waste has the potential to improve hydrogen production with a yield of 0.51 \(\text{LH}_2/\text{gCOD}_{\text{consumed}}\) in 0.5 \(S_0/X_0\), and tests with \(S_0/X_0\) of 1 only revealed a maximum potential of 0.38 \(\text{LH}_2/\text{gCOD}_{\text{consumed}}\).

• **Two-stage anaerobic digestion:**
  1. The benefits of two-stage over single-stage anaerobic digestion from this study included higher biomethane rates and efficiencies, increased net energy production, and total enhancement of the process. The effect of separating the acidogenic and methanogenic stages of anaerobic digestion was demonstrated by improved performance of the second-stage BMP process. Moreover, the feedstock COD removal efficiency was boosted in the second-stage of the BMP process after acidification when compared to the single-stage BMP process.
  2. The optimum experimental range of \(S_0/X_0\) for methane production is 0.5 g COD\(_{\text{substrate}}/g\) VSS\(_{\text{seed}}\) when using ADS as a seed and supernatant as a feed.
However, when using mixed substrate, no a significant difference was observed between different $S_0/X_0$.

3. The potato waste has the potential in improved methane production with a yield of 0.39 m$^3$CH$_4$/kg TCOD$_{removed}$ when using supernatant as a feed, as tests with mixed feed only revealed a maximum potential of 0.35 m$^3$CH$_4$/kg TCOD$_{removed}$.

4. The current study found that the use of two-stage digestion for potato waste led to an increase in the TVFAs-to-TCOD ratio due to the acidification process during hydrogen production in the first stage. The methane yield in the anaerobic digestion stage increased from 0.29 m$^3$CH$_4$/kg TCOD$_{removed}$ in the single-stage process to 0.39 m$^3$CH$_4$/kg TCOD$_{removed}$ in the two-stage process.

5.2. Recommendations
Based on the results of this research, the following suggestions are made:

1. Future research should assess different waste streams, such as food, brewery, and kitchen wastes, as well as starch in biohydrogen production, specifically when using anaerobic digester sludge and acclimatized anaerobic digester sludge.

2. Future studies should compare the use of acclimatized anaerobic digester sludge (AADS) and anaerobic digester sludge (ADS) to determine which is more optimal in the production biohydrogen and biomethane.

3. Future studies should explore the optimum experimental range of $S_0/X_0$ for hydrogen and methane production.

4. Future studies should compare energy outcome from both digestion scenarios.

5. Future studies should study impact of improving the operational conditions for biohydrogen production in the first stage—such as the HRT, SRT, and OLR—on methane production and in the second stage of an anaerobic digestion process using a continuous flow system.

6. Future studies should assess the artificial neural networks for modeling of biohydrogen production for predicting fermentative biohydrogen production in batch studies.
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