Fermentation of Food Waste in a Leach Bed Reactor: Effects of pH and Inoculum to Substrate Ratio

by

Ziyi Xiong

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

A more sustainable method for the treatment of accumulated food waste, instead of landfilling, is in urgently need. Fermentation is one of the most promising waste-to-energy technologies for the energy recovery from food waste. This study investigated food waste fermentation in a leach bed reactor (LBR) under different operating conditions, i.e. pH and inoculum to substrate ratio.

Firstly, for the impact of pH, the food waste fermentation was investigated in a leach bed reactor operated at acidic, neutral and alkaline conditions. Highest solids reduction of 87% was obtained at pH 7 in 14 days of reaction time with minimum mixing. The concentration of volatile fatty acids increased to 28.6 gCOD/L under pH 7, while the highest butyric acid of 16 g COD/L was obtained at pH 6. Bacterial community structure was narrowed down to Bifidobacterium and Clostridium at pH 6, while Bacteroides and Dysgonomonas were identified as main players at both pH 7 and 8. Bacterial populations in the food residue generally reflected those in the leachate, but some bacteria were selectively enriched in the leachate or the food residue. Bacterial community dynamics suggested that biodegradable food waste was first fermented by one of dominant players (e.g., Clostridium) and the other degraded resistant dietary fibers later (e.g., Bifidobacterium, Bacteroides, Dysgonomonas).

Secondly, for the impact of inoculum to substrate ratio, the food waste fermentation was carried out at ISRs of 5%, 10% and 15% (vs/vs). A maximum sCOD concentration of 30-33g/L was obtained under all ISR conditions. Correspondingly, a high degradation efficiency of 85%-91% was obtained at ISR 5%, 10% and 15%, respectively. A faster hydrolysis rate and a shorter reaction time was obtained under ISR 10%, which suggested a higher ISR can accelerate the hydrolysis rate of the food waste, which lead to a decrease in digestion time. The VFA

concentration (24-28g COD/L) and distribution under three ISR conditions had negligible differences, indicating that ISR did not have influence on the acidogenesis of food waste in this study.

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Dedication

To all my family and friends.

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Nomenclature

ABE	Acetone-butanol-ethanol
AD	Aerobic digestion
ASV	Amplified sequence variant
ATP	Adenosine triphosphate
CoA	Coenzyme A
COD	Chemical oxygen demand
sCOD	Soluble COD
tCOD	Total COD
CSTR	Continuous stirred tank reactors
E	Hydraulic pressure head
F/M ratio	Food to microorganism ratio
GHG	Greenhouse gas
HRT	Hydraulic retention time
ISR	Inoculum to substrate ratio
LBR	Leach bed reactor
P	Power
PCR	Polymerase chain reaction
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoates
Q	Flow rate
γ	Energy consumption coefficient
TAE	Tris base, acetic acid and EDTA
TS	Total solid
TSS	Total suspended solid
VFA	Volatile fatty acid
VS	Volatile solid
VSS	Volatile suspended solid

Chapter 1. Introduction

1.1. Background

The rapid increase in the global population and improvement in standard of living has led to excessive municipal solid waste generation. Organic waste, such as food waste, makes up about 40% of all municipal solid waste (Environment Canada, 2013). The most commonly used method for food waste disposal in Canada is landfilling. However, this method has some drawbacks. Landfilling requires a large numbers of land resources, and landfills emits a massive amount of greenhouse gases during the decomposition of food waste (B. Zhang et al., 2017). Food waste is rich in nutrient and highly biodegradable, which can provide value-added products for communities if treated with appropriate technologies (Agler et al., 2011). Thus, anaerobic treatment of organic waste, e.g. fermentation, has gained attention of researchers, since both a reduction in waste quantity and an increase in resource recovery can be achieved simultaneously (Bonk et al., 2015; Demirbas et al., 2011; Stephen et al., 2018). Fermentation is a redox reaction that efficiently breaks down complex organic matter into simple acids (i.e., volatile fatty acids (VFAs)), alcohols and hydrogen gas. These end products are valuable chemicals when separated from the fermented mixture, and can also act as renewable feedstocks to generate higher value-added products in post-treatment processes. For instance, VFA can be processed further to biofuel in the transportation sector in the form of an acetone-butanol-ethanol (ABE) blend (Demirbas et al., 2011; Z. Zhang et al., 2016).

Continuously stirred tank reactors (CSTRs) are the most commonly used bioreactors for biological VFA production from food waste. Due to the high solid content of food waste (20-30%), a variety of pretreatment methods, e.g. dilution, have to be applied to meet the completely mixing

requirement of CSTRs (Jha et al., 2011). However, the operation of a CSTR is energy intensive due to the high solid matter and the viscosity of diluted food waste. Alternatively, dry fermenters, such as a leach bed reactor (LBR), which can carry a higher solid content are more suitable for food waste treatment (Browne et al., 2013; Hussain et al., 2017). The food waste is loaded in the top section of a LBR. Fermented liquid from the food waste slowly leaches out during the fermentation and is collected in the bottom section of the reactor. Instead of intensive mixing, the LBR receives a leachate recirculation from the bottom of the reactor to the top and sprays on the food waste containment vessel uniformly to ensure the contact between microorganisms and the food waste. These designs make LBR an economical and practical method for food waste fermentation.

Different operation parameters have been studied to optimize the yields of end products (e.g., VFA, H2, etc.) in food waste fermentation, including temperature, pH, recirculation rate, inoculum to substrate ratio, and so on (Attero et al., 2000; Hussain et al., 2017; Xu et al., 2012; Zamanzadeh et al., 2016; B. Zhang et al., 2017). Among these parameters, pH is one of the most important factors affecting end product distributions in the fermentative process, because pH influences microbial community and its metabolic pathways (Attero et al., 2000; Hussain et al., 2017; B. Zhang et al., 2017). Microbial community in fermentation with low to moderate organic solids (e.g., animal manure, sludge, etc.), or anaerobic digestion of food waste has been well researched in CSTRs for over decades (Ariunbaatar et al., 2014). However, bacterial community and dynamics in LBRs for food waste fermentation operated at different pHs are not well understood, and conflicting results in limited literature seem to confuse the optimal pH conditions for LBRs. For instance, some works reported better solubilization of food waste in LBRs operated at neutral or alkaline pH, while others showed maximum solid reduction in acidic fermentation of

food waste (Browne et al., 2013; Hussain et al., 2017; Jiang et al., 2013). Thus, the understanding of bacterial community dynamics under different pH is crucial for the optimization of VFA production in LBRs. Inoculum to substrate ratio (ISR) is another critical parameter to determine the fermentation kinetics, solubilization of food waste and its solid reduction (Xu et al., 2012). Literature has commonly reported that a higher production of VFA can be obtained with a higher ISR due to the improved biochemical reactions of bacteria (Zhou et al., 2018). However, the literature mainly focused on the effect of ISR on methane production in anaerobic digestion using different substrates, such as sunflower oil cake and municipal solid waste. In comparison, there is limited information on the impacts of ISR on food waste fermentation (Boulanger et al., 2012; Raposo et al., 2009). Moreover, in several studies, experimental conditions (e.g., substrate, inoculum, pH, temperature, mixing conditions, etc.) in food waste fermentation were inconsistent, and thus it is challenging to determine the optimal ISR of food waste fermentation in LBRs (Zhou et al., 2018).

1.2. Scope and Objectives

The overall objective of this study was to evaluate the influences of two important factors, i.e. pH and ISR, on the hydrolysis and acidogenesis of food waste in a mesophilic LBR. The thesis comprises three specific research areas:

1) The effect of pH (acidic, neutral and alkaline) is studied on the degradation of food waste and the composition of organic compounds produced during the fermentation process in the LBR. Also, the optimum pH condition is determined for the following studies;

2) Dominant bacteria in food waste fermentation at each pH are identified and bacteria community in leachate and food waste is compared to improve understanding on the locality of biochemical reactions in the LBR;
3) The influence of ISR on the hydrolysis of food waste and VFA yield is evaluated in the LBR under the optimum pH condition.

1.3. Thesis Outline

This dissertation is divided into five chapters and references. Chapter 1 provides the current challenges and research innovations on the proposed research and summarizes the research goals of this study. Chapter 2 presents an overview of the available literatures related to the proposed research. Chapter 3 and 4 are the main research outcomes, presented in article format. Chapter 3 investigates the effects of pH on the performance and microbiology of the fermentation of food waste, while Chapter 4 investigates the influence of ISR on the hydrolysis and acidogenesis of food waste. Chapter 5 summarizes research results.

Chapter 2. Background

2.1. General reviews

2.1.1. Introduction

The rapid increase in the global population and improvement in standard of living has led to excessive solid waste generation. Canada is ranked as the highest per capita producer of municipal solid waste amongst 17 OECD countries with an average generation rate of 777 kg per person annually (OECD, 2013). Organic waste, such as food waste, makes up about 40% of all municipal solid waste (Environment Canada, 2013). The accumulation of the waste in landfills without sustainable alternatives has become a significant issue. This organic solid waste consists of various materials, most of which have unexploited value and can be further processed to valued-added products. However, conventional waste management methods are unable to meet the need for waste resource recovery and, of more concern, may cause secondary pollution to the environment.

Currently, landfilling is the most common method of organic waste disposal, but this has significant drawbacks since it can lead to environmental degradation as well as greenhouse gas (GHG) emissions, such as methane production (B. Zhang et al., 2017). Incineration is the second most commonly used waste disposal method, which fully oxidizes organic matter to CO_2. However, incineration requires a massive amount of energy to first dry out high-moisture materials in organic waste (Komemoto et al., 2009) in addition to having high capital and operating costs for incineration facilities. More sustainable methods for food waste treatment, such as curbside recycling and composting, has been well established in developed countries, e.g. Canada (Environment Canada, 2013). However, the digestate leachate from food waste composting requires further handling, and is often directed to a wastewater treatment plant (Environment

Canada, 2013). This post-treatment can increase pressure for existing wastewater treatment facilities, as these leachates usually contain high levels of organic matter. Thus, there is a need for improving the current practices of organic waste treatment from resource consumption to resource circulation directions: a closed-loop system where energy and resources are recovered and reused.

2.1.2. Food waste to energy technologies

Conventional processes for waste disposal treat waste as useless and unwanted and essentially focus on waste quantity reduction or minimizing its adverse effects on human health. However, most organic solid wastes, such as food waste, have potential values. Due to their ready availability, large generation rates, and high biodegradability, they can be used as a raw material for value-added chemicals production or renewable energy generation (Demirbas et al., 2011). Researchers have explored various waste-to-energy conversion technologies which can be classified into physical conversion, thermochemical conversion and biochemical conversion as illustrated in Figure 2.1.

Thermochemical and biochemical pathways for waste-to-energy conversion are also known as biorefinery. Heat, bio-oil, or syngas (carbon monoxide and hydrogen) can be generated directly from thermochemical technologies (i.e., pyrolysis and gasification), where organic waste with low moisture content, such as wood and wood waste, can be treated economically and efficiently. However, the high moisture content in food waste makes it less suitable for thermochemical techniques due to higher energy consumption and lower efficiency. Anaerobic biological treatment, which employs a complex microbial community to break down biodegradable organic matter in the absence of oxygen (Khalid et al., 2011), is more suitable for the treatment of food waste because microorganisms require a high-moisture environment. Fermentation is an anaerobic redox

reaction which well degrades complex organic matter into simple organics, such as alcohols and short-chain fatty acids. Anaerobic digestion includes fermentation and continues the degradation process through methanogenesis, producing biogas, composed mainly of methane (CH_4) and carbon dioxide (CO_2). Literature suggested that the sugar-platform and the carboxylate platform could be the most crucial biorefinery platforms in fermentation (Agler et al., 2011). In the sugar platform, organic waste is converted into pentose and hexose sugars as intermediate chemicals which can be further converted into fuels. In the carboxylate platform, short-chain carboxylates, which are also known as volatile fatty acids (VFAs), are generated as intermediate feedstock for the generation of biofuels. Compared to the sugar platform, the carboxylate platform can be completed with a mixed-culture of microorganisms instead of purified enzymes, making the treatment of food waste more practical and economical (Agler et al., 2011).

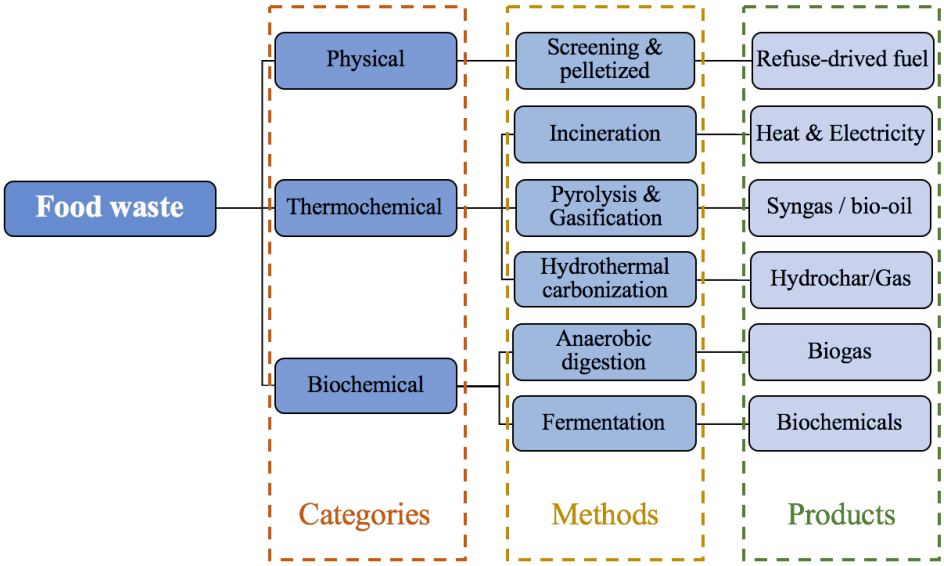


Figure 2.1 *Summary of food-waste-to-energy technologies*

The intermediate VFAs produced from the carboxylate platform are valuable chemicals by themselves when separated from the fermented mixture, and can also act as substrates for further

fermentation reactions, such as ethanol fermentation (Phuong et al., 2015). Thus, food waste can be a sustainable and practical source of VFAs through the carboxylate platform. Since a tipping fee may be earned by a facility receiving food waste, the revenue generated improves the business case for food waste fermentation as compared to current commercial production through chemical processes (Lee et al., 2014).

2.1.3. VFAs in biofuel production

Biofuel refers to solid, liquid, and gaseous fuels that are produced from contemporary biological processes. It has gained attention as an alternative energy source due to its renewability, carbon neutrality, and environmental friendliness. Biofuel can be produced in any regions by converting local organic materials, unlike fossil fuels which can only be extracted in select areas. Among all the forms of biofuel, liquid biofuels such as biodiesel and bio-alcohol are considered to be easier and safer to store and transport over solid and gaseous fuels (Demirbas et al., 2011). Liquid biofuels can also be used with existing infrastructure, such as engines, storage tanks, and pipelines, with only minor modifications, resulting in lower retrofitting costs. Hence, liquid biofuels are considered to be the most promising substitute for gasoline and petroleum in the transportation sector. Moreover, liquid biofuels could contribute to the reduction of GHG emissions by 80% if used in vehicles instead of fossil fuels (Z. Zhang et al., 2016). Biodiesel, bio-ethanol, and bio-butanol are considered as alternatives to gasoline; among them bio-butanol may be more efficient due to its higher energy density (Demirbas et al., 2011).

Bio-butanol can be blended with gasoline to make up to 40% of the fuel mixture and still be used in conventional combustion engines. As compared to gasoline and diesel, bio-butanol can radically reduce the emission of GHG and other tailpipe emissions such as carbon monoxide and

unburned hydrocarbons (Natural Resources Canada, 2015). Bio-butanol can be directly produced through fermentative pathways from organic matters such as corn (Alternative Fuels Data Center, 2018). However, the production of bio-butanol is costly, being highly dependent on the price of raw materials (Demirbas et al., 2011). At present, more than 95% of bio-butanol is derived from edible crops, which might have negative effects on agricultural production by vying for scarce land and water resources (Lashitew, 2011; Yang et al., 2009). For example, it is projected that 5-10% of China's cultivated land will be used to produce raw materials for biofuel production to achieve its production targets by 2020, which could pose significant impacts on China's food supply and trade (Yang et al., 2009). In comparison, volatile fatty acid (VFA) is considered to be an ideal carbon source for bio-butanol production compared with food-derived complex organics for several reasons. Firstly, VFA can be produced from various revenue-neutral or revenue-positive sources such as food waste, which leads to a substantial decrease in biofuel production cost. Secondly, the use of specific, expensive enzymes is not required if VFA is used as carbon source, since VFAs' simpler structures mean they can be directly utilized by microorganisms. Thirdly, VFA provides a higher carbon yield due to less carbon dioxide being released during fermentation (Chang et al., 2010).

2.2. Anaerobic Digestion

Anaerobic digestion, mainly consisting of fermentation and methanogenesis, occurs naturally in swamps, underwater sediments, the intestines of animals, and other anaerobic environments where organic matter is transformed finally to methane and carbon dioxide. Anaerobic digestion is composed of four major steps that are carried out by different groups of microorganisms. Each of these stages produces outputs that will become inputs for the next steps, forming syntrophic interactions. The various microbial steps of anaerobic digestion are illustrated in Figure 2.2.

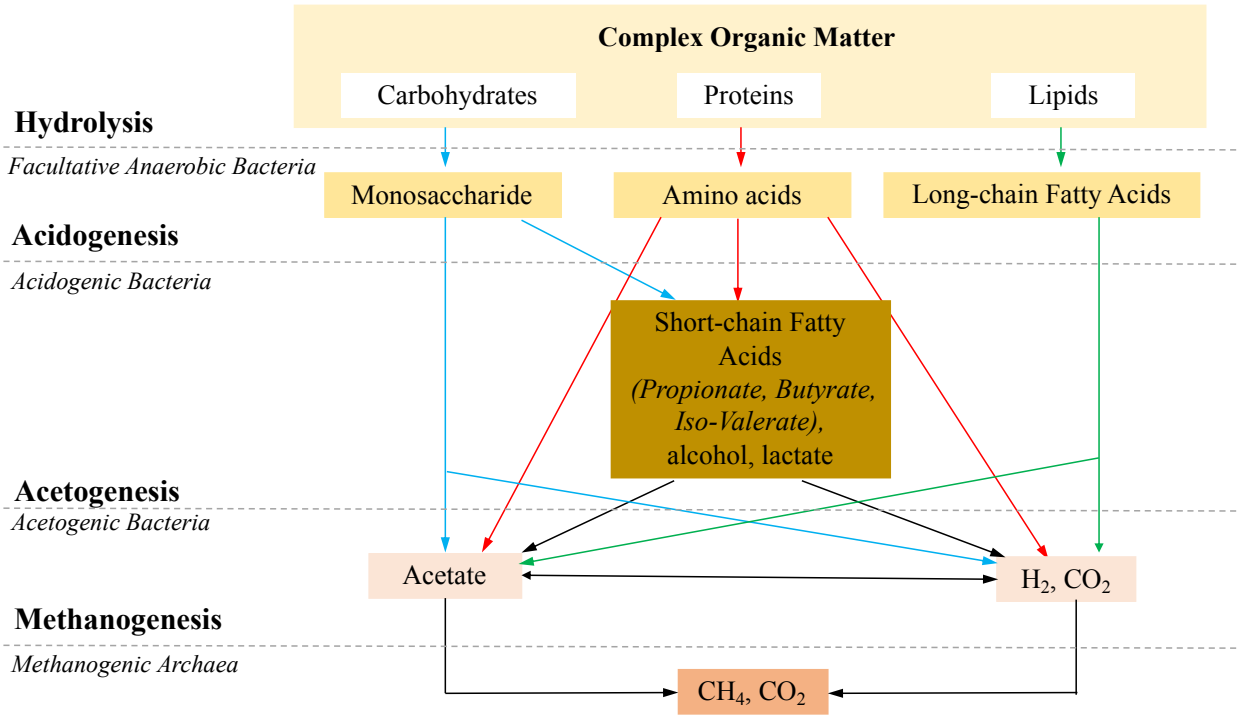


Figure 2.2 *Schematic diagram of anaerobic digestion*

2.2.1. Elementary reactions in anaerobic digestion

2.2.1.1. Hydrolysis

The organic materials present in the food waste usually consist of carbohydrates (cellulose, hemicellulose, starch, etc.), proteins and lipids (oils and fats). During the early stages of anaerobic digestion, hydrolyzing bacteria produce extracellular enzymes that break down these complex organic substrates into monomers by hydrolysis. This transformation makes the substrate assimilable for bacteria and facilitates the penetration of the molecules through the cell walls (Agler et al., 2011; Chang et al., 2010). Compared to the other steps, hydrolysis is considered to be the rate-limiting step (Singhania et al., 2013).

2.2.1.2. Acidogenesis

In this phase, fermenting bacteria break down the compounds produced in the hydrolysis step (i.e., monomers) into simple organic acids, alcohols, hydrogen gas and carbon dioxide (Khalid et al., 2011). Acidogenesis is generally fast because of the high growth rate of acidogenic bacteria (Demirbas et al., 2011). The end products in the acidogenesis of monosaccharides are mainly acetic acid, propionic acid, butyric acid, alcohols (e.g., ethanol, methanol), hydrogen gas, and carbon dioxide. Stoichiometries of the major acidogenesis reactions are described below (Bajpai, 2017):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad (2.1)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (2.2)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow C_4H_7O_2^- + 2HCO_3^- + 3H^+ + 2H_2 \quad (2.3)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2C_2H_5OH + 3HCO_3^- + 2H^+ \quad (2.4)$$

2.2.1.3. Acetogenesis

The acetogenesis step involves the transformation of simple acids and alcohols, such as butyric acid, propionic acid, and ethanol, into acetate, hydrogen gas, and carbon dioxide by acetogenic bacteria (Bajpai, 2017):

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2 \quad (2.5)$$

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2 \quad (2.6)$$

$$C_2H_5OH + 2\ H_2O \rightarrow CH_3COO^- + H^+ + 2H_2 \quad (2.7)$$

2.2.1.4. Methanogenesis

The final stage in anaerobic digestion is to convert acetate, hydrogen gas, and carbon dioxide into methane and carbon dioxide. For these reasons, there are two types of methanogens: acetoclastic

and hydrogenotrophic methanogens (Beyene et al., 2018). The equations below show the production of methane during methanogenesis by acetoclastic methanogens (Equation 2.8) and hydrogenotrophic methanogens (Equation 2.9) (Bajpai, 2017):

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (2.8)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.9)$$

2.3. VFA production optimization in food waste fermentation

2.3.1. Strategies to enhance VFA production

VFAs, such as acetate, propionate, and butyrate, are the main products of food waste fermentation. Based on the stages of anaerobic digestion discussed above, three strategies can be adopted in order to increase the accumulation of higher levels of VFAs: improving the hydrolysis rate, promoting the acidogenesis process, and avoiding the methanogenesis step (Zhou et al., 2018). Factors affecting hydrolysis and acidogenesis of food waste will be discussed in the next section. Moreover, the methanogenesis step must be avoided by inhibiting the activities of methanogens. There are three methods widely used for methanogen inhibition, including thermal pretreatment, pH pretreatment, and short hydraulic retention time (HRT). Thermal pretreatment is to expose methanogen culture at high temperature (75°C-100°C) for 10-20 min to inactivate or kill methanogens (Bonk et al., 2015). For pH pretreatment, the growth of methanogens is seriously inhibited at acidic pH in general (Bonk et al., 2015). Lastly, methanogens are slow-growers, so we can keep a small number of methanogens in continuous bioreactors by keeping short HRTs.

2.3.2. Factors affecting hydrolysis and acidogenesis of food waste

In order to achieve high VFA production from the anaerobic hydrolysis/acidification process of food waste, it is crucial to review the parameters that can influence fermentation performance.

Various factors such as pH, temperature, inoculum to substrate ratio etc. play important roles in the fermentation. Here, I summarized three main parameters affecting the fermentation of food waste.

2.3.2.1. Temperature

Temperature has a significant effect on the biochemical and physicochemical processes of fermentation (Zamanzadeh et al., 2016). Four ranges of temperature had been studied in literature (Lee et al., 2014): psychrophilic condition (4°C-20°C), mesophilic condition (20°C-50°C), thermophilic condition (50°C-60°C) and extreme-thermophilic condition (60°C-80°C). In psychrophilic and mesophilic conditions, an increase in temperature can result in modified enzymatic activity which increases the rate of hydrolysis, normally according to Arrhenius law (Gou et al., 2014; Lee et al., 2014). Arrhenius law would suggest that increasing temperature from mesophilic to thermophilic or extreme-thermophilic conditions would also be beneficial to fermentation. However, inconsistent outcomes are reported in the literature, indicating that high temperature conditions do not always improve fermentation performance (Lee et al., 2014; Zamanzadeh et al., 2016) because microbiological enzymes are not stable in high temperatures (Charlier et al., 2005). Table 2.1 summarizes performance of food waste fermentation under different temperatures.

2.3.2.2. pH

pH is one of the most important factors affecting the distribution of end products in food waste fermentation because microorganisms and metabolic enzymes are controlled by pH (Lee & Rittmann, 2009). Many researchers have carried out various studies on the effect of pH on VFA production in food waste fermentation (Table 2.2). It is generally believed that fermentation can

be carried out at a pH of 4.0 to 11.0, but the adaptability of different microorganisms to a given pH can be entirely different (Khalid et al., 2011). For example, a suitable pH for hydrolyzing fermentative bacteria and hydrogen-producing acetogens is about pH 5.0 to 6.5, and the suitable pH for methanogens fluctuates between a neutral range of pH 7 to 8. Also, when the pH is less than 6.0 or larger than 8.0, the activity of some methanogens can be inhibited (Bajpai, 2017). In the fermentation process, if intermediate VFAs are accumulated, the pH value of the system will be significantly reduced (Lee et al., 2014). Thus, pH controller or buffer solutions are often used in anaerobic digesters to adjust and regulate the acidity and alkalinity of mixed digestate.

Furthermore, literature commonly reported that pH is a key parameter selecting the main metabolic pathway of microorganisms (Cysneiros et al., 2012; Dinamarca et al., 2003; Hussain et al., 2017). The metabolism of microorganisms have two main processes: 1) energy generation, and 2) balance of intracellular reducing powers (Bonk & Hedegaard, 2015). Among the three main VFAs, acetate is the best metabolite in terms of energy generation since bacteria can conserve more ATP from per mol of acetate than that of butyrate or propionate. On the other hand, the generation of butyrate and propionate involves NAD(P)H oxidation per mole of metabolite, which means bacteria can balance intracellular reducing powers through the production of butyrate or propionate (Bonk & Hedegaard, 2015; Zhou et al., 2018). Thus, the changes in VFA distributions indicated that fermentation microorganisms might choose different metabolic pathways under different pHs to meet the requirements of energy generation and the balance of reducing powers. Many previous studies have suggested that the optimum pH of fermentation for VFA production is neutral or acidic. However, the conclusions drawn by different researchers are not consistent since operating conditions (e.g., substrate, inoculum, temperature, etc.) were different.

2.3.2.3. Inoculum to substrate ratio

Inoculum to substrate ratio (ISR) describes the relative amount of anaerobic microbes and food waste existing in reactors. It is essential for the design of dry fermenters, where the volume of food waste is fixed. ISR is the reciprocal of food to microorganisms (F/M) ratio commonly used in bioreactors treating organic waste and wastewater. ISR is calculated as

$$\text{ISR (\%, vs/vs)} = \text{(VS of inoculum (g))} / \text{(VS of substrate (g))}$$

where VS of inoculum is initial volatile solid of the sludge added to the reactor (g); VS of substrate is initial volatile solid of food waste loaded in the reactor (g).

As such, ISR (or F/M ratio) is only a rough estimate of the actual ratio of microorganisms to substrates, but it is nonetheless a widely-used parameter in the optimization of bioreactor operations. An appropriate ISR should be maintained in a bioreactor to improve hydrolysis or fermentation of food waste. An optimal ISR can provide high yields of end products and reduce reaction time (Xu et al., 2012). It is commonly reported that a higher production of VFA can be obtained with a higher ISR due to the increased proportion of active microbes (Zhou et al., 2018). It was reported that the ISR of 4 (vs/vs) was the optimum ratio for biogas production using a mixture of food waste and rice husk as substrate and cow dung as inoculum after testing ISRs of 4, 2, 1, 0.67 and 0.5 (Rizwan et al., 2015). In another study using a leach bed reactor (LBR) treating food waste, an increased hydrolysis rate of protein and carbohydrate was obtained when the ISR was increased from 0.004 to 0.069 (vs/vs). Concomitantly, volatile solid degradation of the food waste was improved from 52.4% to 71.7%, and VFA concentration was doubled (Xu et al., 2012). The literature mainly focused on the effect of ISR on methane production in anaerobic digestion using different substrates, such as sunflower oil cake and municipal solid waste. In comparison, there is limited information on the impacts of ISR on food waste fermentation (Boulanger et al.,

2012; Raposo et al., 2009). In addition, experimental conditions (e.g., substrate, inoculum, and final target products) in food waste fermentation were inconsistent in limited literature, causing a wide range of optimal ISRs (Zhou et al., 2018).

2.4. Different anaerobic technologies in anaerobic treatment of food waste

In order to manage different forms of food waste and obtain particular final bioproducts, a wide variety of operating conditions and types of bioreactors have been studied, which are discussed as follows.

2.4.1. Classification of major reactor operating conditions

Technologies can be divided according to different operating characteristics, which are discussed as follows: dry matter content, feeding mode, and numbers of stages.

2.4.1.1. Dry matter content

Bioreactors can be classified according to the water concentration of feedstock. Wet fermentation has been mainly used for anaerobic digestion of organic materials having a solids content between 5% and 20%. In comparison, dry fermentation is used mainly for treatment of organic materials with solids content from 20% and 50% (Jha et al., 2011).

2.4.1.2. Feeding mode

Three modes of feeding can be applied to food waste treatment: continuous mode, semi-continuous mode and batch mode (Lee et al., 2014). Bioreactors operated in continuous mode are fed continuously, with an incoming quantity of materials equivalent to the discharged volume. All stages of bioreactors take place without interruption, leading to a constant production of biogas and biochemicals (Lee et al., 2014). The second category is batch mode bioreactors. In this type

of reactors, food waste is supplied only at the beginning of the batch. When a sudden decrease or complete stop of the production of biochemicals and biogas is observed in the bioreactors, food waste can be considered as fully degraded. Afterwards, bioreactors are emptied, and new food waste is introduced to start another batch cycle. The last type is the semi-continuous mode, which is a combination of the continuous and batch modes whereby feeding is done continuously but digestate is regularly removed discontinuously (Chen et al., 2013; Lee et al., 2014).

2.4.1.3. Number of stages

There are two broad categories, traditional single-stage bioreactors and more advanced two-stage reactors. The single-stage process has all biochemical reactions done in a single reactor, while different steps of biochemical reactions happen in different reactors in the two-stage process (Jha et al., 2011). Normally, the hydrolysis and acidogenesis phases are carried out in the first reactor then the partially-degraded organic material moves to the second reactor in which acetogenesis and methanogenesis take place (Jha et al., 2011).

2.4.2. Reactor types

Here, continuous stirred tank reactors (CSTR) and leach bed reactors (LBR) are mainly discussed for food waste treatment to focus on the proposed research, although there are more types of bioreactors (Lee et al., 2014). Schematics of CSTR and LBR are provided in Figure 2.3.

CSTRs are one of the most widely used bioreactors in food waste treatment and are applied in wet fermentation in continuous mode using either one or two stages. Their principal advantage is complete mixing of substrate and microorganisms, assuring maximum contact between microorganisms and organic materials and leading to increased biogas and biochemical production (W. S. Lee et al., 2014; Zamanzadeh et al., 2016). However, using a CSTR for food waste treatment

has several drawbacks. Firstly, the operation of a CSTR can be energy intensive due to the continuous mixing of a viscous mixture. Since most of the food waste has a high solid content (>10%) (Jha et al., 2011), CSTRs also require considerable pre-treatment of food waste and may require process water for the dilution. Lastly, there is a lack of efficient separation techniques to recover biochemicals from the mixed fermented liquor, which consists of solid and liquid fractions of food waste as well as microbial biomass.

In comparison, a LBR is a dry fermenter operated under batch mode using a single stage. LBRs have several characteristics which make them amenable to biochemical production from solid food waste. Namely, the high solid content of food waste can be easily handled using LBRs, which are able to treat extremely high solids organic wastes up to 30% solids content (Browne et al., 2013). Secondly, minimum pretreatments are required for LBRs because food waste can be loaded directly into the reactor in bulk. Thirdly, the solid and liquid separation is completed naturally as a result of LBR's configuration. Thus, LBRs overcome the drawbacks of CSTRs in several ways making LBRs potentially more economical and practical for food waste treatment (Browne et al., 2013; Hussain et al., 2017; Jha et al., 2011).

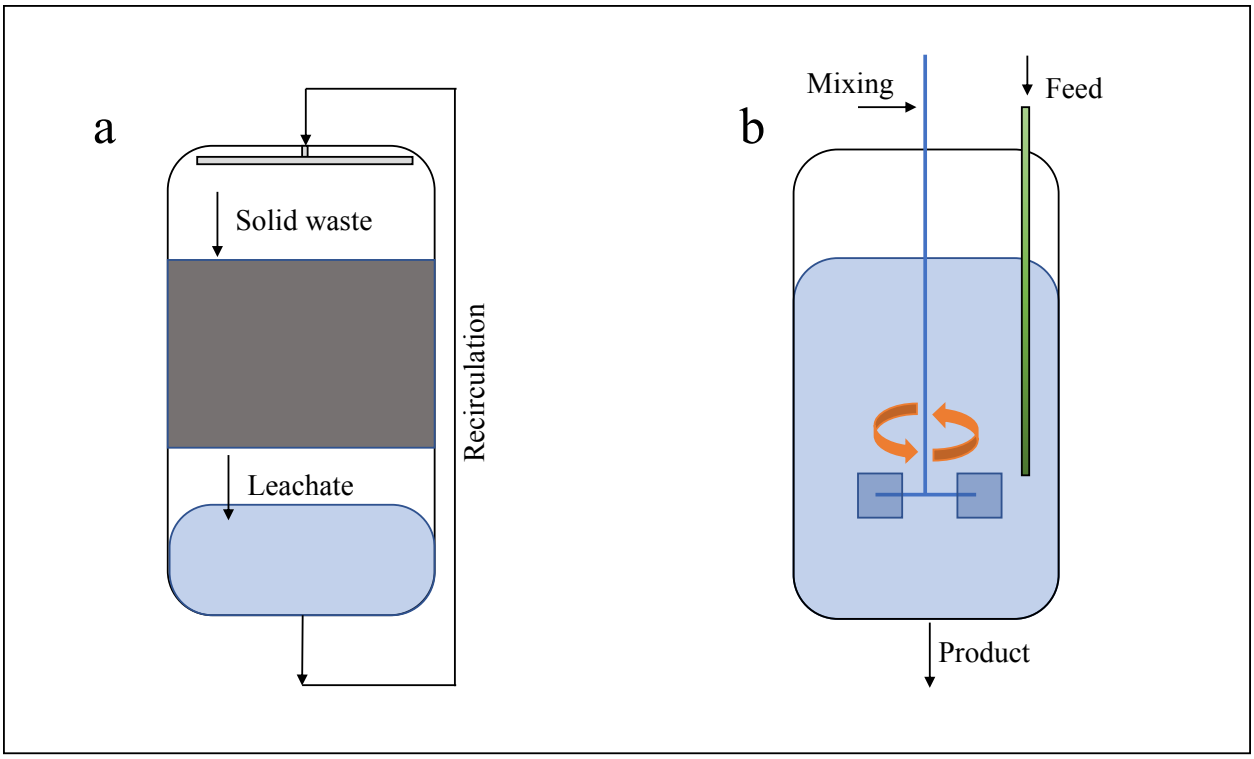


Fig. 2.3 *the configuration of a) LBR and b) CSTR*

Table 2.1 *Comparison of optimal temperature for the production of VFA*

Ref.	Solid wastes	Temperature range studied	Optimal Temperature	Reactor type and operating conditions	VFA production performance	Observation
(Gou et al., 2014)	Food waste	35-55 °C	55°C	Continues stirred tank reactor, waste activated sludge, 188 d	3,550 ± 125 mg/L	Thermophilic condition contributed to the fastest hydrolysis / acidogenesis rate
(Jiang et al., 2013)	Food waste	35-55 °C	45°C	Batch reactor, anaerobic digested sludge, 8 d	47,890 ± 576 mg/L	VFA significantly lower at 55 °C, dominate VFA shifted from acetate to butyrate when increasing temperature
(Zamanzadeh et al., 2016)	Food waste	37-55 °C	55°C	Continuous reactor, food waste sludge, 3 weeks	2,028 ± 864 mg/L	Higher hydrolysis yield but lower acidification yield was obtained with higher temperature
(Dahiya et al., 2015)	Food waste	55-70 °C	55°C	continuous stirred-tank reactor, maize straw sludge, 120 d	17,100 ± 1700 mg/L	Thermophilic condition is more beneficial than hyperthermophilic Since higher efficiencies of hydrolysis, acidification, and hydrogen production was achieved
(Komemoto et al., 2009)	Food waste	15-65 °C	35-45 °C	Serum bottle test, water, 22 d	~8,000 mg/L	Cumulative solubilization rate under mesophilic conditions are higher

Table 2.2 *Comparison of optimal pH for the production of VFA.*

Ref.	Solid wastes	pH range studied	Optimal pH	Reactor type and operating conditions	VFA production performance	VFA distribution
(B. Zhang et al., 2017)	Kitchen waste	5–11	7	Batch reactor, 35 °C, beer wastewater sludge, 32 d	36,000 mg/L	Butyrate dominated at pH 5,7; Acetate and formic acid dominated at pH 9,11
(Jiang et al., 2013)	Food waste	Uncontrolled-7.0	6	Batch reactor, anaerobic digested sludge, 35 °C, 8d	39,460 mg/L	Acetate dominated at pH uncontrolled and pH 5, Butyrate dominated at pH 6 and pH 7
(Chen et al., 2013)	Kitchen waste	4.0-12.0	8	Serum bottle, waste activated sludge, 37°C, 3-9 d	16810 mg/L	Acetate and propionate dominated at optimum pH
(Cysneiros et al., 2012)	Maize	Uncontrolled-6.0	6	Batch reactor, maize digested sludge, 20-65 °C, 28d	26,700 mg COD/L	Butyrate dominated under all pH, but lower pH significantly inhibits the production of acetate
(Attero et al., 2000)	Organic waste	5.0-7.0	7	Batch reactor, water, 28 ± 2 °C, 14d	28,000 ± 1,000 mg COD/L	No data
(Hussain et al., 2017)	Food waste	4.0-7.0	7	Batch reactor, anaerobic digested sludge, 50°C, 14 d	49,000 ± 4,000 mg COD/L	Acetate dominated at pH 4 and 5, Butyrate dominated at pH 6,7
(Wang, Yin, Shen, & Li, 2014)	Food waste	unconrolled-6.0	6	Serum bottle, anaerobic digested sludge, 30 ± 2°C, 20d	52,000mg/L	At pH 5.0 and 6.0, butyric acid was the dominant product, acetic and

						propionic acids dominated at pH 4 and under
(H. Chen et al., 2013)	Food waste	5.0-11.0	9	Semi-continuous serum bottle, anaerobic digested sludge, 35 °C, 9d	25,934 ± 1,485 mg COD/L	No data
(Dahiya et al., 2015)	Food waste	5.0-11.0	10	Serum bottle, anaerobic digested sludge, 28 ± 2 °C, 2d	6300mg/L	At pH 5, 6, 7, butyric acid was the dominant product, acetic dominated at pH 8, 9, 10 and propionate dominated at pH 11

Chapter 3. Understanding of food waste fermentation in a leach bed reactor: reactor performance, and microbial ecology and dynamics

Contributions statement: Ziyi Xiong designed the study, performed all laboratory experiments and analyses, and contributed to data interpretation and manuscript preparation. Roberson Reid fabricated the reactor. Jangho Lee contributed to the microbial community analysis, and Abid Hussain co-supervised this study.

3.1. Introduction

The over-reliance on fossil fuels have caused a drastic increase in global greenhouse gas (GHG) emissions and have strained the existing energy reserves. In light of these events, renewable energy sources such as biofuels are becoming an attractive and sustainable substitute (Coma et al., 2017; Elbeshbishy et al., 2017; Farmanbordar et al., 2018; H. S. Lee et al., 2010). Biofuels refer to the fuel that can be directly derived from contemporary biomass. It can be generated in solid, liquid or gas phases, while liquid phase biofuels are more stable in production processes and have higher energy potential (Stephen & Periyasamy, 2018). At present, the application of organic waste as sustainable feedstock for biofuel generation has gained attention from researchers, since both a reduction in waste quantity and an increase in resource recovery can be achieved simultaneously (Coma et al., 2017; Dahiya et al., 2015). Physicochemical and thermochemical methods, such as pyrolysis of lignocellulosic forest wood, had been adapted to process organic waste into biofuel (Stephen & Periyasamy, 2018). However, these approaches are ineffective when it comes to handling heterogeneous compositions like food waste. Biological methods such as acidogenic fermentation have a high potential in producing liquid biofuel using complex organic matter (e.g.,

food waste) as feedstock (Coma et al., 2017). Volatile fatty acids (VFA), such as acetate, propionate lactate, and butyrate, are the main products of acidic fermentation. Biological VFA platform can give economical and sustainable bioenergy and biochemicals. For instance, VFA can be processed further to be used as biofuel in the transportation sector in the form of an acetone-butanol-ethanol (ABE) blend that has high energy density, low volatility, and good blending properties with gasoline and diesel (Farmanbordar et al., 2018).

Continuously stirred tank reactors (CSTRs) are the most commonly used for biological VFA production from food waste (Browne et al., 2013). Due to the high solid content of food waste (20-30%), a series of pretreatment methods, e.g. dilution, are essential to meet the completely mixing requirement in CSTRs. Alternatively, dry fermenters, such as a leach bed reactor (LBR), can carry a higher solid content directly and thus they are more suitable for food waste treatment (Browne et al., 2013; Hussain et al., 2017). LBRs overcome the drawbacks of CSTRs in several ways. Firstly, the operation of LBR systems is less complicated compared to CSTRs due to the absence of a stirring mechanism. Secondly, LBR design allows food waste treatment in the absence or minimum requirement of water. Third, instead of intensive mixing, LBRs rely on leachate recirculation from the bottom of the leachate bed to the top of the waste assortment to ensure a commensurate conversion rate (Browne et al., 2013; Hussain et al., 2017). These features allow LBRs to be an economical and practical method for food waste fermentation.

Food waste fermentation has been studied to optimize the yields of end products (e.g., VFA, H_2, etc.), including temperature, pH, organic loading rate, and so on (Gou et al., 2014; Hussain et al., 2017; Jiang et al., 2013; Wang et al., 2014). Among the parameters, pH is one of the most important factors affecting end products in the fermentative process because pH is highly influential to microbial community and metabolic pathways (H. S. Lee & Rittmann, 2009; H. S.

Lee et al., 2009). In the LBRs for food waste fermentation, information on microbial community is limited, while microbial community in fermentation of low to moderate organic solids (e.g., animal manure, sludge, etc.), or anaerobic digestion of food waste has been well studied in CSTRs for over decades (Ariunbaatar et al., 2014; Mac & Llabr, 2000). Moreover, bacterial community and dynamics in LBRs for food waste fermentation operated at different pHs are not well understood. Some works reported better solubilization of food waste in LBRs operated at neutral or alkaline pH, while others showed maximum solid reduction in acidic fermentation of food waste (Browne et al., 2013; Hussain et al., 2017; Jiang et al., 2013). The operational characteristics of LBR separate the dilute microbial cocktail of leachate from high solid food waste, potentially causing different populations of microorganisms and related metabolism. However, no study has evaluated microbial communities in the two phases of liquid leachate and solid food waste separately for LBR. In addition, LBRs are operated in batch mode different from CSTRs, which means that the understanding of bacterial community dynamics linked to VFA production is critical for the optimization of organic loading rate and reaction time in LBRs. Despite of this significance, studies on bacterial community dynamics in LBRs for food waste fermentation are minimum.

This study investigated food waste fermentation in a LBR operated at different pHs (acidic, neutral, and alkaline conditions) simultaneously with microbiological and chemical approaches. First, the pH for solubilization of food waste was optimized and operating costs was estimated. Second, VFA produced at three pH conditions were analyzed and the implications of distinctions in VFA distribution were discussed for energy production and balance of reducing powers. Third, dominant bacteria in food waste fermentation at each pH were identified and bacteria community in leachate and food waste was compared to improve understanding on the locality of biochemical

reactions in the LBR (leachate vs. food waste). Finally, bacteria community dynamics in leachate was tracked to better comprehend VFA production with time in the LBR.

3.2. Material and Method

3.2.1. Characteristics of food waste and inoculum

Food waste was collected from a cafeteria at University of Waterloo (Waterloo, ON, Canada). To minimize variations in the food waste composition, approximately 20 kg of the food waste was collected in bulk and used for this study. After collection, the food waste was manually screened for non-biodegradable materials (e.g., egg shells). The sorted waste mainly consisted of vegetables, fruits, bread and pasta. Using a commercial mesh chopper (Starfrit, Canada), the food waste was chopped to an average particle size of ~ 1 cm. Thereafter, the samples were thoroughly mixed and stored at -20ºC in airtight plastic bags (Ziploc bags, SC Johnson, USA) to avoid deterioration of the samples. The samples were defrosted at 4ºC for 24 hours prior to the experiments.

A leach bed reactor (LBR) designed for fermentation of food waste was inoculated with anaerobic digestion sludge monthly sampled from the Kitchener wastewater treatment facility (Ontario, Canada). To kill or inactivate methanogens the sludge was heated to 75 °C for 15 minutes before use. The characteristics of food waste and inoculum used in this study are shown in Table 3.1.

Table 3.1 *Characterization of food waste and inoculum*

Parameters	Food waste	Inoculum
tCOD(g/L)[a]	264±27	10.5±1.2
sCOD (g/L)	/	5.1±0.8
TS(%)	16.5±0.2	0.4±0.1
VS(%)	15.5±0.7	0.3±0.1
VS/TS(%)	94	68
TS (g/L)	165±3	4.6±0.3
VS (g/L)	155±2	3.2±0.2

[a]food waste was blended with a blender after adding deionized water, and COD was measured after appropriate dilution (~350 dilution). tCOD data was averaged in five measurements.

3.2.2. Reactor Design

The cylindrical LBR used in this study was made from acrylic materials and had a total volume of 7.5L with a diameter of 13.5 cm and a height of 69 cm. The reactor consisted of 3 sections: 1) a top section comprising of a removable top cover with o-rings to ensure an anaerobic environment, a headspace to allow for gas collection and a nozzle for leachate recirculation, 2) a middle section comprising of a containment vessel to hold the food waste, and 3) a leachate holding bed with a funnel-shaped bottom (see Figure 3.1). The customized nozzle head was placed on the underside of the removable top cover to drip the leachate evenly over the food waste. The containment vessel, where the food waste is held, had a height of 18cm and an effective volume of 1L. It was composed of polyethylene terephthalate (PET) walls and a 4mm thick mesh bottom that prevents food waste particles from entering the leachate holding bed. The percolated leachate then sits in the 4L capacity leachate holding bed at the bottom of the reactor before it is recirculated by a timer (MODEL XT-4, ChronTrol Corperation, USA) connected to a peristaltic pump (MODEL 115 VAC, Masterflex, Canada). Samples were taken from a recirculation line for routine analysis. A pH controller (MODEL 5656-00, Cole Parmer, USA) was coupled to the leachate bed via a pH probe inserted into the retaining wall. This allowed for continuous monitoring and regulation of

the leachate pH at a desired value with automatic injections of 1M NaOH. A gas counter (MilliGas counter, Ritter Apparatus, Bochum, Germany) was attached to the top cover of the LBR to measure biogas production.

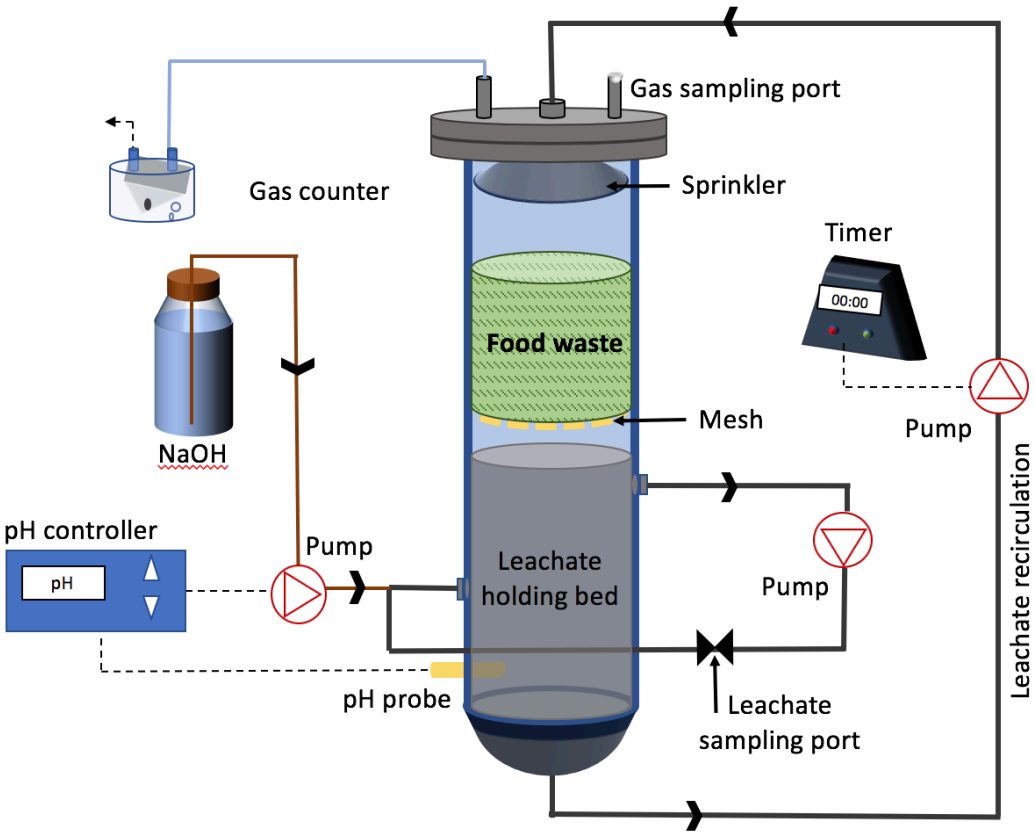


Figure 3.1 Schematic of the leachate bed reactor (LBR).

3.2.3. Experimental set-up

The LBR was continuously operated and monitored in batch mode for 14 days at room temperature (22°C). The food waste and inoculum were added to the LBR in the same manner for each batch. The total solid (TS) and volatile solid (VS) of the food waste before and after fermentation were quantified to determine TS and VS reduction. The reactor was initially loaded with 1 kg of the food waste and 2.5L of the pre-heated AD sludge, which provided an inoculum to substrate ratio

(ISR) of 5±0.5% (g VS of the sludge/g VS of food waste). To ensure a consistent contact between inoculum and food waste, the reactor received an intermittent leachate recirculation from the bottom of the reactor to the top and sprayed on the food waste containment vessel uniformly at a flow rate of 4.4L/h. Leachate samples were collected from the recirculation line at every two days for 14 days. The LBR was operated at controlled pHs of 6, 7 and 8, respectively. The LBR was run at each pH in duplicate and the bioreactor performance was shown as averaged results.

3.2.4. Analytical methods

TS, VS and chemical oxygen demand (COD) were quantified with the Standard Methods (American Public Health Association, 2005). Soluble COD (sCOD) was measured after filtering leachate with membranes (pore size 0.45μm). Volatile fatty acids (VFAs) and simple alcohols in liquid samples were analyzed using a gas chromatograph (HP 5890 Series II, Hewlett Packard, USA) equipped with a flame ionization detector (FID) and a capillary column (30m×0.53mm×0.5μm PAG, Supelco, Bellefonte, PA). The oven temperature for VFA analysis was programed to maintain 150°C for 2 min initially, then increase to 190 °C at a slope of 4°C/min and maintained at 190°C for 3 min. The oven temperature for alcohol measurement was programed to maintain 40 °C for 3 min initially, then increase to 60°C at a slope of 60°C/min and maintained at 60°C for 6 minutes. VFAs in leachate mainly consisted of acetic acid, propionic acid, and butyric acid, so in this study, the total VFA was approximated as the sum of the three acids. Methanol and ethanol were main alcohol products in the leachate, so total alcohol means the sum of methanol and ethanol here.

Gas composition was analyzed by injecting 0.5 mL of gas samples (model 1005 GASTIGHT syringe, Hamilton, Reno, NV) into a gas chromatograph (model 310, SRI Instrument,

USA) equipped with a thermal conductivity detector (TCD) and a 3m × 2.1mm Porapak Q 80-100 mesh column (Supelco, Bellefonte, PA) using argon as the carrier gas. The oven temperature was programed to increase from 50 °C (hold for 1 min) to 110 °C (hold for 1 min) in 8 minutes with an increasing rate of 10 °C/min. All chemical analyses were carried out in triplicate and reported in average data with standard deviations.

3.2.5. Calculation

The mass of cumulative sCOD in fermentation leachate with time was measured once every two days and stabilization of food waste at different pHs was evaluated. The mass of cumulative sCOD was calculated by multiplying volumes of the leachate with measured sCOD concentration.

Hydrolysis of food waste was calculated with Eq. 3.1.

$$\text{Hydrolysis yield(g cumulative sCOD } kg^{-1}VS_{added}) = \frac{\text{cum.sCOD (g)}}{VS_{added}\,(kg)} \quad (3.1)$$

where VS_{added}= initial VS of food waste loaded in the reactor, kg.

VFAs and alcohols were expressed as O_2 equivalent (i.e., theoretical COD) using half reactions of the organics and O_2 (1/4 O_2 + H^+ + e^- = ½ H_2O, 1 mol electrons = 8 g COD). The cumulative VFA with time was calculated as did for cumulative sCOD and was expressed as COD. VFA production efficiency was computed using VFA yield and VFA to sCOD ratio. VFA yield is cumulative VFA per VS of initial food waste (g COD/kg VS_{added}). VFA to sCOD ratio describes the percentage of solubilized matters converted to VFAs.

The energy consumptions for LBR operation was calculated with Eq 3.2 (J. Kim, Kim, Ye, & Lee, 2011).

$$P=Q\gamma E/1000 \quad (3.2)$$

where P is power consumption for pumping leachate (kW), Q is flow rate (m^3 s^{-1}), γ is 9800 N m^{-3}, and E is the hydraulic pressure head (0.15 m for mixing leachate and 1.77 m for circulating leachate to the containment vessel).

3.2.6. Biomass sampling, DNA extraction and 16S rRNA genes sequencing

Leachate samples (10 mL) were collected from the recirculation line every two days for two weeks (8 samples including day 0). Food residues were also sampled from the containment vessel at the end of each experiment cycle (day 14). Food residues were thoroughly mixed and 5g of samples were taken for analysis. Genomic DNA was extracted from the leachate and food residues samples with the Sox DNA Isolation Kit (Metagenom Bio Inc.) according to the supplier's recommendation. PCR was set in triplicates for each sample (25 µl each). Each reaction mixture contained 2.5 µl of 10 × standard Taq buffer, 0.5 µl of 10 mM dNTP, 0.25 µl of BSA (20 mg/ml), 5.0 µl of 1 µM forward primer (Pro341F: CCTACGGGNBGCASCAG), 5.0 µl of 1 µM reverse primer (Pro805R: GACTACNVGGGTATCTAATCC), 5.0 µl DNA, 0.2 µl of Taq DNA polymerase (5u/µl) and 6.55 µl of PCR water. DNA was denatured at 95°C for 5 min, followed by 35 cycles of 95 °C for 30 sec, 30 °C for 30 sec and 72 °C for 50 sec and then extended at 72 °C for 10 min (Takahashi, Tomita, Nishioka, Hisada, & Nishijima, 2014).

The triplicate PCR products were pooled and resolved with 2% TAE agarose gel. PCR products in equal amount of correct amplicons were pooled, gel purified and quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Inc.). Library DNA was sequenced with MiSeq Reagent Kit v2 (2 × 250 cycles). FASTQ files were generated for taxonomic analysis. Demultiplexed sequences were processed using DADA2 v1.6 (Benjamin et al., 2016) managed through QIIME 2 v.2018.2 (Caporaso et al., 2010). In this workflow forward and reverse reads

were truncated to 245 nucleotides, primers were removed, and paired reads were assembled. After chimera filtering an amplified sequence variant (ASV) table was constructed for downstream analysis. Taxonomy was assigned to representative sequences using a naive Bayesian classifier implemented in QIIME 2 trained against SILVA release 132 clustered at 99% identity. Assignments were accepted above a 0.7 confidence threshold.

3.3. Results and Discussion

3.3.1. Solubilization of food waste and energy consumption

Figure 3.2 shows the concentrations of sCOD, total VFA and total alcohol in the leachate with time at different pHs. The concentration of sCOD at pH 8 reached a plateau 2~3 days prior to that of pH 6 and pH 7 (Figure 3.2A). Considering volume changes due to sampling, the solubilization of food waste was accurately calculated with cumulative mass of sCOD. A cumulative sCOD of 118.2g was obtained under pH 8 on the 8^{th} day, as shown in Table 7.1, which accounted for 86.2% of the total sCOD production. In comparison, fractions of the cumulative sCOD of the total sCOD production were less at 69.5% and 67.1% under pH 6 and pH 7 at the same reaction time. This result indicates alkaline pH could accelerate the hydrolysis of food waste, and the literature also reported the same trend (Browne et al., 2013; Dinamarca et al., 2003; Hussain et al., 2017).Table 3.2 summaries solubilization of food waste after 14 days of reaction time. High solid removal and hydrolysis yield was obtained for all pH conditions, while pH 7 and 8 conditions showed slightly better solubilization than pH 6. For all pH conditions, TS and VS removal were 81-87%, and hydrolysis yield ranged from 810 to 883 g cum. sCOD kg^{-1} VS_{added}.

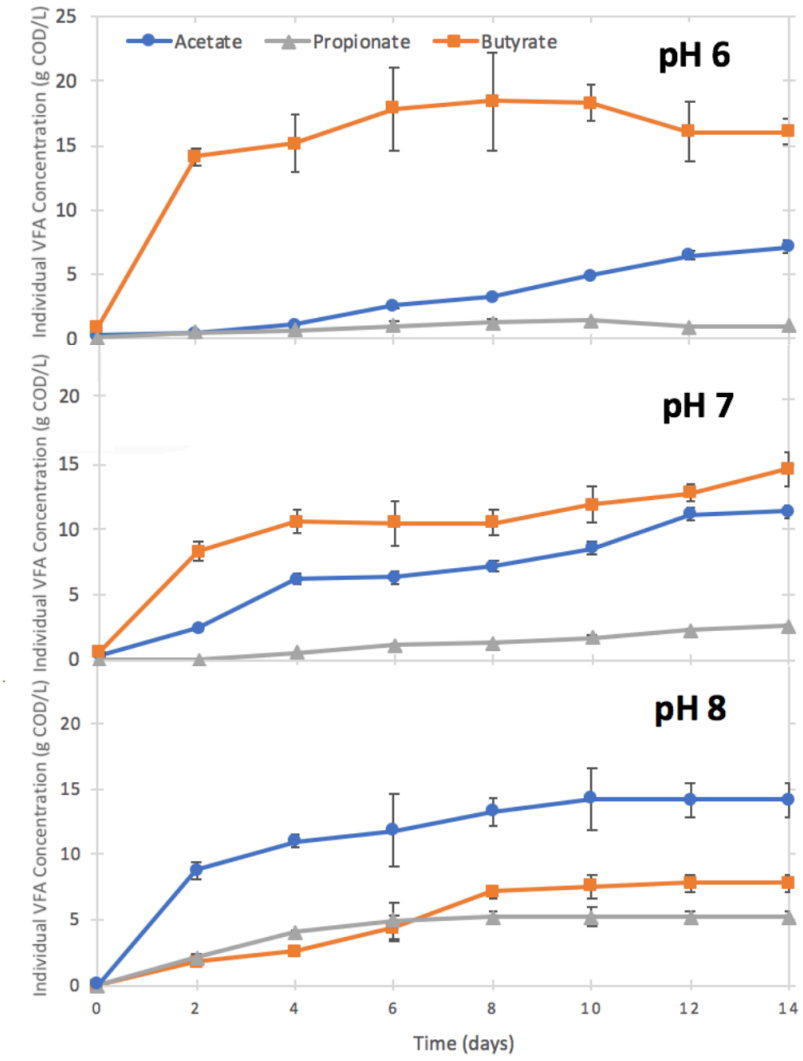


Figure 3.2 *Concentrations of soluble COD (A), VFA (B) and alcohol (C) in the leachate of the LBR operated under different pHs.*

Energy consumption for mixing leachate and circulating it on the top of the LBR was calculated at 0.017 kWh, according to Eq (3.2). VS removal was 0.13-0.14 kg in the LBR, which implies an energy consumption of 124-134 kWh per ton of food waste removed. The operating cost then is calculated at $13-15 per ton of food waste removed based on the electricity cost of $0.11 kWh^{-1} (Ontario hydro, 2018). This cost is much cheaper than $35-46 ton^{-1} (320-417 kWh per ton of food waste removed) of food waste in CSTRs (Moriarty et al., 2013; Naegele et al.,

2012), which supports that the LBR can achieve high solubilization of solid food waste with small energy input.

Table 3.2 *Reactor performance at different pH*

Parameters	pH 6	pH 7	pH 8
Soluble organic matter in the effluent (g sCOD/L)	33±2.2	33±1.1	35±0.8
Hydrolysis yield (g cum.sCOD/kg VS_{added})	811	883	883
Degradation efficiency (VS removed, %)	81	85	87
Degradation efficiency (TS removed, %)	81	84	86
Acetate (gCOD/L)	7±1.0	11±0.8	14±1.2
Propionate (gCOD/L)	1±0.2	2±0.6	5±0.3
Butyrate (gCOD/L)	16±1.0	14±0.6	8±0.2
VFAs (C2-C4) (g COD eq./L)	24±0.2	28±0.6	27±0.2
VFAs (C2-C4) to sCOD ratio (%)	72±1.2	84±2.1	77±1.7
VFA yield (g COD/kg VS_{added})	601	762	686

3.3.2. VFA production and distribution

The concentration of total VFA (sum of butyrate, acetate and propionate) in the leachate is presented in Figure 3.2B. The tendency of VFA production to time was similar to sCOD. At pH 8, VFA production was stabilized on the 8th day, while it was consistently increased at pH 6 and 7 after the 8th day. This is the same trend to sCOD pattern (Figure 3.2A). In 14 days the highest VFA production was obtained at pH 7 with a concentration of 28.6 g COD/L followed by 27.2 g COD/L at pH 8 and 24.3 g COD/L at pH 6. This result means that neutral pH is the optimal condition for VFA production in food waste fermentation in 14 days of reaction time, which was also reported in literature. Hussain et al. (2017) obtained a maximum VFA concentration of 36.5 gCOD/L at pH 7 from food waste in a thermophilic LBR, 40% higher than that obtained at pH 6. Zhang et al. (2017) showed a higher VFA production at pH 7 than at pH 5, 9 and 11 from anaerobic digestion of kitchen waste using a two-phased anaerobic digester. As shown in Table 3.2, the highest VFA yield was observed at pH 7 with 761.8 g COD kg^{-1} VS_{added}, followed by pH 8 and pH 6 with

686.15 and 600.9 gCOD kg^{-1} VS_{added}, respectively. The VFA to sCOD ratio, that showed the same trend to the VFA yield, ranged from 72 to 84%. This means that other products undetectable with the GC-FID would be formed during the fermentation of food waste, such as lactic acid (Tang et al., 2017; W. Zhang et al., 2017), as well as soluble microbial products (An et al., 2013; Daniel et al., 1994; Yeo et al., 2015).

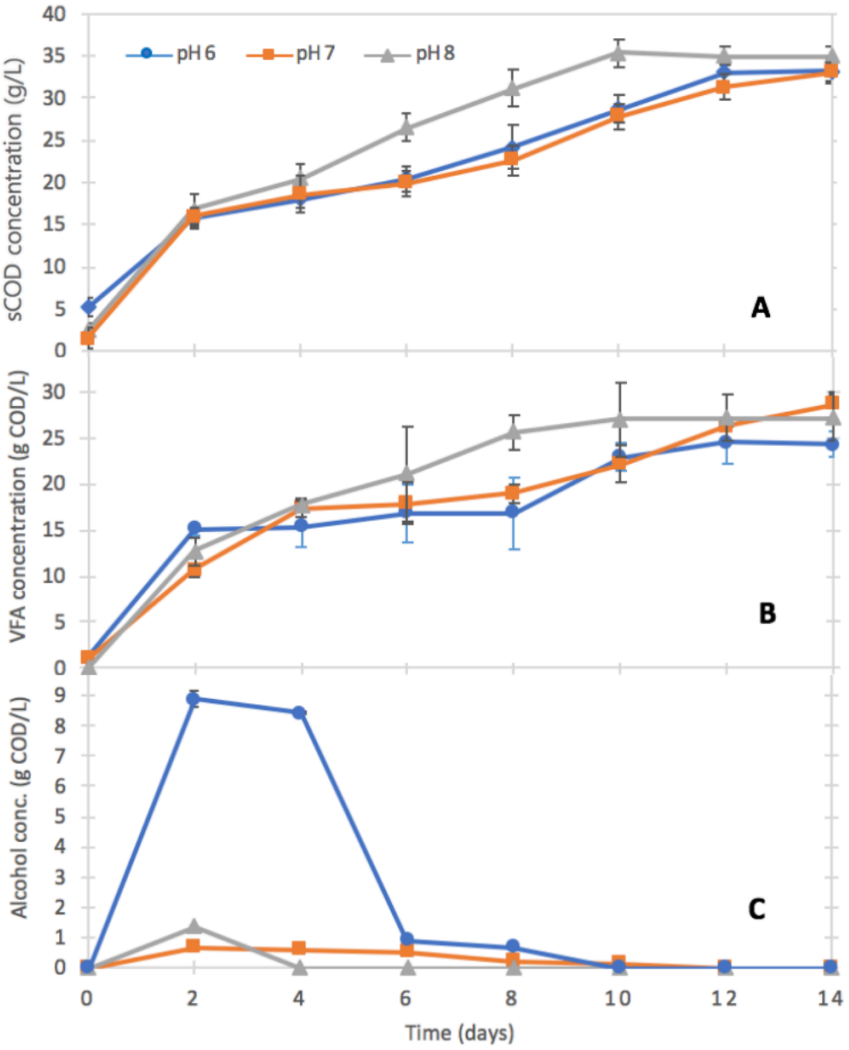


Figure 3.3 *Cumulative VFA concentration (acetate, butyrate, and propionate) in the leachate of the LBR run at different pHs.*

Figure 3.3 shows the concentration of main VFAs (i.e., acetate, propionate and butyrate) with time for each pH condition. For pH 6, butyrate concentration reached at 16 g/L, the highest

acid of the total VFA (70%), followed by acetate (7.1 g/L and 27%) and propionate (0.98 g/L and 4%). Butyrate was consistently the highest of the total VFA at pH 7, but acetate and propionate production increased at the neutral pH. At pH 8, the main VFA shifted from butyrate to acetate, which accounted for 52% of the total VFA (see Table 3.2). Literature commonly reported that pH is a key parameter selecting microbial community and consequently main metabolic pathway (H. S. Lee & Rittmann, 2009; H. S. Lee et al., 2009; H. S. Lee et al., 2008; Yeo et al., 2015): butyrate accumulation at acidic pH but the accumulation of propionate and acetate at neutral pH. These results suggest different microbial and enzymatic activities regulated by pH. Among the three main VFAs, acetate is the best metabolite in terms of energy gain because bacteria can conserve 2 mol ATP mol^{-1} acetate (0.125 mol ATP mol^{-1} electrons). In comparison, butyrate production involves 1 mol ATP synthesis mol^{-1} butyrate (0.05 mol ATP mol^{-1} electrons) and this ATP yield is 2.5-fold less than acetate per mole of electrons. Propionate production would not be coupled to ATP yield or ATP production would be negligible during propionate-producing pathways (Seeliger et al., 2002). The generation of butyrate (20 mol electrons mol^{-1}) and propionate (14 mol electrons mol^{-1}) involves 2 mol NAD(P)H oxidation per mole of metabolite, which means that bacteria can balance intracellular reducing powers through production of butyrate or propionate. In contrast, acetate production from acetyl-CoA does not entail oxidation of intracellular reducing powers (H. S. Lee et al., 2008; Leet et al., 1988; Rittmann et al., 2001). These analyses indicate that fermenting bacteria manage energy production and intracellular reducing powers by regulating VFA distributions (H. S. Lee & Rittmann, 2009; H. S. Lee et al., 2009, 2008). At pH 6, bacteria mainly produced energy and balanced intracellular reducing powers via the butyrate-acetate pathway. At pH 7 and 8 where propionate production increased, bacteria tended to conserve more ATP by increasing acetate production and balance the reducing powers with the combination of butyrate

and propionate.

3.3.3. Alcohol and biogas production

Alcohol (mainly ethanol) concentration was much lower than that of VFAs for all pHs, as shown in Figure 3.2C. Alcohol concentration was very low at pH 7 and 8. In comparison, cumulative alcohol concentration increased by 8.9 g COD/L at day 2 under pH 6, but it sharply decreased down to 0.91 g COD/L at day 6. This result suggests biotransformation of alcohol (mainly ethanol) to other metabolites at this acidic pH.

Biogas production started at day 2, but ceased at day 4 or 6, as shown in Figure 7.1 (Appendix A). The cumulative volumes of H_2 gas were 3.21, 6.97, and 6.40 L, respectively, at pH 6, 7, and 8. These H_2 productions are equivalent to 2.5-5.4 g COD calculated with the ideal gas law (H. S. Lee et al., 2008), which were consistently trivial against sCOD (132-140 g COD) or VFA (96-112 g COD) values in the leachate. In general, biohydrogen yield in mixed culture fermentation is highest at acidic pH between 5-6 (Elbeshbishy et al., 2017; H. S. Lee & Rittmann, 2009; H. S. Lee et al., 2009, 2010; Ranjan et al., 2015), but in this work H_2 production at pH 6 was lower than the other pH conditions. Such small H_2 yield and biotransformation of ethanol at pH 6 imply that the bacterial community at this acidic pH would be different from bacterial community structures generally identified at acidic, mixed-culture fermentation, such as *Clostridium*-enriched culture (Cabrol et al., 2017; H. S. Lee & Rittmann, 2009; S. Rittmann & Herwig, 2012).

3.3.4. Shift of bacterial community: pH effects

Figure 3.4 compared microbial communities in the leachates at pH 6, 7, and 8, which were sampled at day 10 when VFA production stabilized (see Figure 3.3). As expected from different VFA distribution, pH shifted bacterial community significantly. *Bifidobacterium* (75%) was the most

dominant player in the leachate fermented at pH 6, along with *Clostridium*. *Bifidobacterium* can hydrolyze dietary carbohydrates and fibers (resistant starches, cellulose, hemicellulose, glycogen, pectins, etc.) and ferment them into short chain fatty acids (e.g., lactate and acetate) (Pokusaeva & Fitzgerald, 2011). C*lostridium* typically identified as one of the most important bacteria in mixed culture acidic fermentation of organic waste (Cabrol et al., 2017) can ferment carbohydrates into acetate, butyrate and H_2 (H. S. Lee & Rittmann, 2009; Pokusaeva & Fitzgerald, 2011). The dominance of *Bifidobacterium* and *Clostridium* implies a close relationship between them. Rivière et al. (2016) reported the syntrophy, or called cross-feeding, between the two bacteria: *Bifidobacterium* ferments oligosaccharides to acetate, and then *Clostridium* transforms the acetate into butyrate. The butyrate formation via this cross-feeding need reducing powers (e.g., NADH), which might involve H_2 consumption coupled to NADH formation. This proposed coupling reaction between NADH and H_2 might account for low H_2 yield at pH 6.

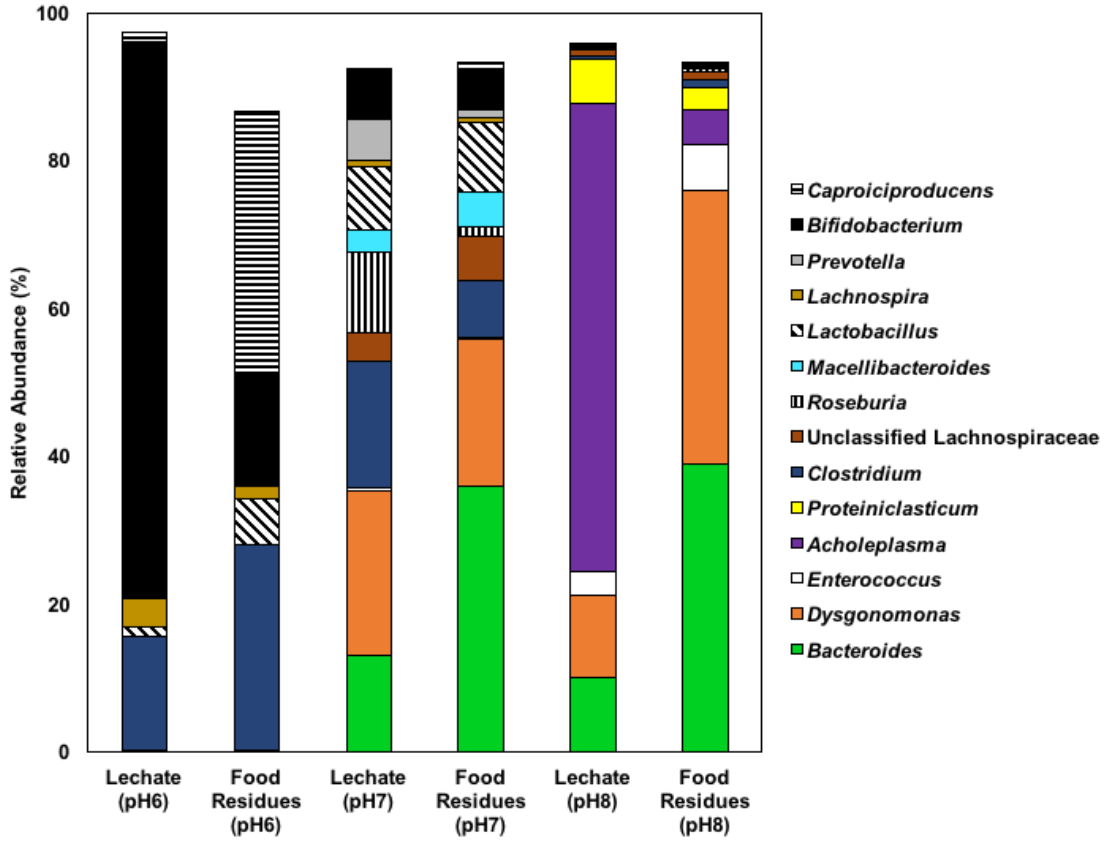


Figure 3.4 *Microbial communities in the leachate and food residues at pH 6, 7 and 8. The leachate was sampled for DNA extraction at day 10 when solubilization of food waste was stabilized. For food residues, DNA extraction was performed at day 14.*

The bacterial community in the leachate became very diverse at pH 7 where *Bacteroides, Dysgonomonas, Clostridium, Bifidobacterium, Roseburia, Lactobacillus,* and *Prevotella. Bacteroides* and *Lactobacillus* are hydrolyzing bacteria found in anaerobic digestion (Wan et al., 2013; Zamanzadeh et al., 2016) operated close to neutral pH. *Bacteroides* is one of the main bacteria in the gastrointestinal tract (Threadgill et al., 2011) and ferments dietary fibers (hemicellulose, pectin, etc.) into acetate (Mirande et al., 2010; Threadgill et al., 2011). *Dysgonomonas* identified in kraft lignin sludge (Duan, Liang, Wang, Du, & Wang, 2016) has lignocellulolytic potential and ferments glucose to propionate, acetate, lactate and succinate (Hofstad et al., 2018; Sun et al., 2015; Y. Yang et al., 2018). *Roseburia,* a genus of butyrate-

producing bacteria, can ferment dietary fibers into butyrate and lactate (Mirande et al., 2010). At pH 8, *Bacteroides* and *Dysgonomonas* genus were still abundant in the leachate, but the population of *Acholeplasma* became as high as 62% at this alkaline pH. Literature reported that *Acholeplasma* grew well at alkaline pH (optimum pH ~8.5) and fermented carbohydrates to acetate, lactate and alcohols (Lelong, 1989).

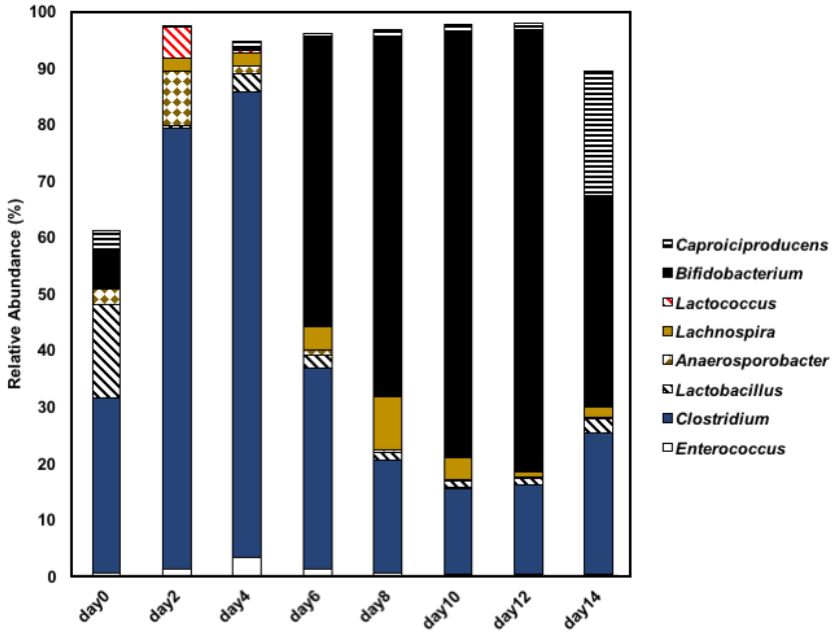
The bacterial populations in the food residues generally reflected the community structure in the leachates. These similar patterns of bacteria community between the leachates and food residues are reasonable because they had been mixed through leachate circulation. However, some bacteria were enriched only in leachates or food residues (see Figure 3.4). For instance, *Caproiciproducens* grouped in *Clostridium* cluster IV (B. Kim, et al., 2018) was as high as 35% in the food residue at pH 6, while it was only 0.4% in the leachate. Metabolic features of *Caproiciproducens* in acidic fermentation of food waste has not studied much, so it is limited to interpret enrichment of *Caproiciproducens* only in the food residue, not in the leachate. One reason for this difference might be longer reaction time in the food residue because the food residue was sampled after 14 days; in comparison, the leachate was sampled in day 10. Secondary fermentation reactions, that can transform simple VFAs into medium chain fatty acids, could occur when production of simple VFAs becomes stable. A stable concentration of cumulative VFAs was observed, as shown in Figure 3.2B. Literature reported that *Caproiciproducens* is a cellulose-hydrolyzing bacterium with extracellular enzymes (Opdahl, 2017), and can produce medium chain fatty acids (Andersen et al., 2017; B. Kim et al., 2018). Thus, Caproiciproducens could be enriched at the food residue in acidic fermentation. At pH 8, Acholeplasma was substantially enriched in the leachate, while *Bacteroides* and *Dysgonomonas* dominated the food waste residue. *Bacteroides* and *Dysgonomonas* can produce extracellular polysaccharides facilitating biofilm formation, grow

well at pH 7-8, and hence they have been often identified from biofilm environments at neutral pH (Chatzidaki-livanis et al., 2008; Cuthbertson et al., 2009). This partially explains the high population of *Bacteroides* and *Dysgonomonas* in the food residues at both pH 7 and 8.

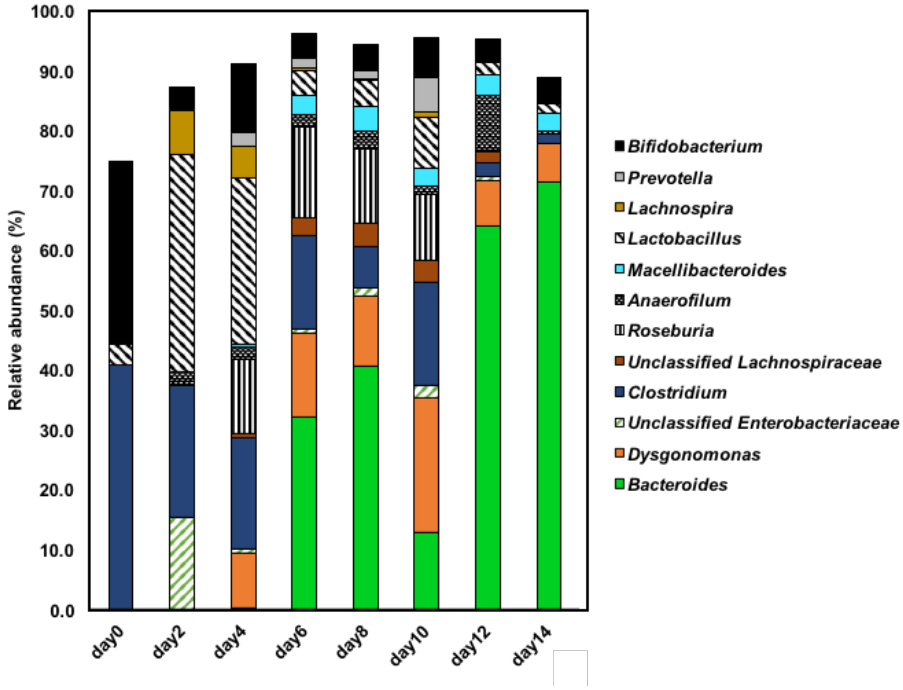
3.3.5. Bacterial community dynamics in the leachates at pH 6 and 7

VFA yield was maximized in food waste fermentation at neutral pH, and hence more detailed information on bacteria community structures at pH 7 was analyzed against acidic fermentation that has been well studied. Figure 3.5a shows the dynamic change of bacterial community in the leachate fermented at pH 6. Relative abundance of *Clostridium* was highly dominant until day 4 when butyrate production was active (see Figure 3.3). The population of *Clostridium* decreased down to 36 % at day 6 from which no significant change of butyrate concentration was observed. From day 6, *Bifidobacterium* gradually increased, and its population became highest at 78% in day 12. The community dynamics suggest that *Clostridium* would mainly ferment biodegradable organics into acetate and butyrate at initial fermentation step (~ 4 days), and *Bifidobacterium* began to ferment resistant dietary fibers (cellulose, hemicellulose, pectins, etc.) mainly into acetate and lactate (Roberfroid et al., 2010). In addition, the change of bacteria community clearly proves the syntrophy between *Clostridium* and *Bifidobacterium*, but their metabolic relationship found in this work seemed different from typical cross-feeding in literature: *Clostridium* synthesize butyrate with the acetate produced by *Bifidobacterium* (Rivière et al., 2016). As shown in Figure 3.3 (pH 6), butyrate formation ceased from day 6 and cumulative butyrate concentration decreased with time. This implies that *Bifidobacterium* might produce acetate from the butyrate generated by *Clostridium*. Literature proves acetate production or consumption via butyryl coenzyme A (CoA) and acetate-CoA transferase (Duncan et al., 2002). This interpretation accounts for decrease in

butyrate from 10 to 14. At day 14, *Bifidobacterium* decreased, and *Caproiciproducens* dramatically increased, suggesting the synthesis of medium chain fatty acids, as discussed early (Andersen et al., 2017).



(a)



(b)

Figure 3.5 *Microbial community dynamics. (a) pH 6 and (b) pH 7.*

At pH 7, *Lactobacillus* (~35%) and *Clostridium* (~20%) were dominant during the first four days of fermentation (Figure 3.5b). *Lactobacillus* and *Clostridium* can hydrolyze particulate carbohydrates and proteins (Aristoy et al., 1999; Xie et al., 2016; Zhang et al., 2005). This dynamic pattern of bacteria community at pH 7 is similar to pH 6, except for relatively high population of *Lactobacillus* which can diversify fermentation products from lactate to acetate, formate, and ethanol at neutral pH or close to it (Rhee et al., 1980; Torino et al., 2001). From day 6 *Bacteroides,* that well ferments dietary fibers into acetate and propionate, gradually dominated bacteria community, along with *Dysgonomonas*, and the exclusive abundance of *Bacteroides* was maintained until day 14. This result indicates that *Bacteroides* and *Dysgonomonas* well ferment dietary fibers resistant to biodegradation by other fermenters (e.g., *Clostridium*).

Chapter 4. Fermentation of Food Waste in a Leach Bed Reactor: Effects of Inoculum to Substrate Ratio

4.1. Introduction

Global economic development and continuous population growth have led to substantial increases in the production of municipal solid waste, about 30% of which are food waste (Zhou et al., 2018). Canada is reported to be among the world's top food waste producers on a per capita basis, with each individual generating on average 396 kg of food waste per year. This harrowing statistic have brought food waste management to the forefront of issues faced by the public and Canadian government (Government of Ontario, 2018). Food waste is both rich in nutrients and highly biodegradable. If treated under appropriate technologies, it can be processed into value-added products that benefit the surrounding community (Agler et al., 2011). Without proper resource recovery technologies for food waste, it can become a serious source of contaminants for the environment. For instance, in its natural environment, food waste generates odor and emits greenhouse gases (GHG) from its decomposition, which contributes to air pollution and climate change. Moreover, concentrated leachate generated during decomposition (Zhou et al., 2018) may seriously contaminate soil and water bodies when discharged without proper treatment. The most commonly used method of food waste treatment in Canada are landfilling and incineration. However, these conventional methods have drawbacks. Landfilling requires a large number of land areas, and can generate GHG, i.e. methane and CO_2, when biodegradable organics break down in the landfill sites due to anaerobic biological reactions (B. Zhang et al., 2017). Incineration requires a massive amount of energy because food waste has a high-moisture content, resulting in intensive energy input during its water vaporization (Zhou et al., 2018). To improve economic

efficiency and sustainability in food waste management, it is essential to develop a more cost-effective and sustainable treatment process.

The fermentation of food waste has attracted increasing attention, since it allows for both the stabilization and generation of valuable products, such as biochemicals (Zhou et al., 2018). Fermentation is a redox reaction that efficiently breaks down complex organic matter into simple acids (i.e., volatile fatty acids (VFAs)), alcohols and hydrogen gas. These end products can be further processed to synthesize valuable chemicals, e.g. butanol and polyhydroxyalkanoates (PHA) (Agler et al., 2011).

Continuous stirred tank reactors (CSTRs) are one of the most widely used bioreactors in food waste treatment. However, CSTRs are not ideal for treating high solid organics such as food waste (20-30% solids) because mechanically stirring high solids requires high-energy inputs. Hence, many studies diluted the solid content of food waste to less than 10% prior to treatments in CSTRs (Browne et al., 2013; Mac et al., 2000; Tang et al., 2017). To resolve this requirement of highly intensive mixing in CSTRs, some works proposed a leach bed reactor (LBR) separating high solid substrate from fermented cocktails. (Brown et al., 2013; Hussain et al., 2017). Briefly describing LBR features, food waste is firstly loaded in the top section of the reactor. Fermented liquid from the food waste slowly leaches out during the fermentation and is collected in the bottom section of the reactor. Instead of intensive mixing, the reactor receives a leachate recirculation from the bottom of the reactor to the top and sprays on the food waste containment vessel uniformly to ensure the contact between microorganisms and the food waste. Due to these features, LBRs are able to treat extremely high solids organic wastes up to 50% of solids content (Jha et al., 2011). In addition, because intensive mixing is not required in LBRs, energy inputs for mixing are minimized.

There are several parameters influencing the performance of LBRs (i.e., solid reduction and VFA production), and among them inoculum to substrate ratio (ISR) is critical for determining the fermentation kinetics, solubilization, and solid reduction of food waste (Xu et al., 2012). Literature has commonly reported that a higher production of VFA can be obtained with a higher ISR due to the improved biochemical reactions of bacteria (Zhou et al., 2018). Various organic wastes including sunflower oil cake and municipal solid waste have been used as feedstock to study the effects of ISR on methane production (Boulanger et al., 2012; Raposo et al., 2009), but the information on ISR effects on fermentation of food waste in LBRs is limited. In several literatures experimental conditions (e.g., substrate, inoculum, pH, temperature, mixing conditions, etc.) in food waste fermentation were inconsistent, and thus it is challenging to determine optimal ISR for food waste fermentation in LBRs (Zhou et al., 2018). The goal of this study is to evaluate the influence of ISR on the hydrolysis of food waste and the VFA yield in a mesophilic LBR, while pH and temperature are fixed during the experiments.

4.2. Material and Method

4.2.1. Characteristics of food waste and inoculum

Food waste was collected from a cafeteria at the University of Waterloo (Waterloo, ON, Canada). To minimize variations in the food waste composition, approximately 20 kg of the food waste was collected in bulk and used for this study. After collection, the food waste was manually screened for non-biodegradable materials (e.g., egg shells). The sorted waste mainly consisted of vegetables and fruits. Using a commercial mesh chopper (Starfrit, Canada), the samples were chopped to an average particle size of ~ 1 cm. After that, the samples were thoroughly mixed and stored at -20ºC in airtight plastic bags (Ziploc bags, SC Johnson, USA) to avoid deterioration of the samples. The

samples were defrosted at 4ºC for 24 h before the experiments.

A leach bed reactor (LBR) designed for fermentation of food waste was inoculated with anaerobic digestion sludge monthly sampled from a full-scale municipal wastewater treatment facility (Kitchener Wastewater Treatment Plant, Region of Waterloo, Ontario, Canada). To kill or inactivate methanogens the sludge was heated to 75 °C for 15 minutes before use. The characteristics of food waste and inoculum used in this study are shown in Table 4.1.

Table 4.1 *Characterization of food waste and inoculum*

Parameters	Food waste	Inoculum
tCOD(g/L)[a]	264±27	10.5±1.2
sCOD (g/L)	/	5.1±0.8
TS(%)	16.5±0.2	1.5±0.5
VS(%)	15.5±0.7	1.2±0.2
VS/TS(%)	94	75
TS (g/L)	165±3	15.6±1.3
VS (g/L)	155±2	11.7±0.2

[a]food waste was blended with a blender after adding deionized water, and the COD was measured after appropriate dilution (~350 dilutions). tCOD data were averaged in five measurements.

4.2.2. Reactor Design

The cylindrical LBR used in this study was made from acrylic materials and had a total volume of 7.5L with a diameter of 135mm and a height of 690mm. It comprised of 3 parts: Firstly, a removable top cover with o-rings to ensure an anaerobic environment, a headspace to allow for gas collection and a nozzle attached to the removable cover; Secondly, a middle section comprising of a containment vessel to hold the food waste; And thirdly, a leachate holding bed with a funnel-shaped bottom, which is shown in Figure 4.1. A customized nozzle head was placed on the underside of the removable top cover to drip the leachate evenly over the food waste below. The containment vessel, where the food waste was held, had a height of 180mm and an effective volume of 1L. It was fabricated from polyethylene terephthalate (PET) walls and was evenly

divided into three sections horizontally with three pieces of thick mesh (pore size 4mm) that prevented food waste particles from entering the leachate holding bed. The percolated leachate then was collected in the 4L capacity leachate holding bed at the bottom of the reactor before it was recirculated by a timer (MODEL XT-4, ChronTrol Corporation, USA) activated peristaltic pump (MODEL 115 VAC, Masterflex, Canada). Samples were taken from this recirculation line for routine analysis. A pH controller (MODEL 5656-00, Cole Parmer, USA) was coupled to the leachate holding tank via a pH probe inserted into the retaining wall, allowing for continuous monitoring and regulation of the leachate pH with automatic injections of 1M NaOH. The reactor was equipped with a gas counter (MilliGascounter, Ritter Apparatus, Bochum, Germany) attached to the top cover to analyse biogas production.

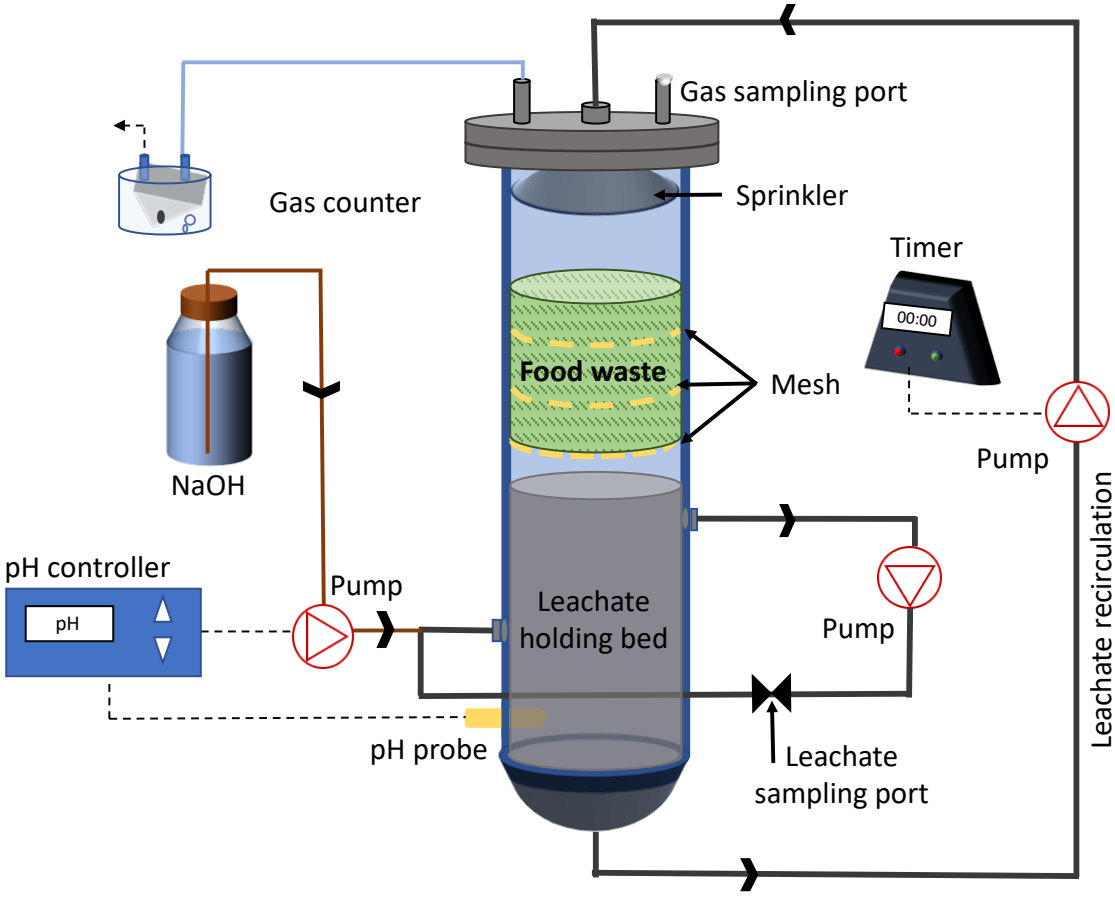


Figure 4.1 *Schematic of the leachate bed reactor (LBR).*

4.2.3. Experimental set-up

The LBR was continuously operated and monitored in batch mode for 14 days at room temperature (22°C). The food waste and inoculum were added to the LBR in the same procedure for each batch. The total solid (TS) and volatile solid (VS) of the food waste before and after fermentation were quantified to determine TS and VS reduction. The reactor was initially loaded with 1 kg of the food waste (wet weight). Three volumes of pre-heated AD sludge, i.e. 0.6L, 1.3L, and 2L, was added to the reactor initially, providing an ISR of 5±0.5%, 10±0.6%, and 15±0.5% (g VS of the sludge/g VS of food waste), respectively. In addition, DI water was added with the inoculum to make an equal startup inoculum volume of 2.5L. A pH of 6 was monitored and maintained in the leachate throughout the experiments using a pH controller. To ensure a consistent contact between inoculum and food waste, the reactor received an intermittent leachate recirculation from the bottom of the reactor to the top and sprayed on the food waste containment vessel uniformly at a flow rate of 4.4L/h. Leachate samples were collected from the recirculation line at every two days for 14 days. The leachate was tested for total chemical oxygen demand (tCOD), soluble chemical oxygen demand (sCOD), and VFAs.

The effect of ISR on the hydrolysis and acidogenesis of acidogenic fermentation from the food waste was examined by operating the LBR at controlled ISR of 5%, 10% and 15% (vs/vs), hereafter called ISR 5%, ISR 10%, and ISR 15%.

4.2.4. Analytical methods

The TS, VS and chemical oxygen demand (COD) were quantified with the Standard Methods. The soluble COD (sCOD) was measured after filtering leachate with membranes (pore size 0.45μm).

Volatile fatty acids (VFAs) in liquid samples were analyzed using a gas chromatograph (HP 5890 Series II, Hewlett Packard, USA) equipped with a flame ionization detector (FID) and a capillary column (30m×0.53mm×0.5μm PAG, Supelco, Bellefonte, PA). The oven temperature for VFA analysis was programed to maintain 150°C for 2 min initially, then increase to 190 °C at a slope of 4°C/min and maintained at 190°C for 3 min. VFAs in leachate mainly consisted of acetic acid, propionic acid, and butyric acid, so total VFA was calculated through the combination of these three acids in this study.

Gas composition was analyzed by injecting 0.5 mL of gas samples (model 1005 GASTIGHT syringe, Hamilton, Reno, NV) into a gas chromatograph (model 310, SRI Instrument, USA) equipped with a thermal conductivity detector (TCD) and a 3m × 2.1mm Porapak Q 80-100 mesh column (Supelco, Bellefonte, PA) using argon as the carrier gas. The oven temperature was programed to increase from 50 °C (hold for 1 min) to 110 °C (hold for 1 min) in 8 minutes with an increasing rate of 10 °C/min. All chemical analyses were carried out in triplicate and reported in average data with standard deviations.

4.2.5. Calculation

The mass of cumulative sCOD in fermentation leachate with time was measured on a bi-daily basis and can evaluate the stabilization of food waste at different ISRs. The mass of cumulative sCOD was calculated by multiplying volumes of the leachate with measured sCOD concentration. Hydrolysis of food waste was calculated with Eq. 4.1.

$$\text{Hydrolysis yield}(\text{ g cumulative sCOD } kg^{-1}VS_{initial}) = \frac{\text{cum.sCOD (g)}}{VS_{initial}\,(kg)} \quad (4.1)$$

where $VS_{initial}$= initial VS of food waste loaded in the reactor, kg.

VFAs were expressed as O_2 equivalent (i.e., theoretical COD) using half-reactions of the

organics and O_2 (1/4 O_2 + H^+ + e^- = ½ H_2O). The cumulative VFA with time was calculated in the same manner as that of cumulative sCOD and was expressed in COD equivalent. VFA production efficiency was computed using VFA yield (cumulative VFA/$VS_{initial}$) and VFA to sCOD ratio. VFA yield (g COD/kg VS_{added}) stands for the VFA production as g COD per the VS of food waste added to the reactor initially. VFA to sCOD ratio describes the percentage of solubilized matters converted to VFAs.

4.3. Results and Discussion

The LBR was operated for 14 days at 22°C under controlled ISR conditions with target values of 5%, 10% and 15%. The actual ISR values were 5±0.5%, 10±0.6%, and 15±0.5% respectively.

4.3.1. Hydrolysis and substrate degradation

In the hydrolysis step, complex compounds such as carbohydrates, proteins and lipids are broken down into soluble sugar, amino acid and fatty acids. Thus, the status of food waste hydrolysis was represented by the sCOD concentration in the leachate. The sCOD concentrations that were observed versus time at ISR 5%, 10% and 15% are shown in Figure. 4.2 A. A maximum sCOD concentration of 30-33g/L was obtained under all ISR conditions. Correspondingly, the cumulative sCOD was 119.5±2.8g, 130.6±0.7g and 129.9±1.6g at ISR 5%, 10% and 15% respectively. The results indicate that the increase of ISR from 5% to 15% did not have significant influences on the extent of organic solid particle solubilization, suggesting that the food waste was almost fully digested under the lowest ISR. However, the concentration of sCOD at ISR 10% reached a plateau for 2~3 days prior to that of ISR 5% and ISR 15%, which implied that a higher IS ratio, i.e. from 5% to 10% and 15%, may enhance the reactor performance of food by reducing the required solids retention time. However, a relatively slower rate of hydrolysis was obtained at ISR 15% compared

to that of ISR 10%, which is the result of the high concentration of inoculum at ISR 15%. It was observed that under ISR 15%, the texture of the leachate became very viscous, resulting in an unstable performance of the reactor. Similar outcomes were reported in other literature as well (Lim, 2008; Zhou et al., 2018). The same trend of sCOD leaching can be observed in Table 8.1 that presents the cumulative COD masses (see Table 8.1 in Appendix B). A cumulative sCOD of 122.3±1.5g was obtained at ISR 10% on the 8th day, which accounted for approximately 93% of the total sCOD production. Correspondingly, around 76% and 82% values were observed under ISR 5% and ISR 15%, which were 10-20% lower than ISR 10%. Therefore, it appears that the increase of ISR from 5% to 10% accelerated the hydrolysis rate of the food waste, which lead to a decrease in digestion time.

The degree of food waste solubilization was also reported as the hydrolysis yield and VS degradation efficiency. The hydrolysis yield (g cum. sCOD kg-1 VSadded) was defined as the ratio of the cumulative sCOD production to the initially loaded VS of the food waste. The degradation efficiency (%) was determined as the percentage of VS removed from the substrate to the VS added initially to the reactor. The hydrolysis yields were slightly higher at ISR 10% (837) and ISR 15% (833) compared to that of ISR 5% (767), while a similar degradation efficiency of 85%, 91% and 90% was obtained at ISR 5%, 10% and 15%, respectively. The increase of ISR from 5% to 10% resulted in an enhancement of VS degradation of food waste. Thus, the results indicated that higher ISR conditions could improve the hydrolysis of food waste, which has been observed in previous studies as well. Xu et al. (2012) obtained an improvement of 60% in hydrolysis rate and efficiency by increasing the ISR from 0% to 6.9% (vs/vs) in a leach bed reactor. Rizwan et al. (2015) investigated the hydrolysis efficiency of the co-digestion of food waste and rice husk under ISR 0.5, 0.67, 1, 2, and 4 (vs/vs) in laboratory glass bottles and reported the highest

TS and VS degradation at the highest ISR value of 4. The better performance of organic waste hydrolysis under higher ISR conditions may due to the increased amount of active microorganisms presenting in the food waste holding vessel.

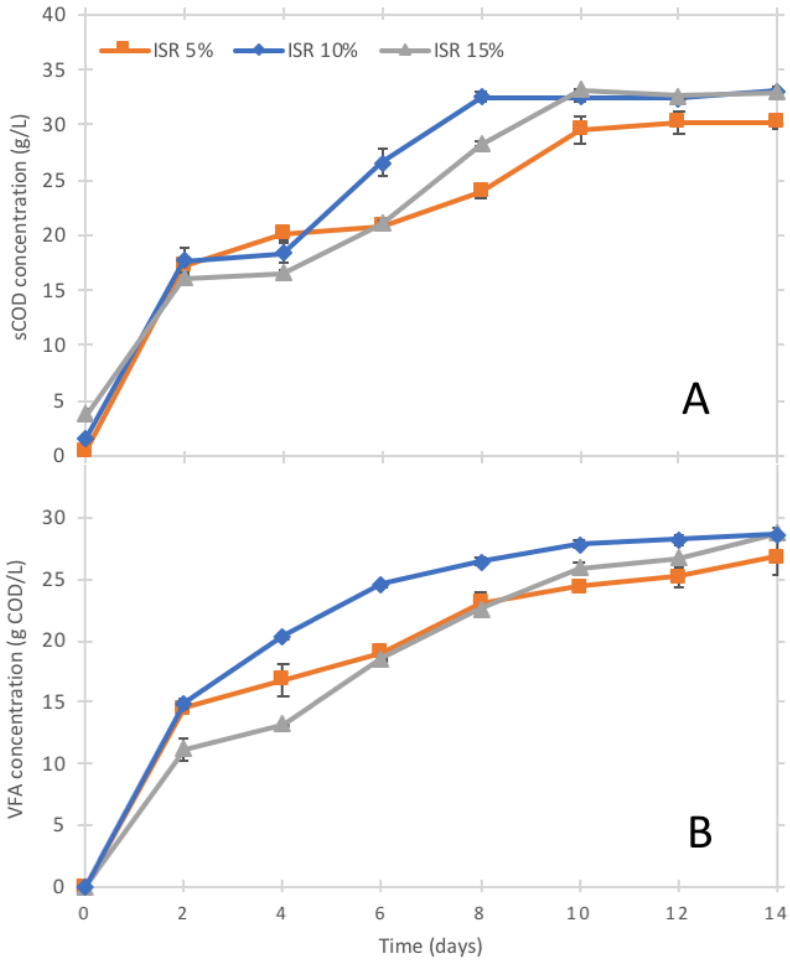


Figure 4.2 *Concentrations of soluble COD (A) and VFA (B) in the leachate of the LBR operated under different ISRs.*

4.3.2. VFA production

VFAs represent one of the main products of the acidogenesis process. The concentrations of total VFAs (as COD) measured in the leachate over the fermentation period (14 days) are shown in

Figure. 4.2 B. The trends in VFA production against time were similar to that of sCOD. VFA concentration started from 0-1 g/L as COD initially and experienced a rapid increase during the first two days for all the ISRs. Afterwards, the acidogenesis rate slowed down and remained relatively stable till the end of all experiments. It was observed that the increase of VFA concentration at ISR 10% experienced a higher rate compared to that of ISR 5% and 15%. At ISR 10%, 77% of the total VFAs production was achieved on the 6^{th} day, while the VFAs production accounted for 64% of the total VFAs under ISR 5% and ISR 15% on the same day. However, the VFA productions under ISR 5%, 10%, and 15% at the endpoint of the 14-days experiments were comparable with concentrations of 26±0.8 g/L as COD, 28±0.4 g/L as COD, and 28±0.2 g/L as COD, respectively. The corresponding cumulative VFA production values were 105±1.5 as COD, 104±0.2 as COD, and 108±0.5g as COD.

The acidogenesis efficiency of anaerobic digestion can also be determined by VFA yield (g COD/kg VSadded) and the VFA to sCOD ratio (%). As shown in Table 4.2, high VFA yields and VFA to sCOD ratios were obtained under all ISR conditions. VFA to sCOD ratios were 84-87%, and VFA yields ranged from 669 to 695 g cum. g COD kg-1 VS_{added}. These results indicated that the increase in ISR did not influence the acidogenesis of food waste in this study. Literatures commonly reported the production rate of VFA closely reflects the growth of acidogenic bacteria presenting in reactors (Guo et al., 2014; Xu et al., 2012). The rapid increase of VFA concentration during the first 2-4 days indicated a high growth rate of acidogenic bacteria under all conditions, while ISR 10% showed slightly better acidogenesis than ISR 15% and ISR 5%. However, the VFA production rate slowed down for the following days of the fermentation periods, may because the acidogenesis step was limited by the hydrolysis, since the sCOD and VFAs leaching shared the similar tendency under all ISR conditions. The same observations were reported in previous

studies. Hydrolysis is often the rate-limiting step for anaerobic fermentation, while acidification is the fastest reaction in anaerobic fermentation due to the high growth rate of acidogenic bacteria (Ma et al., 2013).

Table 4.2 *Reactor performance at different ISR values*

Parameters	ISR 5%	ISR 10%	ISR 15%
Soluble organic matter in the effluent (g sCOD/L)	30±0.7	33±1	32±0.4
Hydrolysis yield (g cum.sCOD/kg VSadded)	767	837	833
Degradation efficiency (VS removed, %)	85	91	90
Degradation efficiency (TS removed, %)	86	92	88
Acetate (gCOD/L)	7±0.8	7±0.3	8±0.2
Propionate (gCOD/L)	1±0.6	1±0.1	2.5±0.1
Butyrate (gCOD/L)	17±0.3	20±0.5	18±0.4
VFAs (C2-C4) (g COD eq./L)	26±0.8	28±0.4	28±0.2
VFAs (C2-C4):sCOD (%)	86	84	87
VFA yield (g COD/kg TVSadded)	678	669	695

4.3.3. VFA distribution

The VFAs produced in this study mainly consisted of acetate, butyrate and propionate. The analysis of individual VFAs was considered to be crucial since differences in the VFA composition can strongly affect the utilization of the fermented leachate. The concentration of individual VFAs against time are presented in Figure. 4.3. Butyrate was the most prevalent VFA accounting for more than 60% of the total VFA at the end of the experiments under all ISRs. The second most common VFA was acetate, which represented for more than 25% of the VFA mix and followed by 5-10% of propionate. The highest concentration of butyrate was obtained at ISR 10% with 20±0.5g COD/L, while the highest acetate and propionate concentration was observed under ISR 15% with 8±0.2 g COD/L and 2.5±0.1 g COD/L, respectively. However, the differences of individual VFAs productions between these three ISRs were little, indicating that the influence of ISR on VFA distributions was negligible. This observation is consistent with previous studies (Guo

et al., 2014; Xu et al., 2012). Furthermore, a low propionate concentration was obtained at all ISRs, showing that acetate-butyrate pathway was the major metabolic pathway in this study. This VFA profile is similar to other fermentation studies with acidic pH conditions (pH 4-6) (Dinamarca et al., 2003; Hussain et al., 2017), which means pH is a key parameter selecting microbial community and consequently main metabolic pathway, resulting in particular VFA distributions (H. S. Lee & Rittmann, 2009; Zhou et al., 2018).

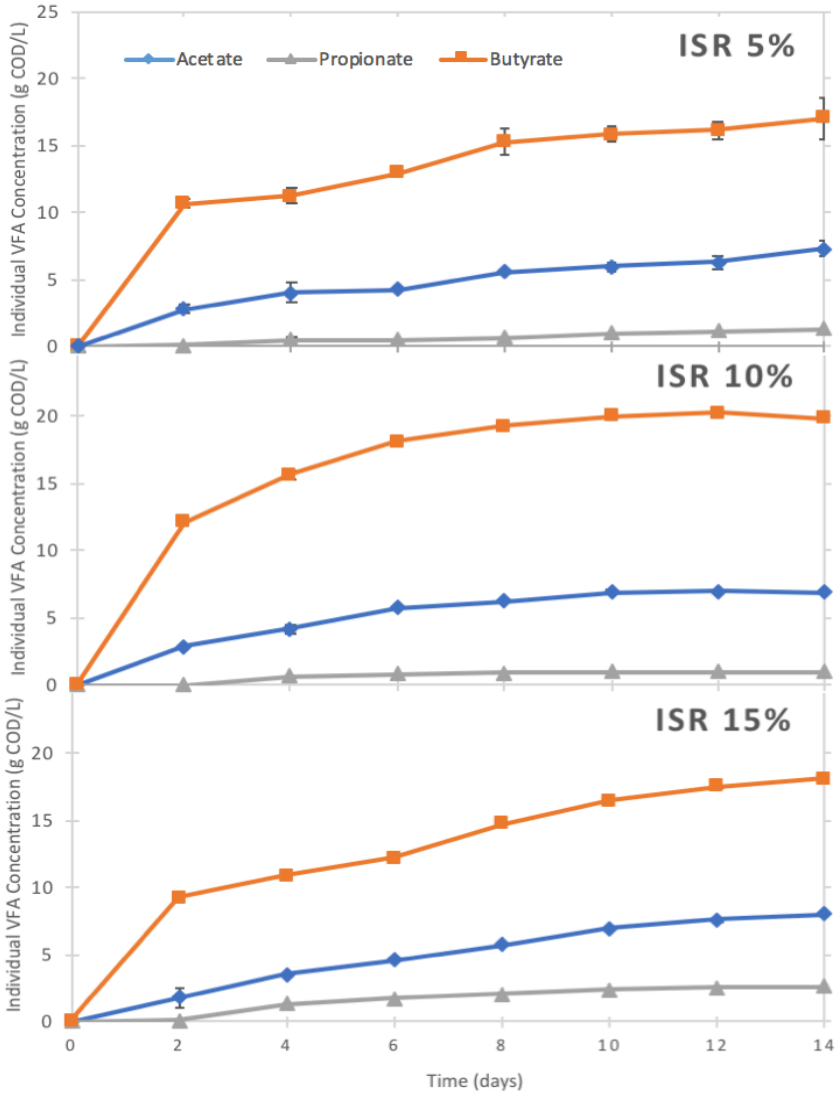


Figure 4.3 *VFA concentration (acetate, butyrate, and propionate) in the leachate of the LBR run at different ISRs.*

4.3.4. Biogas production

Biogas production started at day 2 but ceased at day 4 or 6 (see Figure 8.1). The cumulative volumes of H_2 gas were 6361±487, 16208±1574, and 17850±1445 mL, respectively, at ISR 5%, 10%, and 15%. The H_2 productions under ISR 10% and 15% were equivalent to around 12 g COD calculated with the ideal gas law, which was significantly higher than ISR 5% with ~4.5g COD equivalent.

Chapter 5. Conclusion

This study aimed to assess the impacts of pH and ISR on solid solubilization and VFA production in a mesophilic LBR treating food waste. The study tested a range of pH (i.e. pH 6, 7 and 8) and ISR (i.e. ISR 5%, 10% and 15%) with a fixed fermentation period of 14 days, and showed that a high hydrolysis and VFA yield can be achieved over all the operating conditions. These results demonstrated the potential of the LBR technology to accomplish resource recovery and generate value-added products from food waste treatment.

The specific conclusions are as follows:

Effect of pH on the fermentation of food waste in a LBR and its microbial community analysis: The LBR reduced food waste by 87% in 14 days with small energy input, and the treatment cost of food waste was only $13-15 (electric energy consumption) per ton of food waste removed in the LBR. VFA concentration was increased by 28.6 g COD/L, and butyric acid concentration was as high as 16 g COD/L in the leachate. Bacterial community dynamics suggested that resistant dietary fibers would be degraded after fermentation of biodegradable food waste and this sequential fermentation would be carried out by different groups of bacteria. This result implies that enriching fiber-degrading bacteria could be one of the key factors improving food waste removal.

Effect of ISR on the fermentation of food waste in a LBR: With an increase in ISR from 5% to 10%, the hydrolysis of food waste was improved in the LBR. In comparison, the increase of the ISR from 5% to 10% had negligible effects on VFA production and VFA distribution, suggesting that microbial concentration is saturated for acidogenic fermentation at 5% ISR. The highest ISR

of 15% reduced the hydrolysis efficiency of food waste treatment, and thus the ISR of 10% (vs/vs) is recommended for mesophilic food waste fermentation at pH 6 in the LBR.

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Appendix A: Supplementary data for chapter 3

Table 0.1 *Cumulative sCOD production and its portion in total sCOD production at different pHs*

Time	Cumulative sCOD production					
Day	pH 6		pH 7		pH 8	
	g	%	g	%	g	%
0	12.8±0.1	10.1%	3.6±0.6	2.6%	6.0±0.1	4.4%
2	48.2±0.6	38.1%	52.4±0.3	37.8%	52.7±2.3	38.3%
4	58.9±0.4	46.6%	66.7±3.8	48.5%	72.2±2.1	52.6%
6	71.3±1.4	56.4%	76.7±2.0	55.7%	92.9±2.1	67.6%
8	87.9±5.0	69.5%	92.5±2.9	67.1%	118.2±4.4	86.2%
10	104.0±1.8	82.3%	114.2±1.7	83.0%	133.4±2.3	97.0%
12	125.8±1.8	99.5%	131.4±2.1	95.4%	137.4±0.1	100.0%
14	126.4±0.7	100.0%	137.6±0.8	100.0%	137.4±0.1	100.0%

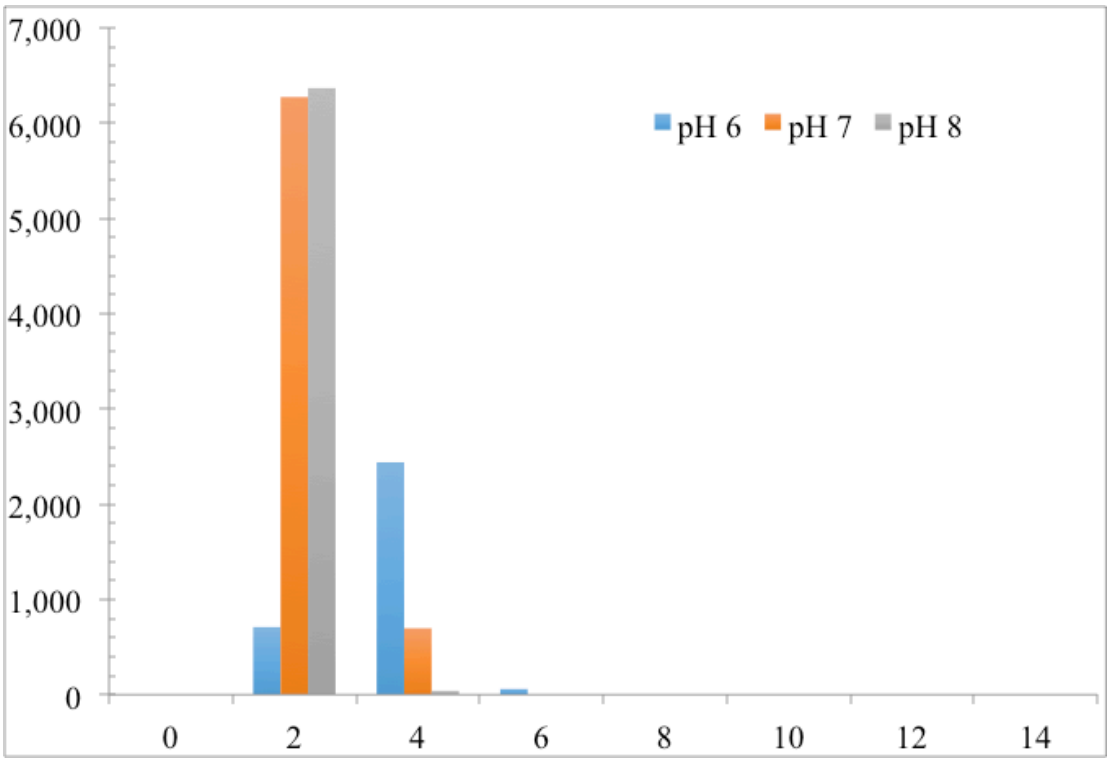

Figure 0.1 *H2 production in the LBR operated at pH 6, 7, and 8. Data was averaged in duplicate tests.*

Appendix B: Supplementary data for chapter 4

Table 0.1 *Cumulative sCOD production with time under different ISRs*

Time	Cumulative sCOD production					
Day	ISR 5%		ISR 10%		ISR 15%	
	g	%	g	%	g	%
0	1.3±0.6	1.1%	4.1±0.6	3.1%	9.4±1.4	7.2%
2	55.6±0.8	46.5%	57.0±4.2	43.7%	52.0±1.3	40.1%
4	70.0±2.1	58.6%	63.6±3.0	48.7%	57.4±1.2	44.2%
6	75.4±0.1	63.1%	95.8±4.5	73.4%	76.0±1.3	58.5%
8	90.5±2.2	75.7%	122.3±1.5	93.6%	106.2±1.0	81.8%
10	114.8±4.6	96.1%	126.2±1.1	96.7%	128.4±0.5	98.8%
12	118.9±3.7	99.5%	127.7±0.6	97.9%	127.8±0.5	98.4%
14	119.5±2.8	100.0%	130.6±0.7	100.0%	129.9±1.6	100.0%

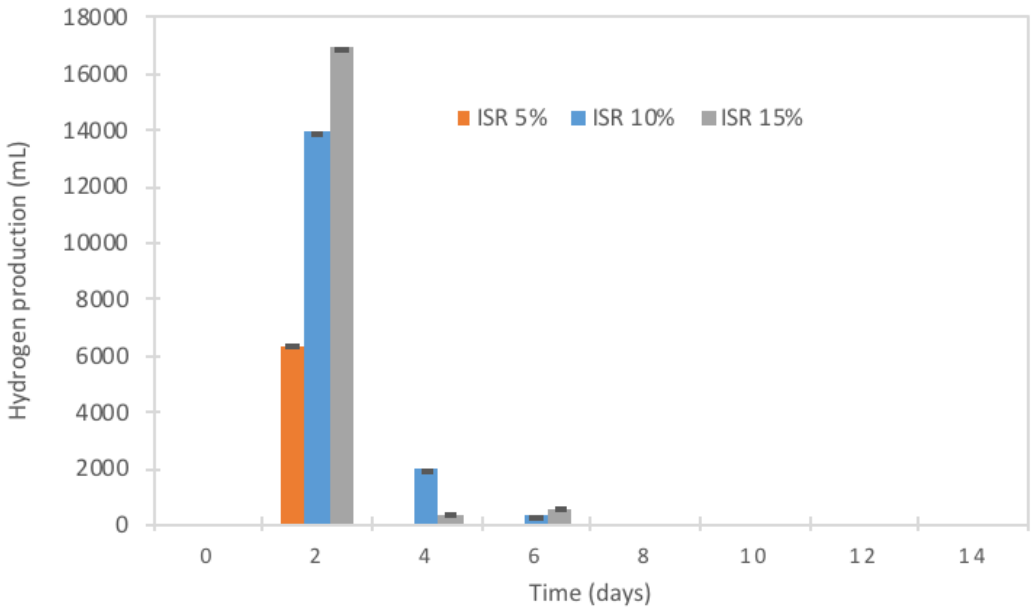

Figure 0.1 *Hydrogen production from the LBR under different ISR.*