Acidogenic Fermentation of Food Waste in a Leachate Bed Reactor at High Organic Loading: Effect of Granular Activated Carbon (GAC) and Inoculum

by

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Abstract

Food waste forms a major fraction of municipal solid waste worldwide, constituting 15-30% of municipal solid waste. If the post-harvest losses are included, the global food waste generation exceeds 1.3 billion tons a year, with economic losses exceeding $1 trillion. Landfilling is the primary method of food waste disposal in most countries, resulting in the loss of a valuable resource (food waste) and causing adverse health and environmental effects such as greenhouse gas emissions, pollution of the subsurface environment, and loss of habitat. To ensure environmental and public health, sustainable and cost-effective approaches for food waste stabilization are being intensively researched. Technologies that can stabilize and transform food waste into valuable products present a tangible solution to this challenge. Acidogenic fermentation is emerging biotechnology that transforms food waste to high-value biochemicals such as short-chain fatty acids (SCFAs); thereby combining sustainable management of food waste with resource recovery. Dry fermenters such as leachate bed reactors (LBRs) have received a lot of attention as an economical platform for acidogenic fermentation of food waste. However, LBRs have many operational challenges that need to be resolved including low product yields at high volumetric organic loading, and long fermentation times. This study evaluated the effects of granular activated carbon (GAC) and different inoculum type/enrichment methods to improve the hydrolysis and acidogenesis of food waste in LBRs operated at high volumetric loading (49 g $VS/L_{reactor}$).

First, the effects of four different GAC loadings of 0, 0.25, 0.38, and 0.51 g GAC/g $VS_{foodwaste}$ on the performance of LBRs (hydrolysis and acidogenesis) was evaluated. High GAC loading of 0.51 g GAC/g $VS_{foodwaste}$ achieved hydrolysis yield of 620 g SCOD/kg VS_{added} and acidification yield of 507 g COD_{SCFA}/kg VS_{added}, which were the highest

amongst all the GAC loadings. GAC loading also impacted the SCFA composition. For GAC loadings of 0.38-0.51 g GAC/g $VS_{foodwaste}$, butyrate was the dominant SCFA by constituting 57-60% of the SCFA produced, whereas the composition of acetate (38-40% of total SCFA) and butyrate (36-38% of total SCFA) was similar at low or no GAC loadings (0-0.25 g GAC/g $VS_{foodwaste}$).

The second study investigated the effects of inoculum type (return activated sludge and anaerobic digestion sludge), followed by inoculum enrichment on hydrolysis and acidogenesis of food waste. Return activated sludge as inoculum resulted in a 28-48 % higher hydrolysis and acidification yields than anaerobic digestion sludge as inoculum. Inoculum also affected the SCFA composition. Butyrate composed the major fraction (60%) of SCFA with return activated sludge, whereas acetate (36% of total SCFA) and butyrate (37% of total SCFA) had almost similar composition with anaerobic digestion sludge. Enrichment of return activated sludge further enhanced the hydrolysis yield of 683 g SCOD/kg VS_{added} and acidification yield of 617 g COD_{SCFA}/kg VS_{added}, which is amongst the highest reported in the literature.

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Abbreviations

ABE	Acetone-Butanol-Ethanol
AD-sludge	Anaerobic Digestion Sludge
AF	Acidogenic Fermentation
CEPT	Chemically Enhanced Primary Treatment
COD	Chemical Oxygen Demand
CO_2	Carbon Dioxide
Cum. SCOD	Cumulative Soluble Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
DLC	Demolition, Land clearing, and Construction
FID	Flame Ionization Detector
FW	Food Waste
GAC	Granular Activated Carbon
GHG	Greenhouse gas
3HB	3-Hydroxybutyrate
ICI	Institutional, Commercial, and Industrial
ISR	Inoculum to Substrate Ratio
LBR	Leachate Bed Reactor
LRR	Leachate Recirculation Rate
MSW	Municipal Solid Waste
mL	Milliliter
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PLA	Polylactate
RAS	Return Activated Sludge
ROPEC	Robert O. Pickard Environmental Centre
SBR	Sequential Batch Reactor
SCFA	Short-Chain Fatty Acid
SCOD	Soluble Chemical Oxygen Demand
TS	Total Solid
US$	United State Dollar
VS	Volatile Solid

Chapter 1. Introduction

1.1 Background

Increase in population and rapid urbanization have led to a massive increase in the generation of municipal solid waste (MSW) (Tiseo, 2021; Zhou et al., 2018). Over 2.01 billion tons of MSW was generated globally in 2016, which is anticipated to increase by 69% and reach 3.40 billion tons by 2050. Food waste can constitute 15-30% of the national MSW generation (Kaza et al., 2018; Arras et al., 2019). The food waste generated is even higher if agricultural post-harvest losses are included in the estimates, thus making food waste as the single most common type of waste generated.

Canada is amongst the highest food waste generating countries in the world. Food waste generation is approximately 0.5 kg of food waste per capita per day. The resulting economic losses are estimated to be a staggering $3 billion per year (Commission for Environmental Cooperation, 2017; Ministry of the Environment and Climate Change, 2020). Landfilling is the primary method of food waste disposal in Canada which poses serious risks to the environment and public health including greenhouse gas (GHG) emissions, and contamination of soil and groundwater. Moreover, waste and landfill management costs the Canadian taxpayers over $3 billion annually, which is projected to further increase with population growth, waste generation and economic growth trends (Statistics Canada, 2011). To reduce such environmental and economic impacts of waste disposal, and meet the climate change targets, municipalities across Canada have committed to divert food waste from landfills by 2030-2050 (National Zero Waste Council, 2018; Ministry of the Environment and Climate Change, 2017). Similar efforts are being pursued in other countries, which has spurred national and international interest in

developing technologies for the sustainable management of food waste. Anaerobic biotechnologies such as acidogenic fermentation that can simultaneously treat and recover value-added products from food waste represent a sustainable solution to this challenge.

The conversion of organics to carboxylates through acidogenic fermentation is an emerging concept for resource recovery from food waste. Carboxylates refer to a highly valued mixed spectrum of dissolved short-chain fatty acids (SCFAs), mostly acetate (C2), propionate (C3), and butyrate (C4). These carboxylates are currently mass-produced by the reformation of petrochemicals and are used as a precursor for many petrochemical-derived materials and fuels (Chang et al., 2010; Coma et al., 2017; Bastidas-Oyanedel et al., 2015; Arras et al., 2019). The price for carboxylates ranges between US$ 2000–2500/ton for butyrate, US$ 1500–1700/ton for propionate, and US$ 400–800/ton for acetate – comparably higher than US$ 500-800/ton for gasoline (Zacharof & Lovitt, 2013; Bastidas-Oyanedel et al., 2015; Bruni et al., 2021). With an annual market growth projection of 4-15%, the transformation of food waste to carboxylates could establish an eco-friendly alternative for food waste management by promoting resource recovery and establishing a circular economy.

Three broad steps are involved in acidogenic fermentation: hydrolysis, acidogenesis, and acetogenesis. Sustainable production of carboxylates from food waste requires the development of specialized bioreactors in which hydrolysis and acidogenesis can proceed at a high rate and without any inhibition. Wet fermenters such as continuously stirred tank reactor (CSTR) currently used to produce carboxylates are energy-intensive and require extensive pre-treatment of food waste to prevent inhibition of hydrolysis and acidogenesis, which makes them unsuitable for large-scale operations (Shewa et al., 2020;

Browne et al., 2013; Saha & Lee, 2020; Xiong et al., 2019a). Dry fermenters such as leachate bed reactors (LBRs) in contrast are less energy-intensive and do not require extensive pre-treatment of the food waste. Moreover, the operating characteristic of LBRs readily separates the food waste from the carboxylates-containing leachate. Thus, the leachate is suitable for downstream processing without the costly solid-liquid separation (Hussain et al., 2021; Saha & Lee, 2020; Browne et al., 2013). In addition, the leachate at the bottom of the LBR is recycled to the top and sprinkled over the food waste bed, thereby eliminating the need for continuous mixing (i.e., less energy required) as in CSTR. Moreover, due to batch operation, the leachate accumulates a consistently high concentration of carboxylates towards the end of operation, which is ideal for industrial applications. These advantages significantly improve the energy efficiency and scalability of LBR and allow it to be more competitive. However, two critical research and development gaps need to be addressed to advance LBR for sustainable carboxylates production: 1) improving volumetric organic loading, and 2) reducing the fermentation time.

The volumetric organic loadings (g VS/$L_{reactor}$) in LBRs have remained between 18-23 g VS/$L_{reactor}$, which is comparatively lower than that reported in CSTRs (Greses et al., 2021; Jiang et al., 2013; Lim et al., 2008). This is attributed to the reduction in permeability of the food waste bed at high volumetric organic loading. In practice, large components of the food waste are disintegrated into smaller components during acidogenic fermentation. As a result, the permeability of the food waste bed is reduced, which restricts the flow of the leachate through the food waste bed. The leachate gradually starts to accumulate on top of the food waste bed and inhibits the hydrolysis and acidogenic process

leading to poor reactor performance (André et al., 2015; André et al., 2018; Shewani et al., 2015). Many studies have mixed exogenous materials such as woodchips, sawdust, corncob, etc., to maintain the permeability of the waste bed (Han et al., 2015; Xu et al., 2011; Demirer & Chen, 2008). However, these materials begin to degrade over time (Chang & Chen, 2010; Han et al., 2015; Zhao et al., 2011) and cannot retain their physical structure, eventually resulting in a reduction of the waste bed permeability. Inorganic materials such as plastic particles, plastic hollow spheres, rubber have also been tested, but they are not sustainable and proved to be costly (Xu et al., 2011; Chakraborty et al., 2021). Carbonaceous materials such as granular activated carbon (GAC) are derived from organic waste biomass and can be a sustainable and inexpensive option. Literature has also shown the positive impact of GAC as a support media for microbial attachment in anaerobic reactors producing methane, and hydrogen (Bertin et al., 2010; Guo et al., 2008; Lutpi et al., 2015). Thus, the mixing of GAC with food waste in LBR could provide dual benefits: (1) maintaining permeability at high volumetric organic loading – GAC does not degrade during acidogenic fermentation and can thus maintain the permeability of the food waste bed in LBR, (2) reducing fermentation time – GAC has a microporous surface, which provides the immobilization sites for the hydrolytic and acidogenic bacteria and thereby could reduce the fermentation time. However, improvement in LBR performance with the application of GAC is yet to be experimentally elucidated.

The starting inoculum is another important operational parameter that could play an important role in improving the biochemical reaction kinetics and reducing the fermentation time in LBR. Variability in hydrolytic and acidogenic microbial populations among different inoculums could lead to distinct process performance of acidogenic

fermentation. Anaerobic digestion sludge (AD-sludge) has been widely used as an inoculum for acidogenic fermentation of food waste in LBR (Yin et al., 2016; Xiong et al., 2019a; Xiong et al., 2019b). Studies on other anaerobic biotechnologies have used different inoculums such as return activated sludge (RAS) and have reported high hydrolytic efficiency due to the higher hydrolytic activity of inoculums sourced from an aerobic environment (Gao et al., 2014; Saad et al., 2019; Li et al., 2020). Information on the potency of RAS as an inoculum in LBR to produce carboxylates at higher volumetric organic loading is sparse and needs to be experimentally investigated. Using enriched inoculum may further reduce the fermentation time. Enriched inoculum contains a higher proportion of the desired microbial populations and is better adapted to the operating conditions (Sukphun & Sittijunda, 2021; Shewa et al., 2020). Hence, enriched microbial culture could further improve the process kinetics along with the product yields. Thus, a study focusing on the impact of enriched inoculum on the hydrolysis and acidogenesis of food waste in LBR at high volumetric organic loading is warranted.

1.2 Scope and objectives

The overarching goal of this study was to optimize the acidogenic fermentation of food waste in LBR at high volumetric organic loading (49 g $VS/L_{reactor}$). To this end, three specific objectives were defined:

1. To evaluate the impact of different GAC loadings (0, 0.25, 0.38, 0.51 g GAC/g $VS_{foodwaste}$) on the hydrolysis and acidification of food waste in LBR.
2. Impact of inoculum type (AD-sludge vs RAS) and sequential enrichment procedure on the hydrolysis and acidification yields and fermentation time.
3. Identification of dominant microbial populations in LBR at different conditions.

1.3 Thesis outline

This thesis has five chapters:

1. Chapter 1 introduces acidogenic fermentation as a sustainable biotechnology for food waste management and recovery of value-added products. It also summarizes critical challenges pertaining to acidogenic fermentation of food waste in a LBR and outlines the research objectives of this thesis.
2. Chapter 2 gives an overview of food waste generation in Canada and Ontario. The environmental impact of food waste disposal is provided. Acidogenic fermentation as a sustainable pathway for resource recovery from food waste and the importance of LBRs as cost-effective bioreactor platforms is reviewed. Critical research and knowledge gaps on LBR, which form the crust of this study are reviewed.
3. Chapter 3 is presented in an article form. This chapter evaluates the impact of different GAC loadings on acidogenic fermentation of food waste in a LBR at high volumetric organic loading.
4. Chapter 4 is also written in an article form. This chapter investigates the effect of different inoculums on acidogenic fermentation of food waste.
5. Chapter 5 summarizes the significant results/outcomes obtained in chapters 3 and 4 and provides recommendations for future work.

Chapter 2. Literature review

2.1 Food waste generation and management in Canada

Canada's growing population and economic growth have resulted in a 16% increase in the generation of MSW over the past two decades (Yunis & Aliakbari, 2021). In 2016, Canadians generated 34.7 million tons of MSW. Food waste accounts for 6.3-30.9% of MSW, varying with the provinces (Table 2.1). For example, food waste accounted for 30.9% of MSW in Manitoba, 26.4% of MSW in Alberta, and 24.2% of MSW in Ontario (Ministry of the Environment and Climate Change, 2020).

Table 2.1 MSW and food waste generation in different provinces of Canada (Ministry of the Environment and Climate Change, 2020)

Province	Population	*Quantity of MSW generated (tons/year)	Food waste composition of total MSW (%)	Quantity of food waste generated (tons/person/year)
Manitoba	1314139	1185109	30.9	0.279
Alberta	4196061	5057867	26.4	0.318
Ontario	13875394	12788799	24.2	0.223
Saskatchewan	1135987	1067410	24.1	0.226
Quebec	8225950	7759218	22.5	0.212
Nunavut	36975	N/A	22	N/A
Yukon	38547	N/A	22	N/A
British Columbia	4859250	4381516	19.3	0.174
Northwest Territories	44649	N/A	18.9	N/A
Newfoundland and Labrador	529426	434728	17.5	0.144
Nova Scotia	942790	668456	9.4	0.067
New Brunswick	763350	654398	9.3	0.080
Prince Edward Island	146969	108590	6.3	0.047

*Quantity of MSW generated is a sum of MSW from residential, ICI (Institutional, Commercial, and Industrial), and demolition sectors.

Figure 2.1 depicts the food waste generation (%) by different sectors in Canada. About 66% of the food waste generated in Canada originates from residential and ICI sectors, while the remaining 34% is generated from other sectors like food processors and farms (Ontario Ministry of the Environment and Climate Change, 2018). Most of the food waste from residential and ICI sectors is being landfilled in Canada. This landfilling of food waste leads to the generation of over 56 million tons of GHG emissions annually (Nikkel et al., 2019) and accounts for 20% of the national methane and about 3% of the GHG emissions (Prairie Climate Centre, 2017). Provinces across Canada spend a staggering $200-400 million annually on waste and landfill management, with collective nationwide expenses exceeding $3 billion (Statistics Canada, 2011). This alarming environmental and economic impact, along with the commitment to divert the organic waste fraction (food waste, paper, yard waste, etc.) from landfills by 2030-2050, has spurred national interest in developing high-performance and cost-effective solutions for sustainable management of organic waste such as food waste

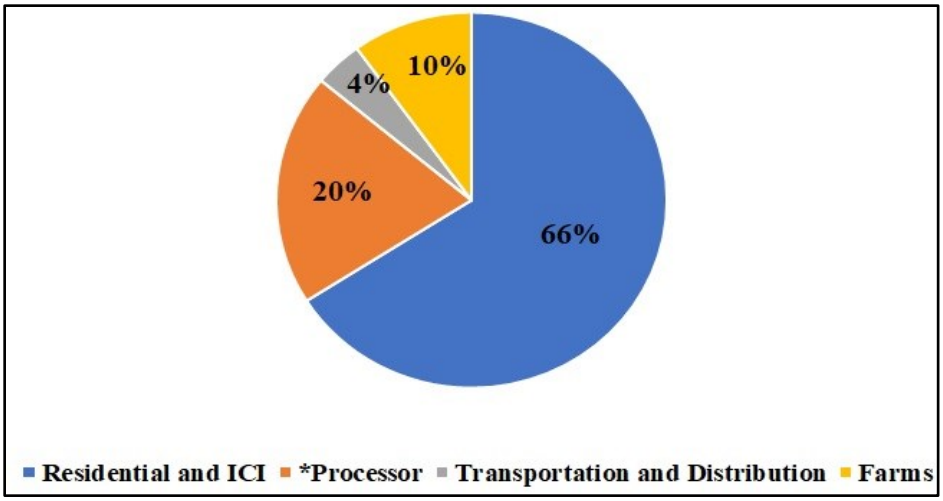


Figure 2.1 Percentage share of different sectors in food waste generation in Canada (Ontario Ministry of the Environment and Climate Change, 2018).*Some proportions of the processing waste are also being landfilled.

2.1.1 Food waste generation and disposal in Ontario

In Canada, Ontario has the highest food waste generation amongst all the provinces. Ontario generated 3.09 million tons of food waste in 2016, which was 2-3 times higher than Alberta and British Columbia (Ministry of the Environment and Climate Change, 2020). This variation in food waste generation among the provinces could be attributed to a difference in population in the provinces (Table 2.1). For example, Ontario has the highest population among all the provinces, resulting in the highest food waste generation in Ontario. Similar to other provinces, food waste in Ontario is generated from residential and ICI sectors such as grocery stores, food processing units, and so on (Ministry of the Environment and Climate Change, 2017). Currently, food waste is being treated as residual waste in Ontario. Hence, it is disposed of in landfills with other wastes. About 2.5 million tonnes of food and yard waste is landfilled in Ontario annually, which is approximately 80% of total food and yard waste generated annually in the province. This quantity of food and yard waste landfilled is much higher than other waste materials landfilled by the province (Figure 2.2) (Ministry of the Environment and Climate Change, 2020).

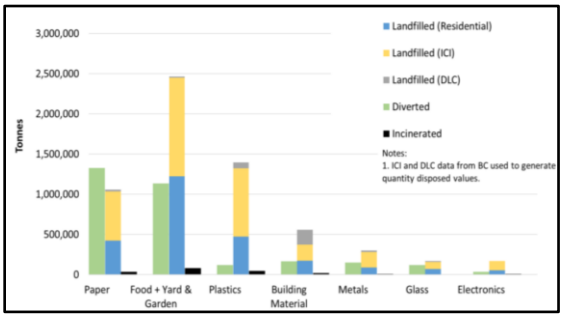


Figure 2.2 Quantities of selected materials (i.e., paper, food waste, etc.) of MSW landfilled, incinerated, and diverted in Ontario. (Ministry of the Environment and Climate Change, 2020). Where, Landfilled (Residential) – waste coming from the residential sector is disposed in landfill, Landfilled (ICI) – waste coming from the ICI sector is disposed of in landfill, and Landfilled (DLC) – waste coming from demolition, land clearing, and construction (DLC) sector is disposed of in landfill

Food and other organic wastes are responsible for most of the waste-derived GHG emissions which are estimated at 5.3 % of the total GHG emitted by Ontario (Figure 2.3) (Ontario Waste Management Association, 2015). Moreover, waste and landfill management in Ontario costs taxpayers over $400 million annually with one estimate by Statistics Canada placing the yearly expenditure to be over $1 billion (Statistics Canada, 2011). Given the projected population growth, waste generation, and economic trends, current landfills in Ontario are estimated to reach their capacity by 2030, if no progress is made to keep food and organic wastes out of the landfills. It is also estimated that Ontario would need 16 new landfill sites if landfilling continues at the same rate (Ministry of the Environment and Climate Change, 2017). Acquisition and preparations of new landfill sites could cost the province over a billion dollars.

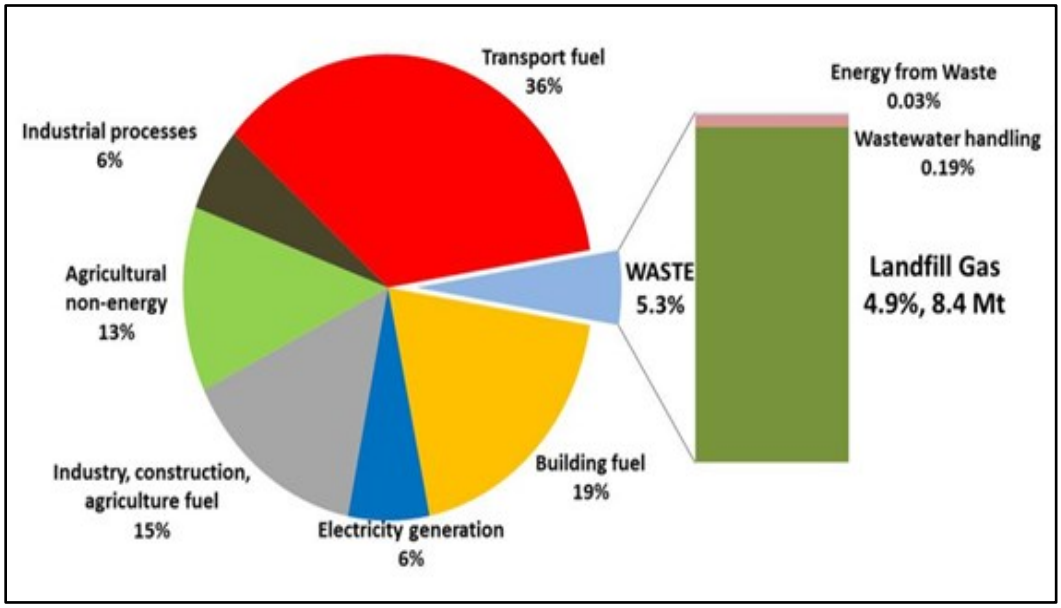


Figure 2.3 Ontario's GHG emission from different sectors (Ontario Waste Management Association, 2015).

To reduce the environmental and economic impacts of waste disposal, and meet its climate change mitigation targets, Waste Free Ontario Act was enacted by the Government of Ontario in 2016. This act has brought the vision of building up a circular and low carbon economy to make Ontario waste-free by 2050. Under this plan, Ontario has committed to at least 50% waste diversion and a complete food waste disposal ban from landfills by 2030. Thus, research and development of innovative and cost-effective solutions for sustainable management of food waste are placed at a high priority under the Waste Free Ontario Act and the Climate Change and Low-carbon Economy Act of 2016 (Ministry of the Environment and Climate Change, 2017).

Anaerobic biotechnologies that can simultaneously treat and recover high-valued chemicals such as carboxylates from food waste represent a promising solution for the management of food waste at the provincial and the national level.

2.2 Carboxylate platform for resource recovery from food waste

Food waste is a highly degradable material rich in carbon and nutrients. These characteristics along with the high quantity of food waste generated make food waste a suitable feedstock for producing biomaterials/bioproducts, thus transforming food waste into a resource (Dahiya et al., 2015; Yin et al., 2016). The food waste can be converted to biomaterials either through the thermochemical or the biochemical route (Figure 2.4). However, the biochemical route is better suited due to the high moisture content of the food waste (70-80%). The thermochemical route requires the removal of the moisture which leads to high-energy consumption (Agler et al., 2011; Holtzapple & Granda, 2009). Moreover, the requirement of expensive chemical catalysts further adds to the production cost, making the process economically unfeasible.

There are two major platforms under the biochemical route: (1) the sugar platform, and (2) the carboxylate platform (Figure 2.4). Sugar platform entails the enzymatic hydrolysis of particulate organics into five and six-carbon sugars (e.g., pentose, hexose) as intermediate chemicals (Agler et al., 2011; Dahiya et al., 2018). However, the sugar platform requires expensive enzymes and is better suited for feedstocks with a more homogeneous composition. Conversion of heterogeneous mixtures such as food waste through the sugar platform is costly and results in low product yields (Holtzapple et al., 2022).

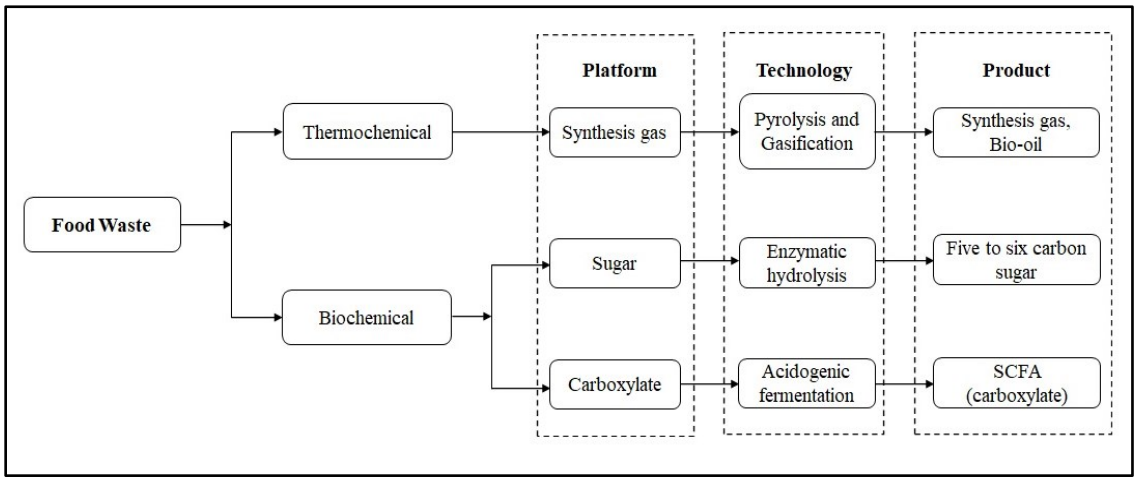


Figure 2.4 Various pathways to valorize the food waste

The carboxylate platform is a new platform for the transformation of biomass into value-added chemicals such as carboxylates. Carboxylates are SCFA containing at least one carboxyl group and two to six carbon atoms, such as acetate, propionate, and butyrate which can be distilled at atmospheric pressure (Agler et al., 2011; Lee et al., 2014). These chemicals are currently derived from non-renewable petrochemicals which pose two major drawbacks: 1) depletion of fossil fuel deposits and, 2) GHG emissions during the synthesis of carboxylate from fossil fuels; for instance, 3.3 tons of CO_2-equivalent GHG/ton of

acetate production is emitted during acetate production (Atasoy et al., 2018; Pandey et al., 2022). Production of carboxylates from food waste through the carboxylate platform offers a more sustainable approach and supports a circular economy.

The carboxylate platform has the lowest capital costs amongst the biomass conversion processes and results in higher product yields than other platforms. The production of SCFA in the carboxylate platform can be achieved via the acidogenic fermentation process. Acidogenic fermentation is performed by a mixed microbial community, which eliminates the requirement for sterile conditions, and can degrade most of the components in a heterogeneous substrate like food waste (Zhou et al., 2018; Jones et al., 2021). The SCFA produced through the carboxylate platform are used as chemicals by itself. Acetate, propionate, and butyrate are classified as bulk chemicals as they are used in large quantities in different industries. In addition, they are used as precursors to produce complex biofuels and materials as summarized in section 2.3 below.

2.3 Market value and applications of carboxylates

Carboxylates demand a high market value. Bulk price and production quantity of individual components of carboxylate are listed in Table 2.2 and uses are shown in Figure 2.5.

Table 2.2 Market value and market size of individual components of carboxylates

Component of Carboxylates	Chemical Formula	Market Value[a] US$/ton	Market Size[b] ton/year
Acetate	CH_3COOH	400-800	3,500,000
Propionate	CH_3CH_2COOH	1500-1700	1,80,000
Butyrate	$CH_3(CH_2)_2COOH$	2000-2500	30,000

[a]Market value (Zacharof & Lovitt, 2013; Bastidas-Oyanedel et al., 2015; Bruni et al., 2021).
[b]Market size (Bastidas-Oyanedel et al., 2015; Zacharof & Lovitt, 2013).

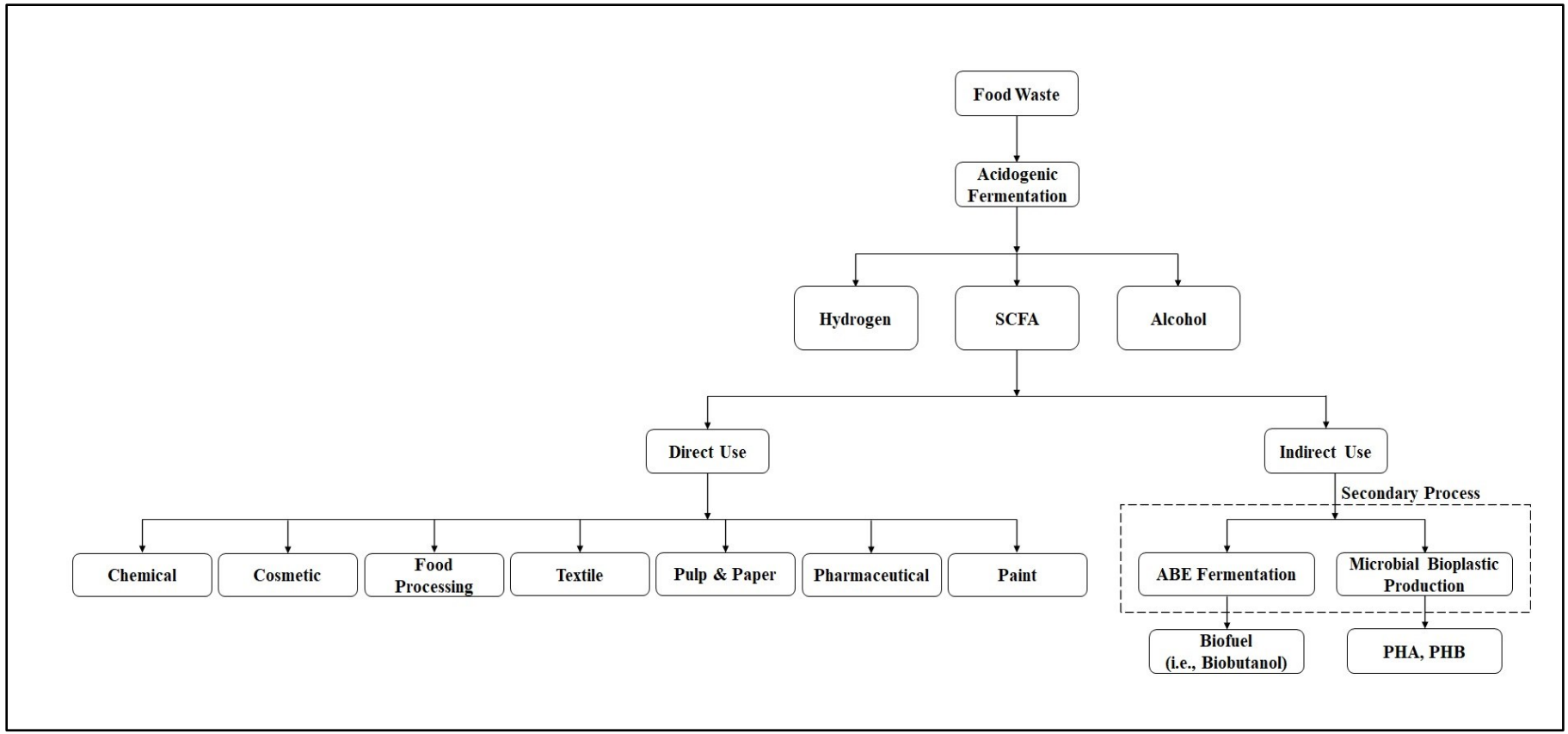


Figure 2.5 Use of carboxylates.

2.3.1 Production of biopolymers

Biopolymers are an eco-friendly alternative to conventional petro-derived plastics. A mixture of carboxylates is used as a substrate in microbial production of different biopolymers including polyhydroxyalkanoates (PHA), polyhydroxybutyrate (PHB), and polylactate (PLA) (Bhatt et al., 2020; Talan et al., 2022). The composition of carboxylates has a direct impact on the formation of the monomers of biopolymers and their physical properties (elasticity, crystallinity, and stiffness). For instance, carboxylates mixture containing high acetate and butyrate composition would yield 3-hydroxybutyrate (3HB) monomers (Bruni et al., 2021). High acetate and butyrate composition is achieved in food waste fermentation; thus it is a suitable process for the production of 3HB. 3HB has better characteristics: 1) it is a thermoplastic biopolymer that can withstand 30˚C - 120˚C of temperature, and 2) it is a non-toxic biopolymer. In addition, during its degradation, 3-hydroxybutyric acid is produced, which is a common component of the human blood. As a result, it can be used in biotechnology such as in surgery as seam threads and surgical implants (Alves et al., 2017; Asiri et al., 2020; Iordanskii & Bonartsev, 2014).

Furthermore, individual component of carboxylates is used as a precursor in the production of cellulose-based plastic, hydrophobic and lipophobic papers, and so on (Murali et al., 2017). It is also used to enhance the physical properties of the polymers in some cases. For example, butyrate is applied as an additive to the fibers in the production of the polymer to improve the resistivity to heat and sunlight (Zhang et al., 2009).

2.3.2 Biofuel production

Biofuel refers to biomethanol, bioethanol, and biobutanol (Jin et al., 2011). Bioethanol and biobutanol can be produced by microbial reduction of a mixture of carboxylates (Steinbusch et al., 2008). At present, bioethanol is widely used by blending with gasoline (Demirbas et al., 2011). However, bioethanol is more corrosive, and it has a lower heating value as compared to gasoline (Cho et al., 2018). Hence, biobutanol can be a better alternative due to its higher heating value and viscosity, along with lower volatility and ignition problem. Moreover, biobutanol can be blended with gasoline at any proportion (Kujawska et al., 2015). Biobutanol can be produced using mixed carboxylates through acetone-butanol-ethanol (ABE) fermentation by clostridium bacteria. ABE fermentation consists of two main phases: acidogenesis and solventogenesis. Therefore, it is required to obtain better acidogenesis performance to further improve biobutanol production. A high concentration of butyrate in carboxylates composition would help to produce more butanol, resulting in optimal acetone to butanol ratio (Cho et al., 2018; Stein et al., 2017). For example, Lee et al. (2008) demonstrated improvement in butanol production and acetone to butanol ratio by continuously supplying butyrate to fermentation medium during ABE fermentation.

2.3.3 Carboxylates as a platform chemical in different industries

Carboxylates have a wide range of applications as a building block in manufacturing of products in different industries such as pharmaceutical, food processing, textile, and cosmetics among others. Such applications of carboxylates in different industries are summarized below:

2.3.3.1 Pharmaceutical industry

Carboxylates are used as precursors in the production of different medicines. For example, (1) acetate is used in the generation of aspirin, beta and oxacillin antibiotics, antiepileptic drugs (Bastidas-Oyanedel et al., 2015), (2) propionate is a vital chemical to synthesize various medications (e.g. antibiotics, cholesterol-lowering drugs, etc.) (Murali et al., 2017; Bruni et al., 2021; Technavio Research), and (3) butyrate has anticancer properties; hence it is widely used to cure cancer such as colorectal, oral, etc. Moreover, it is utilized in the production of several medicines for gastrointestinal and neurological disorders (Hajjar et al., 2021; Bedford & Gong, 2018; Miki et al., 2007). Mixture of carboxylates are also now being used in the construction of modules for prosthetic surgery (Zacharof & Lovitt, 2013).

2.3.3.2 Food processing industry

Carboxylates and their derivatives have a wide range of applications in the food processing sector. Acetate, propionate, and their derivatives (sodium acetate, sodium propionate, calcium propionate) can inhibit bacterial and fungal growth, hence it is used as a preservative to improve the shelf life of food grains, animal fodder, processed foods, and bakery products. Moreover, acetate is used as a vinegar, acidity regulator, and desalting agent to enhance the taste and flavor of food. Esters of propionate and butyrate (e.g., geranyl propionate, methyl butyrate, and ethyl butyrate) are used as an artificial flavoring agent in food and beverage preparation (Zacharof & Lovitt, 2013; Bastidas-Oyanedel et al., 2015; Mitsubishi Chemical Corporation). Moreover, butyrate has antimicrobial potential. Therefore, it is being increasingly used in animal food as a substitute for antibiotics (Bauwens, 2016; Deepa et al., 2018).

2.3.3.3 Other industrial applications

Carboxylates are used in various industries (i.e., pulp and paper, paint, textile, chemical, etc.) as a mixture or as an individual component to produce solvents, plasticizers, dyes, biosurfactants, bioflocculants, pesticides, and herbicides. For instance, acetate is used in the production of solvents such as ethyl acetate, butyl acetate, and propyl acetate. Derivatives of butyrate such as calcium butyrate are used as a solvent in the leather tanning process (Bhatt et al., 2020; Zhang et al., 2009; Bhatia & Yang, 2017). In addition, around half of the global production of butyrate is used as butyl acrylate and butyl methacrylate to synthesize latex surface coatings, enamels, adhesives, sealants, and elastomers (Demirbas et al., 2011). Furthermore, acetate and propionate are used in the manufacturing of moisturizers and as a skin-lightening agent, and butyrate is used as a fragrant in cosmetic and perfume industries (Zacharof & Lovitt, 2013).

2.4 Acidogenic fermentation and elementary reactions

Acidogenic fermentation (AF) is an important bioprocess within the carboxylate platform. AF is carried out by a consortium of bacteria (mixed microbial culture) under anaerobic conditions to produce carboxylates from complex organics by multi-step concurrent biochemical reactions (Figure 2.6). These reactions are similar to those in the anaerobic digestion process but with the important omission of methanogenesis to ensure the formation of soluble products (e.g., SCFA) instead of biogas. The elementary reactions consisting of hydrolysis, acidogenesis, and acetogenesis are summarized below:

2.4.1 Hydrolysis

Hydrolysis is the first step of AF, which involves hydrolytic bacteria excreting enzymes (such as lipase, protease, cellulase) to break down the particulate organic matter into

soluble monomers. The main components of food waste such as carbohydrate, protein, and lipids are transformed to glucose, amino acids, long chain fatty acids, respectively (Figure 2.6). These hydrolysates serve as a substrate for acidogenic bacteria in the next stage of acidogenesis (Singhania et al., 2013; Shen et al., 2017). Compared to other steps, hydrolysis is widely reported to be the rate-limiting step. The below equation shows the chemical reaction associated with the hydrolysis process (Anukam et al., 2019; Deepanraj et al., 2014):

$$(C_6H_{10}O_5)\,n + nH_2O \rightarrow n(C_6H_{12}O_6) + nH_2 \quad (2.1)$$

2.4.2 Acidogenesis

At this stage, hydrolysates produced in the hydrolysis step are converted to carboxylates (or SCFA), alcohol, carbon dioxide (CO_2), and hydrogen (H_2) by acidogenic bacteria. Acetate, propionate, and butyrate are the main carboxylates produced through acidogenesis (Strazzera et al., 2018; Singhania et al., 2013). Due to easy availability of soluble monomer as a substrate to acidogenic bacteria, this process is comparatively faster. The below equations demonstrate the key chemical reactions that occur during the acidogenesis stage (Deepanraj et al., 2014):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + CO_2 \quad (2.2)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O \quad (2.3)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2CH_2COOH + 2H_2 + 2CO_2 \quad (2.4)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (2.5)$$

2.4.3 Acetogenesis

During acetogenesis, carboxylates and alcohol such as propionate, butyrate, ethanol are further converted to acetate, hydrogen, and carbon dioxide by acetogenic bacteria. The main chemical reactions associated with this stage are described below (Deepanraj et al., 2014):

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2 \quad (2.6)$$

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (2.7)$$

$$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2 \quad (2.8)$$

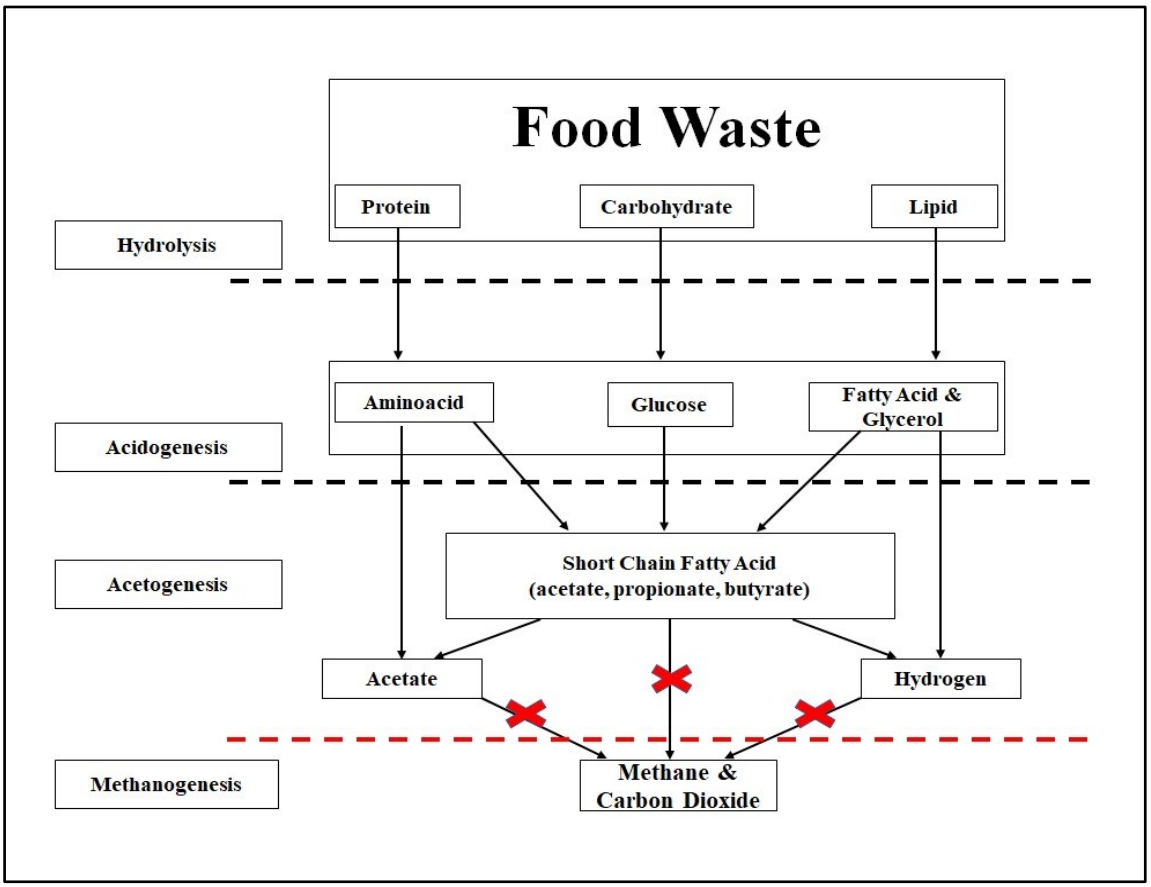


Figure 2.6 Schematic process flowchart of anaerobic digestion. (Red line and cross marks indicate the stage where anaerobic digestion is stopped to accumulate carboxylates).

2.5 Leachate bed reactor for dry acidogenic fermentation

Bioreactor systems for acidogenic fermentation can be divided into two broad categories based on requirement of process water: "wet" and "dry" systems. Wet systems are primarily CSTRs in which the food waste has to be pulped and slurried with process water to have a TS of ≤ 15% (Figure 2.7A). Whereas, dry (or high solid) systems such as LBR can theoretically carry TS content up to 40%, so food waste does not need to be diluted with process water and requires smaller bioreactor volume (Hussain et al., 2017; Kakuk et al., 2017; Jansson et al., 2019; Yang et al., 2015; Browne et al., 2013; Khalid et al., 2011). The food waste and inoculum (leachate) are placed in separate zones namely food waste holding basket and leachate holding bed, respectively (Figure 2.7B). The food waste holding basket has a perforated bottom (e.g., mesh), therefore the hydrolytic and fermented products (i.e., carboxylates) continuously leach into the leachate holding bed. The leachate drained at the bottom of the LBR is recycled to the food waste holding basket and sprayed over the food waste bed. This eliminates the need for continuous mixing as in CSTR, making LBR less energy-intensive. Another advantage is that the carboxylates containing leachate can be directly used for downstream processes without the need for a costly solid-liquid separation unit, contrary to what one would have with a CSTR as the primary reactor (Xiong et al., 2019b; Jha et al., 2011; Sun et al., 2021; Hussain et al., 2017; Shewa et al., 2020). Also, LBR is operated in batch mode, thus maintaining a relatively constant concentration of carboxylates towards the end of batch process. These advantages significantly improve the energy efficiency and scalability of LBR for carboxylates production from food waste. However, some challenges such as low volumetric organic loading, long fermentation time, reduction in permeability in the food waste holding basket,

and other operational complexities (liquid mixing in leachate holding bed and leachate recirculation) need to be addressed for large scale applications.

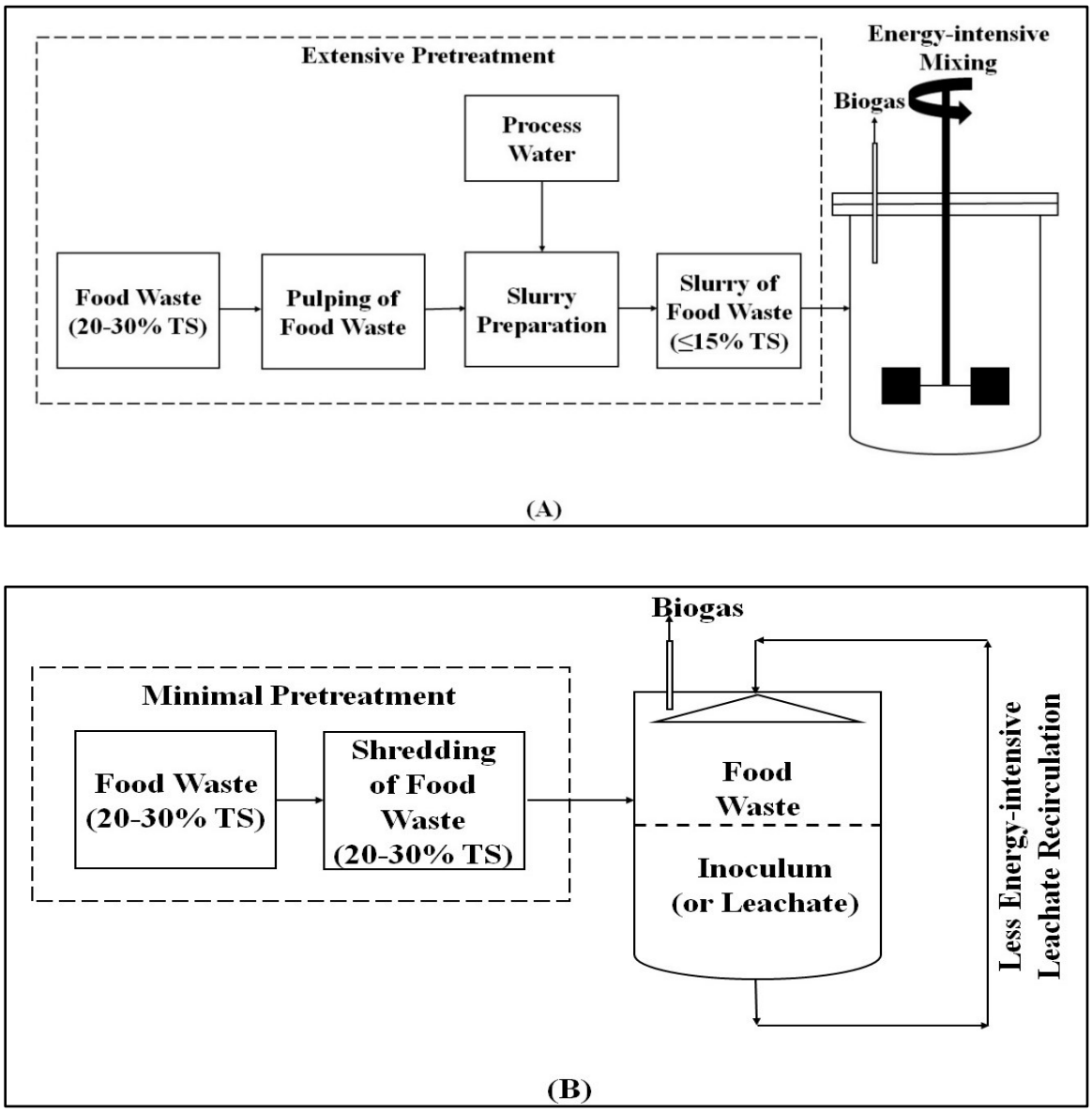


Figure 2.7 Schematic diagram of (A) CSTR, and (B) LBR.

2.6 Process parameter affecting the acidogenic fermentation in LBR

Various factors affect the AF of food waste including pH, inoculum type, temperature, volumetric organic loading, fermentation time, inoculum to substrate ratio, and leachate recirculation rate (Figure 2.8). Factors important for this study are discussed below.

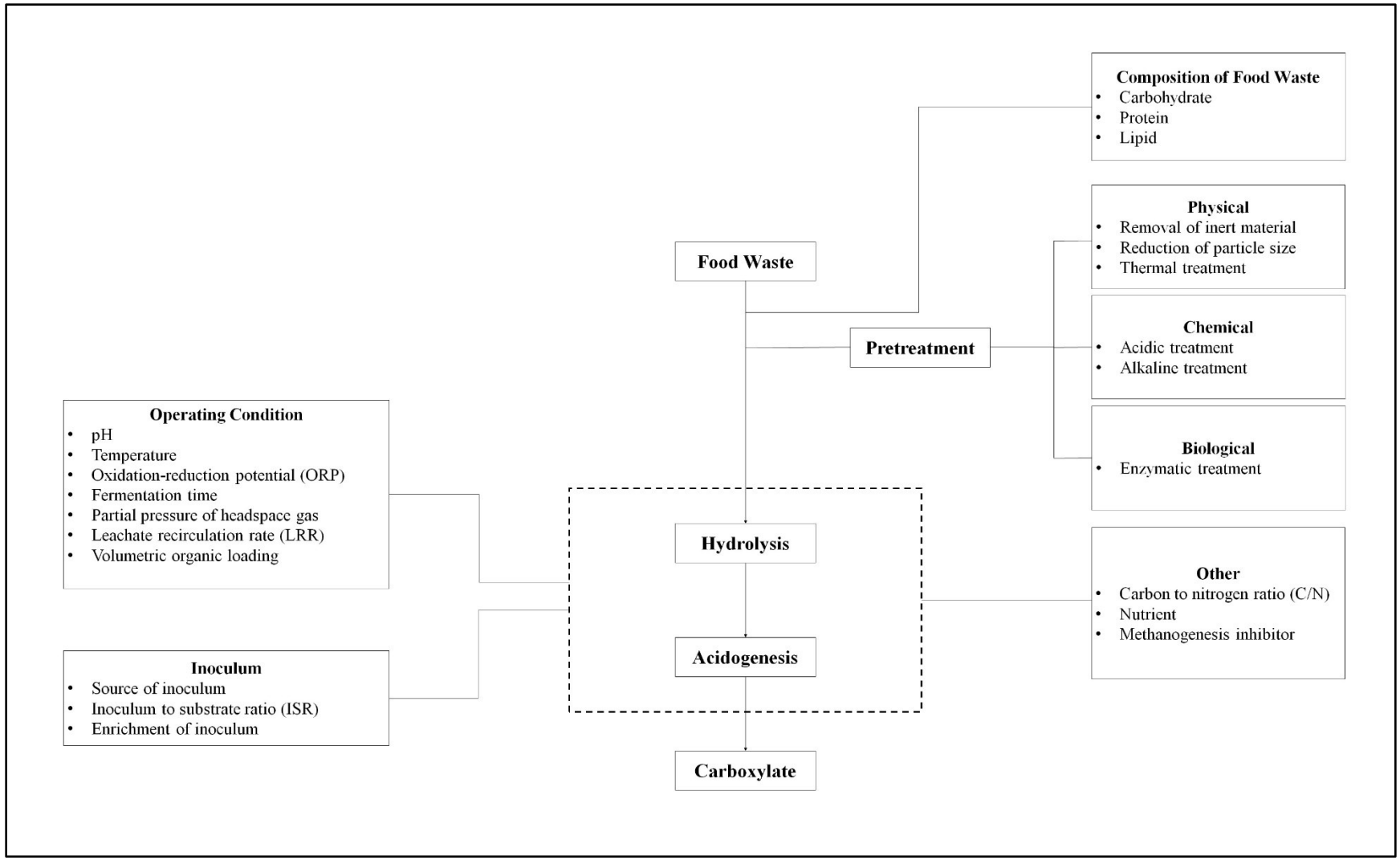


Figure 2.8 Factors affecting carboxylates (or SCFA) production from food waste.

2.6.1 Volumetric organic loading and fermentation time

Volumetric organic loading is defined as the mass of volatile solid (VS) added per unit volume of a bioreactor (equation-2.9). Fermentation time is the duration of time that the substrate (food waste) remains in the bioreactor during the fermentation. Volumetric organic loading and fermentation time, both affect the efficiency of carboxylates production and design of the bioreactor. For efficient AF, the fermentation time should be long enough for solubilization of complex organic matter, but not long enough to activate the methanogens and lead to the consumption of carboxylates (Strazzera et al., 2018; Wainaina et al., 2019). The efficiency of carboxylates production at different volumetric organic loading and fermentation times can be determined from three important process performance parameters: (1) hydrolysis yield (g SCOD/kg VS_{added}), (2) acidification yield (g COD_{SCFA}/kg VS_{added}), and (3) VS destruction efficiency (%). Hydrolysis yield shows the transformation of initially added VS into SCOD (soluble chemical oxygen demand). Acidification yield (or carboxylate yield) indicates the total carboxylates produced from the VS added into the reactor. VS destruction efficiency describes the same fact as hydrolysis yield, but it considers destruction of initial VS added into the bioreactor.

$$\text{Volumetric organic loading (g VS/L}_{reactor}) = \frac{\text{mass of VS added to bioreactor (g VS)}}{\text{total volume of bioreactor (L}_{reactor})} \quad (2.9)$$

Theoretically, LBR can accommodate higher solid (or organic) loading than CSTR (Menzel et al., 2020; Chakraborty et al., 2021; Yang et al., 2020). However, in practice, LBR has been operated at a similar or even lower volumetric organic loading than CSTRs. The volumetric organic loading for LBR on food waste has generally ranged between 18-

23 g VS/$L_{reactor}$ with a fermentation times of 6-14 days to achieve stable process performance at mesophilic temperature (Shewa et al., 2020; Xiong et al., 2019a; Xiong et al., 2019b). For example, Xiong et al. (2019b) operated a LBR at a volumetric organic loading of 21.73 g VS/$L_{reactor}$ with a long fermentation time of 14 days at room temperature (22˚C). High hydrolysis yield and acidification yield of 883 g SCOD/kg VS_{added} and 761 g COD_{SCFA}/kg VS_{added} were obtained, respectively. However, poor process performance has been reported when volumetric organic loading was higher than 18-23 g VS/$L_{reactor}$. To compare, Saha & Lee (2020) operated a LBR at a volumetric organic loading of 32.34 g VS/$L_{reactor}$ and obtained 25% lower acidification yield than that reported by Xiong et al. (2019b) despite of the same operating temperature (22˚C) and fermentation time (14 days). This deterioration of process performance at a high volumetric organic loading could be attributed to the accumulation of leachate in the food waste holding basket because of the reduction in permeability of the food waste bed (discussed in section 2.7).

Shorter fermentation times of 5-7 days have been reported for CSTR operated at volumetric organic loading of 18-23 g VS/$L_{reactor}$ (Arras et al., 2019; Dahiya & Mohan, 2019; Jiang et al., 2013). Reduced fermentation time of 6-7 days at low volumetric organic loading of < 20 g VS/$L_{reactor}$ has been reported for LBR but required the use of enriched inoculum. Shewa et al. (2020) obtained hydrolysis yield and acidification yield of 693 g SCOD/kg VS_{added} and 649 g COD_{SCFA}/kg VS_{added}, respectively with a fermentation time of 6 days at volumetric organic loading of 18.17 g VS/$L_{reactor}$ by using enriched inoculum consisting of a mixture of enriched biomass and fresh AD-sludge. Operation at high volumetric organic loading is vital to improve the applicability of LBR for carboxylates production from food waste.

2.6.2 Inoculum

For efficient carboxylates production, it is important that the inoculum contains balanced hydrolytic and acidogenic microbial populations. Imbalance between these microbial populations can hinder or limit the fermentative reactions, resulting in low product yields (Arras et al., 2019; Dahiya et al., 2015). Very few studies have investigated the impact of different inoculums on the AF of food waste in LBR. Most of the studies on LBR have utilized AD-sludge as an inoculum (Selvam et al., 2010; Zhang et al., 2020; Xiong et al., 2019a). The hydrolysis yield and acidification yield with AD-sludge have generally ranged between 510-767 g SCOD/kg VS_{added} and 429-761 g COD_{SCFA}/kg VS_{added}, respectively with a fermentation time of 14 days at volumetric organic loading of 18-23 g $VS/L_{reactor}$ (Xiong et al., 2019a; Xiong et al., 2019b; Shewa et al., 2020)

There are only three studies that have tested the impact of inoculum type (e.g., enriched inoculum and cow manure) on AF of food waste in LBR. Shewa et al. (2020) compared the process performance of LBR with two different inoculums: AD-sludge and enriched inoculum (consisting of a mixture (1:1) of enriched biomass and AD-sludge). A 36% higher hydrolysis yield and 51% higher acidification yield were obtained with enriched inoculum than AD-sludge. Similarly, in another study, a high hydrolysis yield of 774 g SCOD/kg VS_{added} and acidification yield of 697 g COD_{SCFA}/kg VS_{added} were obtained by using enriched inoculum at a fermentation time of 10 days (Hussain et al., 2021). Improvements in LBR performance with enriched inoculum could be attributed to higher hydrolytic and acidogenic activity, thus resulting in improved hydrolysis and acidification yields along with shorter fermentation time. Yan et al. (2014) compared cow manure with AD-sludge as an inoculum in LBR and observed 16% improvement in hydrolysis yield

with cow manure as compared to AD-sludge due to the higher hydrolytic activity of the cow manure. Therefore, appropriate selection of inoculum is critical for the operation of LBR at high volumetric organic loading.

2.6.3 Leachate recirculation rate (LRR)

LRR represents the frequency at which a certain volume of leachate is recirculated from the leachate holding bed to the food waste holding basket. LRR is a critical parameter to ensure proper contact between bacteria and food waste as well as to maintain the moisture content of the food waste. In addition, recirculation of the leachate distributes the enzymes, allowing enzymes to access the surface of food waste readily. Leachate recirculation replenishes the microenvironment and delivers the nutrients, thus promoting the flourishment of the microbial community (Browne et al., 2013; Cadavid-Rodríguez & Horan, 2014). LRR can be calculated as follows:

$$\text{LRR } (L/L_{bed}\cdot day) = \frac{\text{volume of leachate recirculated(L)}}{\text{volume of leachate holding bed}(L_{bed}) \cdot \text{interval between two immediate recirculations (day)}} \quad (2.10)$$

Some studies have demonstrated the impact of LRR on hydrolysis and acidification of food waste. In a LBR operated at thermophilic temperature (50˚C) with volumetric organic loading of 46 g VS/$L_{reactor}$, 16% increase in VS destruction efficiency and 33% improvement in acidification yield were achieved when LRR was increased from 1.7 $L/L_{bed}\cdot$day to 2.6 $L/L_{bed}\cdot$day (Hussain et al., 2017). However, such improvements at high volumetric organic loading have only been observed at high temperature due to faster degradation of food waste resulting in proper leachate percolation through the food waste bed. At mesophilic temperature with such high volumetric organic loading, increasing LRR

may negatively impact the LBR performance. For example, 10% reduction in VS degradation efficiency was obtained by increasing the LRR from 15.64 L/L_{bed}·day to 46.93 L/L_{bed}·day in LBR with high volumetric organic loading of 32.34 g $VS/L_{reactor}$ at mesophilic temperature (Saha & Lee, 2020). The lowered VS destruction efficiency at higher LRR was due to improper leachate percolation through the food waste bed at high volumetric organic loading. Hence, LRR needs to be optimized for every LBR depending on other operating parameters (e.g., temperature, volumetric organic loading, etc.).

2.6.4 pH

pH is a key process parameter that affects the enzymatic and metabolic activity of the hydrolytic and acidogenic bacteria (Moretto et al., 2019; Wang et al., 2014). Literature has commonly reported that slightly acidic to neutral pH (pH 6-7) improved hydrolysis and acidification yields during food waste fermentation in LBR (Xiong et al., 2019b; Hussain et al., 2017; Chakraborty et al., 2021; Xu et al., 2012). Hussain et al. (2017) obtained a hydrolysis yield of 478-530 g SCOD/kg VS_{added} at pH 6-7, which was higher than that obtained hydrolysis yield of 227-405 g SCOD/kg VS_{added} at pH 4-5. As a result, two to five-fold higher acidification yield was achieved at pH 6-7 than pH 4-5. Similarly, Xu et al. (2011) reported 72% improvement in hydrolysis yield and 66% increase in acidification yield at pH 6 as compared to pH<4 in LBR. Furthermore, Xiong et al. (2019b) compared the process performance of LBR at pH 7 and pH 8 and observed 11% higher acidification yield with pH 7 than at pH 8, regardless of similar hydrolysis yield at pH 7 and pH 8. Overall, slightly acidic to neutral pH (pH 6-7) has been demonstrated as optimal pH to achieve high carboxylates production from food waste in LBR.

Accumulation of SCFA results in a drop of pH to acidic conditions (pH<3). At these acidic conditions, the bulk liquid has a higher concentration of undissociated ions that can easily permeate into the cytoplasm through the membrane. These undissociated ions release proton ions by dissolution because of the relatively alkaline environment inside the cell, resulting in a lower pH and an imbalance in the proton motive force. Hence, the cell needs to expend more ATP to keep the neutral condition inside the cell, which may lead to cell inactivation (Infantes et al., 2012). Therefore, it is required to maintain the pH in a slightly acidic to neutral range.

Moreover, it is commonly reported that pH impacts the composition of SCFA (Zhou et al., 2018; Atasoy et al., 2019; Jankowska et al., 2017). When pH was maintained at slightly acidic to neutral pH (pH 6-7), acetate and butyrate were the major fraction of SCFA as compared to pH 4-5. At pH 6-7, acetate and butyrate collectively constituted 49-97% of SCFA, whereas it was 59-62% at pH 4-5. Furthermore, the remaining proportion of SCFA was composed of other constituents (e.g., propionate). The higher composition of acetate and butyrate at pH 6-7 could be attributed to the pH of 6-7 favors the acetate-butyrate metabolic pathway in bacteria (Xiong et al., 2019b; Hussain et al., 2017).

2.6.5 Temperature

Temperature has a direct impact on the growth and metabolism of bacteria thus, it is one of the crucial factors affecting AF. Each microbial taxon has its own optimal range of temperature for replication. Hence, the fluctuation in operating temperature leads to a change in the microbial structure of the microbiome engaged in AF. In addition, the temperature affects enzymatic activity and biochemical reaction kinetics. Therefore, the

temperature affects hydrolysis and acidogenesis in terms of production rate and product yield (Arras et al., 2019; Zhou et al., 2018; Strazzera et al., 2018; Jiang et al., 2013).

Many researchers have studied the effect of temperature on hydrolysis and acidogenesis during AF. It has been demonstrated that thermophilic to hyper-thermophilic temperature (>50˚C) resulted in a faster hydrolysis rate and higher hydrolysis yield, owing to the thermochemical breakdown and solubilization of macromolecules into soluble monomers (Arras et al., 2019; Wainaina et al., 2019). However, the increase in hydrolysis rate does not necessarily result in higher acidification yield because acidogenic bacteria are sensitive to high temperatures. For example, Zhang et al. (2020) achieved about 10% higher hydrolysis yield at thermophilic temperature (55˚C) as compared to mesophilic temperature (35˚C), still acidification yield was two-fold higher at mesophilic temperature than the thermophilic temperature. To maintain high acidification rates at thermophilic temperature, enrichment of inoculum at high temperature is required. Maintaining the reactor at thermophilic or hyper-thermophilic conditions requires higher energy input. Therefore, the net economic gain with increased product yields at high temperatures needs to be evaluated.

Studies have reported comparable hydrolysis and acidification yields at mesophilic temperature (22-35°C) to those obtained at high temperature (>50 ˚C) by improving the LBR design, operating at low volumetric organic loading, and using enriched inoculum (Browne et al., 2013; Chakraborty et al., 2021; Saha & Lee, 2020; Shewa et al., 2020; Xiong et al., 2019a). For instance, Xiong et al. (2019a) operated LBR at room temperature (22°C) and obtained a hydrolysis yield of 837 g SCOD/kg VS_{added} and acidification yield of 669 g COD_{SCFA}/kg VS_{added} by improving the LBR design, which was comparable to that

reported by Hussain et al. (2017) (hydrolysis yield of 565 g SCOD/kg VS_{added} and acidification yield of 330 g COD_{SCFA}/kg VS_{added}) with LBR operated at 50°C. Similarly, Shewa et al. (2020) reported a high hydrolysis yield of 693 g SCOD/kg VS_{added} and acidification yield of 649 g COD_{SCFA}/kg VS_{added} at room temperature with the use of enriched inoculum. These results indicate that high hydrolytic and acidification yields can be obtained with LBR at mesophilic temperatures but requires careful optimization of reactor design and operating parameters. Mesophilic operation would require lower energy input in comparison to thermophilic operations and may be better suited for regions with colder climates.

2.6.6 Inoculum to substrate ratio (ISR)

ISR can be computed as the ratio of the mass of VS of inoculum to the mass of VS of food waste, added to the bioreactor (equation 2.11).

$$\text{ISR (\%)} = \frac{\text{mass of VS of inoculum (g VS)}}{\text{mass of VS of food waste (g VS)}} \quad (2.11)$$

A high ISR means a higher relative abundance of bacteria (hydrolytic and acidogenic) capable of decomposing the food waste. Consequently, faster hydrolysis and acidogensis of food waste would occur, resulting in a shorter fermentation time. However, LBR has two major difficulties at a high ISR: 1) inoculum would be concentrated (high quantity of VS) to maintain the reactor volume small, which may cause improper leachate percolation in the food waste holding basket, and 2) volume of LBR needs to be increased to eliminate the use of concentrated inoculum, which is not economical. Hence, it is desirable to maintain low ISR in LBR operations. Literature also suggested that an ISR of 4-6% is optimal for high carboxylates production from food waste in LBR. When the ISR

was increased from 0.4% to 6.9%, 23% improvement in hydrolysis yield and two times higher acidification yield were achieved using food waste in LBR (Xu et al., 2012). In another research study on AF of food waste using LBR, hydrolysis yield and acidification yield were higher by 37% and 50%, respectively at ISR 5% as compared to ISR 10%. The poor performance at ISR 10% was due to improper leachate percolation through the food waste bed in the food waste holding basket (Xiong et al., 2019a). Therefore, LBR should be operated at ISR of 4-6% rather than higher ISR (e.g., 10%) during food waste fermentation, to avoid deterioration of reactor performance.

2.7 Summary of research needs to improve carboxylates production in LBR

As discussed in section 2.6, the two major inter-related limitations for LBR operation on food waste are: 1) low volumetric organic loading, and (2) long fermentation time. LBR operation with high volumetric organic loading and shorter fermentation time is essential to improve treatment capacity and increase the carboxylates production per unit volume of the bioreactor - all of which impact the economic viability and sustainability of the carboxylate platform.

Literature has repeatedly reported low hydrolysis and acidification yields at a high volumetric organic loading in LBR (Xu et al., 2012; Xu et al., 2014a; Xu et al., 2014b). To shorten the fermentation time, studies even tested high ISR and LRR while maintaining low volumetric organic loading. However, low product yields were reported in such cases as well (Xiong et al., 2019a; Saha & Lee, 2020). The sub-optimal performance of the LBR, especially at high volumetric organic loading is due to reduction in permeability of the food waste bed during fermentation (André et al., 2015; André et al., 2018; Shewani et al., 2015). The permeability is primarily governed by the macro-porosity of the food waste bed. As

shown in Figure 2.9A, at the initial stage of fermentation, the food waste bed has high macro-porosity because of the large particle size of the food waste. As a result, the leachate can easily percolate through the food waste bed. However, as the fermentation proceeds, the large food waste particulates disintegrate into smaller fragments, which reduces macro-porosity of the food waste bed, thus making it less permeable to the leachate (Figure 2.9B). Moreover, the accumulation of the carboxylates along with biomass growth makes the leachate more viscous (or concentrated), which further restricts the leachate percolation through the food waste bed. Consequently, the leachate gradually accumulates on top of the food waste bed (Figure 2.9C). This poor leachate percolation limits the contact between the bacteria (hydrolytic and acidogenic) and the food waste, thus resulting in low hydrolysis and acidification yields.

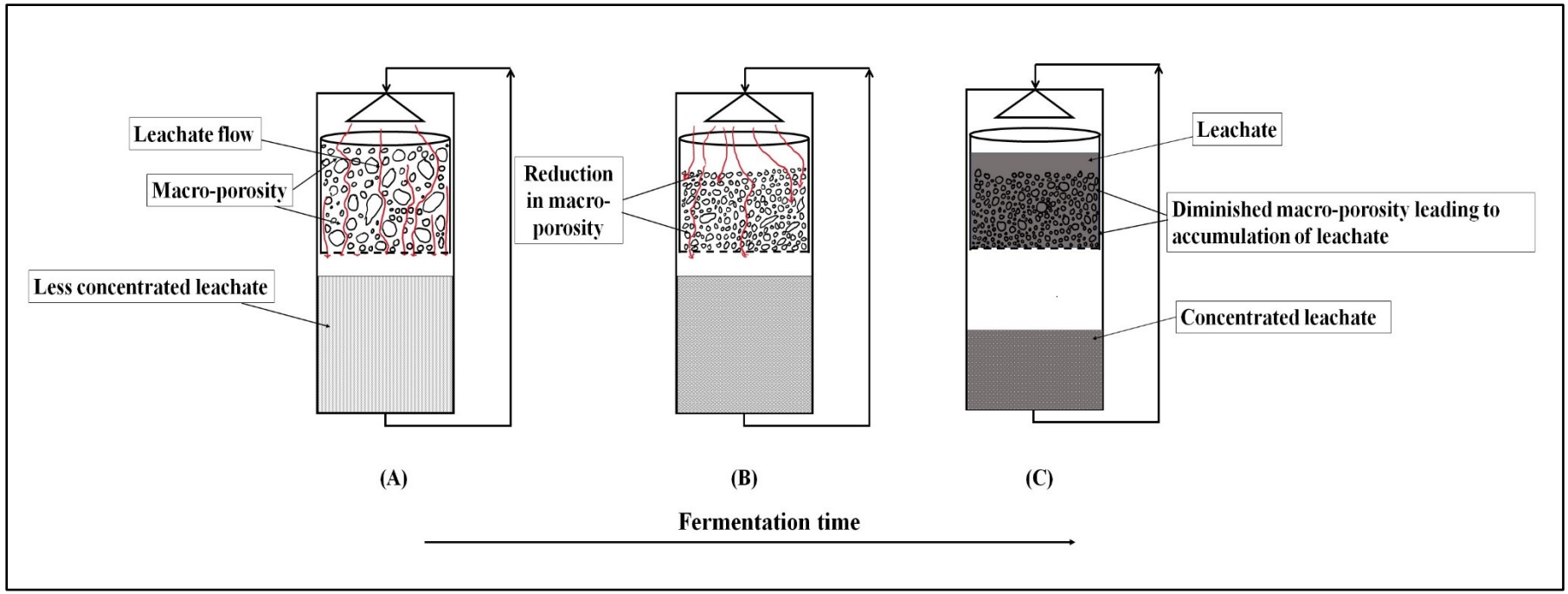


Figure 2.9 Leachate percolation in LBR at high volumetric organic loading; (A) LBR at initial stage, (B) LBR after some period of decomposition (reduction in macro-porosity), (C) LBR with diminished macro-porosity.

GAC for LBR operation at high volumetric organic loading:

Researchers have explored various strategies to operate LBR at high volumetric organic loading including dilution of the leachate by addition of water (to reduce the viscosity of leachate) and flushing of waste bed (addition of water from top-to-down or/and down-to-top of waste bed) to improve the leachability of waste bed in LBR (Yan et al., 2014; Xu et al., 2011; Xu et al., 2014a; Cysneiros et al., 2012; Uke & Stentiford, 2013). However, all these approaches make the fermentation process more complex. Also, the addition of dilution water dilutes the concentration of the carboxylates in the leachate which is undesirable if carboxylate is the final product.

The addition of exogenous materials such as sawdust, woodchips, and corn cob to the waste bed has been studied to maintain the permeability of the waste bed (Han et al., 2015; Xu et al., 2011; Demirer & Chen, 2008; Selvam et al., 2010; Xu et al., 2012; Yan et al., 2016). However, such exogenous materials are biodegradable and thus degrade over the time, resulting in loss of their physical structure and so the permeability of the waste bed (Chang & Chen, 2010; Han et al., 2015; Zhao et al., 2011). As an alternative, studies have tested the application of non-biodegradable materials such as plastic particulates, plastic hollow spheres, and plastic beads to maintain the permeability (by improving the macro-porosity) of the waste bed and improve the performance of the LBR (Xu et al., 2011; Chakraborty et al., 2021). However, such materials are costly and unsustainable.

Recyclable carbonaceous materials such as GAC can be a sustainable and inexpensive option to maintain the permeability of the waste bed. GAC can be derived by pyrolysis of the organic waste biomass with low production costs. Research studies have

also reported the positive impact of GAC as a support media for microbial attachment in anaerobic bioreactors producing hydrogen, and methane (Bertin et al., 2010; Lutpi et al., 2015; Mohan et al., 2008; Guo et al., 2008; Wang et al., 2020). For example, Guo et al. (2008) operated an expanded granular sludge bed reactor packed with GAC at high volumetric organic loading to produce hydrogen from molasses-containing wastewater and showed high hydrogen production with better operational stability at a shorter fermentation time. Similarly, Wang et al. (2020) reported 12.14% higher methane production by using GAC as a support media in anaerobic co-digestion of municipal sludge and food waste. Thus, the mixing of GAC with food waste in LBR could provide dual benefits: 1) maintaining permeability at high volumetric organic loading – GAC does not degrade during the fermentation and can thus maintain the permeability of the food waste bed in LBR, (2) reducing fermentation time – GAC has a microporous surface, which provides the immobilization sites for the hydrolytic and acidogenic bacteria and thereby it could reduce the fermentation time. However, improvement in LBR performance with the application of GAC is yet to be experimentally elucidated.

Inoculum optimization for LBR operation at high volumetric organic loading:

As discussed in section 2.6, AD-sludge has been extensively utilized as an inoculum for carboxylates production from food waste in LBR (Xiong et al., 2019a; Xiong et al., 2019b; Hussain et al., 2017). On the other hand, there are other potential inoculums in literature such as RAS, which have shown to improve hydrolytic and acidogenic activity in anaerobic bioreactor but are yet to be studied for AF of food waste in LBR (Gao et al., 2014; Wang et al., 2014). For example, Saad et al. (2019) investigated the effect of three different inoculums: aeration tank sludge, RAS, and anaerobic sludge on co-digestion of food waste

and chicken manure in a CSTR. It was observed that RAS performed better among all three inoculums in terms of biogas production (hydrogen and methane). Higher biogas production was a result of higher hydrolysis and acidogenic activity with RAS. Similarly, high carboxylates production was reported for co-digestion of kitchen waste and waste activated sludge using CSTR (Chen et al., 2013). Hence, the impact of RAS as inoculum on carboxylate production from food waste in LBR is warranted.

Several studies have demonstrated improved carboxylate yields in CSTRs using enriched inoculum (Chang et al., 2018; Lukitawesa et al., 2019). Serial enrichment/acclimatization of the inoculum results in a higher abundance of the desired fermentative microbial populations. Consequently, resulting in improved process performance in terms of shortening the fermentation time, and enhancing the hydrolysis and acidification yields (Sukphun & Sittijunda, 2021; Shewa et al., 2020). Lukitawesa et al. (2019) reported an increase in SCFA production from 0.45 g/L to 16 g/L on food waste by using enriched inocula instead of a fresh inoculum in a CSTR. Likewise, Chang et al. (2018) reported 19% higher production of carboxylates from food waste by using enriched microbiome rather than fresh microbiome in a CSTR. Higher carboxylates production was attributed to a shift of the microbial community towards carboxylates production. Thus, the use of enriched inoculum could lead to higher carboxylates production and reduced fermentation time for LBR operated at high volumetric organic loading.

Chapter 3. Acidogenic fermentation of food waste in a leachate bed reactor at high volumetric organic loading: Effect of different GAC loadings

3.1 Abstract

This study investigated the impact of different GAC loadings (0, 0.25, 0.38, 0.51 g GAC/g $VS_{foodwaste}$) on hydrolysis and acidogenesis of food waste in a LBR operated at high volumetric organic loading of 49 g $VS/L_{reactor}$. The highest hydrolysis yield of 620 g SCOD/kg VS_{added} and acidification yield of 507 g COD_{SCFA}/kg VS_{added} were obtained for the LBR with the GAC loading of 0.51 g GAC/g $VS_{foodwaste}$. Butyrate dominated the SCFA composition by constituting 57-60% of total SCFA at high GAC loadings of 0.38-0.51 g GAC/g $VS_{foodwaste}$, while similar composition of acetate (38-40%) and butyrate (36-38%) were obtained at low or no GAC loadings (0-0.25 g GAC/g $VS_{foodwaste}$).

3.2 Introduction

Concerns regarding climate change and energy security are driving efforts to replace petrochemical-derived products with more renewable and sustainable products such as products derived from waste biomass (i.e., bioproducts). The waste biomass can be transformed into various bioproducts through the biorefinery platform. Analogous to a petrochemical refinery where fossil fuels are converted to different materials and energy, a biorefinery refers to the production of bioenergy, biochemicals, and biofuels from various biomass sources (Coma et al., 2017; Igbokwe et al., 2022; Bhatt et al., 2020). The typical biorefinery platforms include the sugar and synthesis gas platform (Holtzapple et al., 2022; Molina-Peñate et al., 2022). Recently, a new platform, the carboxylate platform has been introduced into the biorefinery platform and has the lowest capital costs amongst the various platforms (Dahiya et al., 2018; Jones et al., 2021; Atasoy et al., 2018; Ramos-

Suarez et al., 2021). Carboxylates are SCFAs containing two or six carbon atoms with at least one carboxyl group, such as, acetate, propionate, and butyrate. Carboxylates have a wide range of applications as a building block in different industries (i.e., pharmaceutical, chemical, food processing, etc.), and can also be used for the production of biofuels such as bioethanol and biobutanol (Uçkun Kiran et al., 2015; Yin et al., 2016; Moretto et al., 2019; Dahiya et al., 2015). These carboxylates can be produced from waste biomass by acidogenic fermentation, which is driven by diverse hydrolytic and acidogenic bacteria (Xiong et al., 2019b; Strazzera et al., 2018). Waste biomass such as food waste presents a sustainable feedstock for carboxylates production since it constitutes between 30-60 % of organic fraction of MSW generation globally (Kaza et al., 2018; Arras et al., 2019).

Dry fermenters such as LBRs have been increasingly utilized for SCFA production from food waste. LBR has unique operational features which are better suited to produce SCFA from food waste. These features include the physical separation of the food waste from the carboxylates-containing leachate, which allows the leachate to be utilized without the need for costly solid-liquid separation for downstream processing. In addition, the food waste can be added to the LBR without dilution with process water, and also no continuous stirring of the food waste and leachate is required, allowing for an economical and energy-efficient bioreactor system (Browne et al., 2013; Cysneiros et al., 2012; Xiong et al., 2019b).

To achieve high carboxylates production in LBR, optimization of operational parameters including pH, temperature, LRR, and ISR have resulted in a two-fold increase in LBR performance (Saha & Lee, 2020; Xiong et al., 2019a; Xiong et al., 2019b; Hussain et al., 2017; Xu et al., 2014a; Xu et al., 2012; Xu et al., 2011). However, one of the major

limitations of LBR – the low volumetric organic loading is yet to be addressed. As shown in Table 3.1, LBRs have been operated at a similar or even lower volumetric organic loading than CSTRs for acidogenic fermentation of food waste, which is due to the decrease in permeability (or macro-porosity) of the food waste bed as the acidogenic fermentation of food waste proceeds. This reduction in permeability of the food waste bed restricts the flow of the leachate through the food waste bed, inhibiting the hydrolysis and acidogenesis of the food waste. To operate LBRs at high volumetric organic loading, different approaches to improve leachability and maintain the permeability of waste bed have been researched (Table 3.1). For instance, flushing of the waste bed and dilution of leachate have been performed to improve the leachability of the waste bed in LBRs (Cysneiros et al., 2012; Uke & Stentiford, 2013; Xu et al., 2014a; Xu et al., 2014b; Yan et al., 2014). However, such approaches dilute the concentration of the carboxylates (desired product) in leachate and negate the very advantage of LBRs i.e., high concentration of carboxylates in the leachate. The addition of exogenous materials such as sawdust, wood chips, and corn cob were used to improve the leachability of the waste bed. However, these materials are organic in nature and degrade over time. (Chang & Chen, 2010; Han et al., 2015; Zhao et al., 2011). Consequently, these materials lose their structure and are unable to maintain the permeability of the waste bed

. On other hand, several studies have applied inorganic materials (e.g., plastic particles, plastic hollow spheres, plastic beads, etc.) (Xu et al., 2011; Chakraborty et al., 2021). However, these materials are costly and not the most sustainable from an environmental standpoint. Carbonaceous materials such as GAC produced from waste biomass could be a sustainable and cost-effective option. Studies on anaerobic bioreactors

have reported the positive impact of GAC as a support media for microbial attachment, resulting in increased product yields such as hydrogen and methane (Bertin et al., 2010; Guo et al., 2008; Lutpi et al., 2015). Thus, the mixing of GAC with food waste in LBR could provide dual benefits: 1) maintaining permeability at high volumetric organic loading – GAC does not degrade during acidogenic fermentation and can thus maintain the permeability of the food waste bed in LBR, (2) reducing fermentation time – GAC have a microporous surface, which could provide the immobilization sites for the hydrolytic and acidogenic bacteria and thereby reduce the fermentation time. However, improvements in LBR performance with the application of GAC is yet to be experimentally elucidated.

This study systematically evaluated the impact of GAC on the hydrolysis and acidogenesis of food waste in a LBR operated at high volumetric loading of 49 g VS/$L_{reactor}$. Firstly, hydrolysis rates and yields were compared for four LBRs with different GAC loadings including a control reactor with no GAC. Secondly, acidification yields and composition of SCFA were analyzed for different LBRs to investigate the impact of GAC on SCFA production (i.e., carboxylates production).

Table 3.1 Comparison of volumetric organic loading in LBR and CSTR for AF of food waste

Substrate	Inoculum	Reactor Type	Volumetric Organic Loading (gVS /$L_{reactor}$)	Fermentation time (day)	Temperature (˚C)	pH	ISR (%) (VS basis)	Hydrolysis yield (g SCOD/ kg VS_{added})	Acidification yield (g COD_{SCFA}/ kg VS_{added})	Strategy	Mode of Operation	Reference
**FW	AD-sludge	CSTR	81	27	25	5.5 - 6	-	-	-	-	Batch	(Greses et al., 2021)
**FW	Enriched inoculum	LBR	21.7	10	22	6	4	774	697	-	Batch	(Hussain et al., 2021)
**FW	AD-sludge + enriched inoculum	LBR	18.1	6	22	7	4	693	649	-	Batch	(Shewa et al., 2020)
**FW	AD-sludge	LBR	32.3	14	22	6	10	-	571	-	Batch	(Saha & Lee, 2020)
**FW	AD-sludge	LBR	21.7	14	22	6	5-10	767	637	Fractionalized LBR	Batch	(Xiong et al., 2019a)
**FW	AD-sludge	LBR	22.7	14	22	6	10	837	669	Fractionalized LBR	Batch	(Xiong et al., 2019a)
**FW	AD-sludge	LBR	21.7	14	22	7	5	883	761.81	-	Batch	(Xiong et al., 2019b)

**FW + @CEPT Sludge	AD-sludge	CSTR	99.1	20	35	6.5 - 7	-	760	-	-	Batch	(Chakraborty et al., 2018)
**FW	AD-sludge	LBR	46	14	50	7	4	565	330	-	Batch	(Hussain et al., 2017)
**FW	Cow manure	LBR	65.16	18	-	6	-	570	-	^WC as a bulking agent, 1 L water added at day 0, and %Dilution	Batch	(Yan et al., 2014)
**FW	AD-sludge	LBR	61.31	17	35	6	-	240.16	82.08	^WC as a bulking agent, and %Dilution	Batch	(Xu et al., 2014a)
**FW	AD-sludge	LBR	59.80	17	35	6	-	-	390	^WC as a bulking agent, 1 L water added at day 0, and %Dilution	Batch	(Xu et al., 2014b)
**FW	AD-sludge	CSTR	80*	5	35	6	-	-	411	-	Semi-continuous	(Jiang et al., 2013)

**FW	AD-sludge	LBR	62.43	17	35	6	6.9	640	180	^WC as a bulking agent	Batch	(Xu et al., 2012)
**FW	AD-sludge	LBR	59.80	17	-	6	-	620	101	^WC as a bulking agent, 1 L water added at day 0, and %Dilution	Batch	(Xu et al., 2011)
**FW	AD-sludge	CSTR	72*	8	35	5.5	-	-	350	-	Semi-continuous	(Lim et al., 2008)

Volumetric organic loading*(g VS/$L_{reactor}$) = Organic loading rate (g VS/$L_{reactor}$/day) × hydraulic retention time (day)
@CEPT Sludge = Chemically enhanced primary treatment sludge
**FW = Food Waste
^WC = Wood Chips
%Dilution = daily 50% replacement of leachate by tap water

3.3 Material and method

3.3.1 Characteristics of food waste, inoculum, and GAC

Simulated food waste was used in this study because of the closure of cafeterias and restaurants due to the Covid-19 pandemic. The simulated food waste consisted of carrot, potato, bread, and pet food accounting for 40, 35, 15, and 10% respectively, (wet basis). This composition of simulated food waste was similar to the real food waste reported in the literature (Xiong et al., 2019a; Xiong et al., 2019b; Hussain et al., 2017). The pet food was used to increase the VS content of the simulated food waste as pet food contains a high VS. Moreover, it also contains high proportions of carbohydrates, proteins, and lipids (Fernández et al., 2005; Xu & Li, 2012). The individual components were stored at -10˚C to maintain the quality of the simulated food waste. The required quantity of individual components of the simulated food waste was thawed at room temperature (22˚C) for two hours prior to the experiment and then immediately diced to an average particle size of ~1 cm by using a mesh chopper (Starfrit, Canada). Required amounts of GAC pellets were well mixed with the simulated food waste before loading into the LBR. The GAC pellets (CNZ, USA) had a cylindrical shape with lengths of 7 – 25 mm and a diameter of 4 mm. This dimension of GAC pellets was used since the improper leachate percolation was observed with smaller GAC pellets of 5 – 7 mm in food waste holding basket. GAC is not degraded during AF of food waste despite having a high VS content. It could be due to GAC contains complex macromolecules that are not destroyed even during thermal pyrolysis in manufacturing of the GAC. Hence, GAC is resistant to chemical and microbial degradation (Altamirano-Corona et al., 2021). RAS was used as an inoculum, which was collected from the Robert O. Pickard Environmental Centre (ROPEC) wastewater

treatment facility (Ottawa, Ontario, Canada) and stored at 4°C prior to experimental use. The characteristics of simulated food waste, inoculum, and GAC pellets used in this study are described in Table 3.2.

Table 3.2 Characteristics of simulated food waste, inoculum, and GAC pellets

Parameters	Simulated Food Waste	Inoculum	GAC Pellets
TS (%)	36.4 ± 0.5	0.69 ± 0.03	99.4 ± 0.06
VS (%)	34.8 ± 0.4	0.46 ± 0.01	73.0 ± 1.0
TS (g/kg)	364 ± 5	6.9 ± 0.3	994 ± 0.6
VS (g/kg)	348 ± 4	4.6 ± 0.1	730 ± 10
VS/TS (%)	96	67	73
SCOD (g/L)	-	1.4 ± 0.2	-

3.3.2 LBR configuration

The LBR had a cylindrical shape and was fabricated using acrylic tubes. The reactor had a height of 390 mm and a diameter of 110 mm, resulting in a total volume of 3.7 L. The LBR was encompassed of three different compartments (Figure 3.1): 1) headspace with the gas collection and sprinkling system in the top compartment, 2) acrylic-made food waste holding basket in the middle compartment, and 3) leachate holding bed in the bottom compartment. The headspace of the LBR had a detachable cover with a gas collection port and sprinkler nozzle. The volume of the headspace was 0.4 liter to retain the gas generated during the AF. The gas collection port was connected to a gas collection bag (MODEL 22052, Restek, Canada). The sprinkling system consisted of a customized shower head with pores of 3 mm to spray the leachate over the food waste at some time interval. The food waste holding basket had a volume of 1.2 L and featured a perforated base consisting

of a 3 mm mesh to prevent food waste from seeping into the leachate holding bed. Leachate was accumulated in the 2.1 L leachate holding bed. LBR had a sampling port to collect the liquid samples for analysis. The pH probe was inserted into the retaining wall of LBR at the bottom to monitor the pH by a pH controller (MODEL MC122, Milwaukee, USA). To maintain the pH of leachate at the desired level, the pH controller was connected to a dosing pump (MODEL 7553-80, Cole Parmer, USA) to inject neutralizing agent through the injection port. Leachate was mixed thoroughly by using a pump (MODEL 07559-00, Cole Parmer, USA) in the leachate holding bed. To recirculate the leachate from the leachate holding bed to the food waste holding basket, the bottom of the LBR was linked to the shower head nozzle on top by PVC tubing and a pump (MODEL 300308P, Burcam). The recirculation and mixing of leachate both were performed at specified periods using a timer (MODEL XT-4, ChronTrol, USA). The joints of the LBR had O-ring to ensure anaerobic condition inside the LBR. The schematic diagram and set-up of LBR are shown in Figure 3.1 and Figure 3.2 respectively.

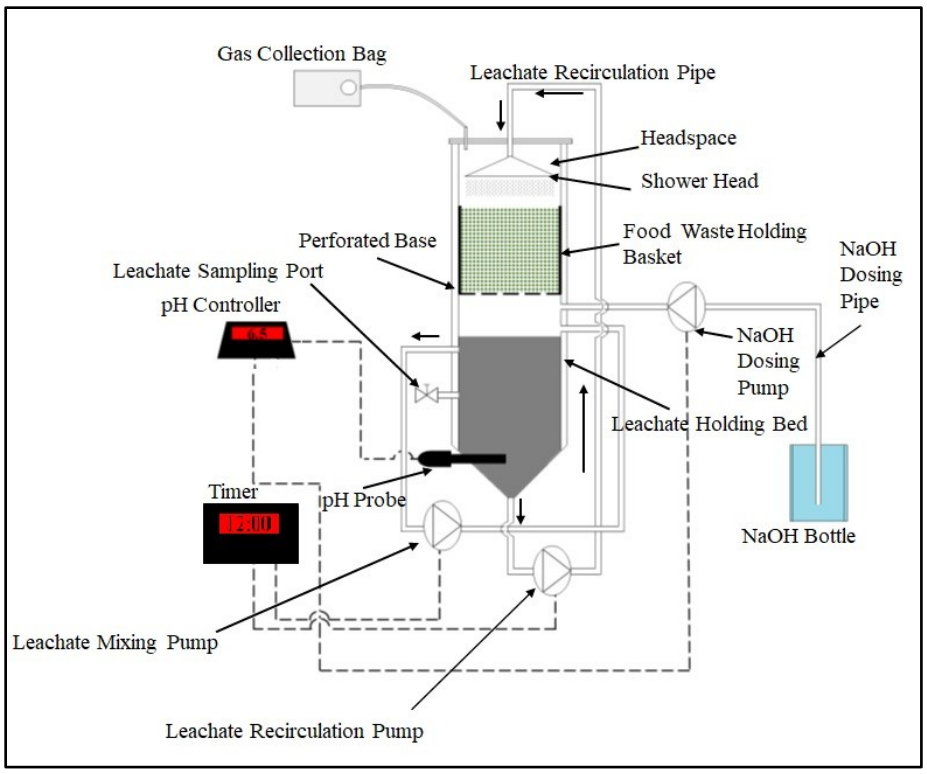


Figure 3.1 Schematic diagram of LBR.

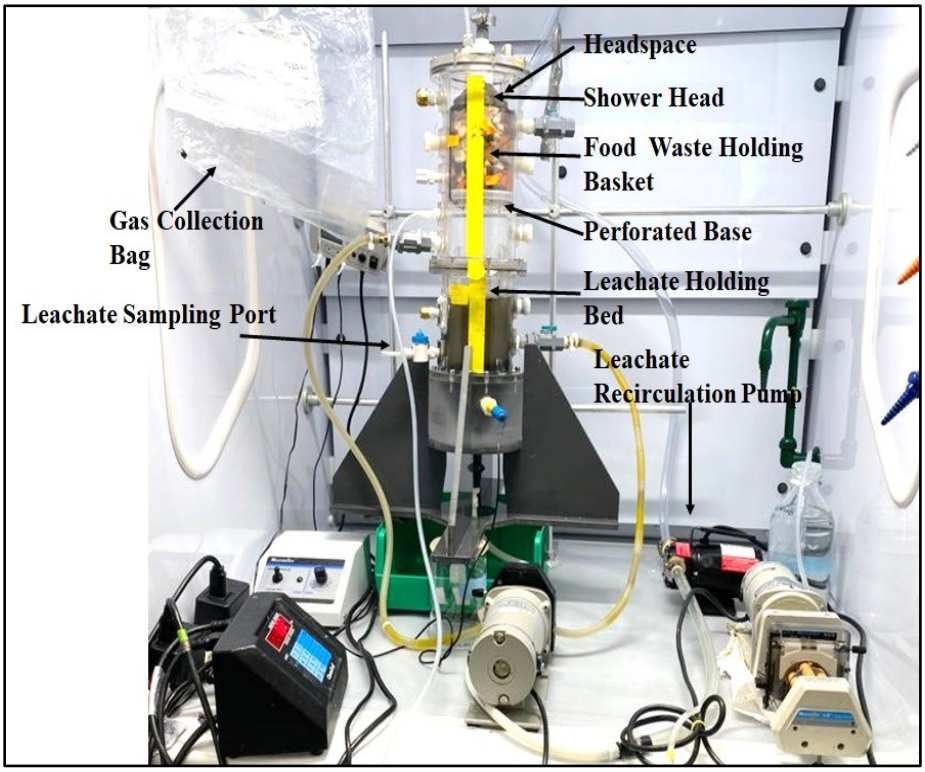


Figure 3.2 Set-up of LBR.

3.3.3 Pre-treatment of GAC

Prior to each experimental run, GAC pellets were washed repeatedly with distilled water to remove powdery residue as shown in Figure 3.3, followed by heating at 105 °C for 24 hours in an oven (Precision, Thermo Scientific) to remove the excess moisture. Figure 3.4 shows the pretreated GAC before and after drying.

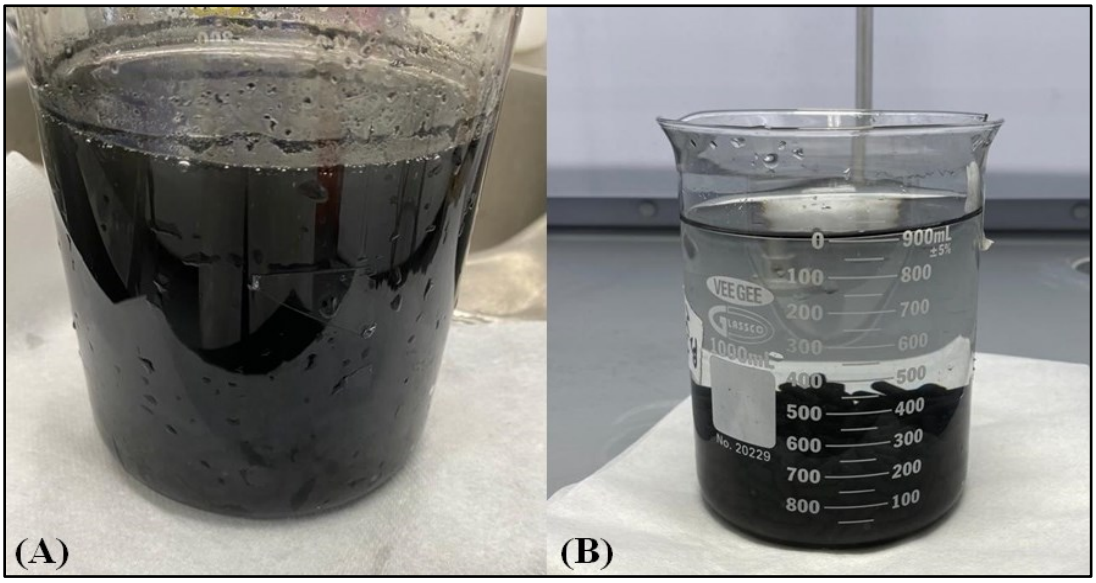


Figure 3.3 GAC pellets (A) at the time of first washing, (B) after washing repeatedly.

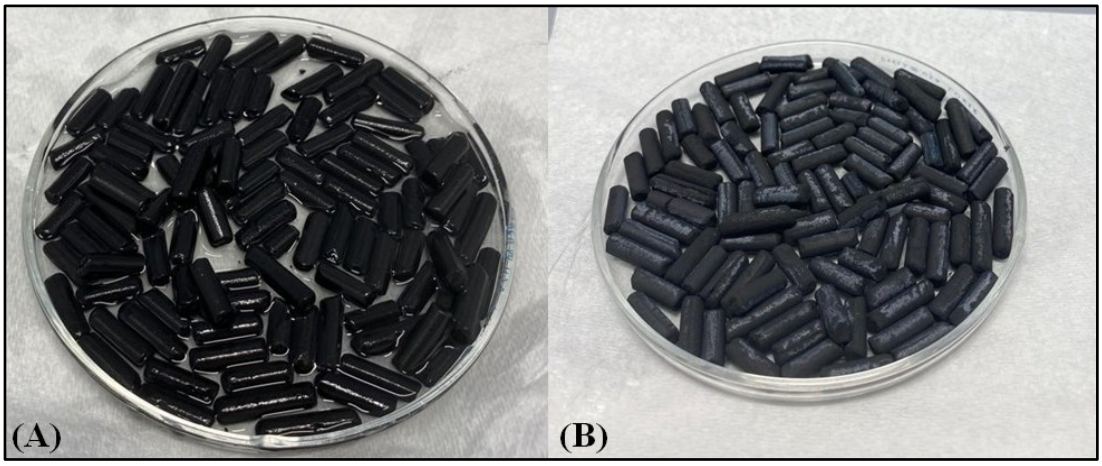


Figure 3.4 GAC pellets (A) before drying, (B) after drying.

3.3.4 LBR operation and GAC loading

All LBR experiments followed the same procedure unless specified. The LBR was operated at room temperature (22°C) in a fixed batch cycle of 14 days. The food waste basket was loaded with 500 g of simulated food waste along with the desired loading of pretreated GAC pellets (g GAC/g $VS_{foodwaste}$). The leachate holding bed was loaded with 1.5 L of RAS as inoculum to achieve an ISR of 4%. The food waste and inoculum had an average VS of 348 g/kg and 4.6 g/kg (Table 3.2), giving a volumetric organic loading of 49 g $VS/L_{reactor}$. The LBR was loaded with four different GAC loadings: 0 g GAC/g $VS_{foodwaste}$ (designated as LBR-C), 0.25 g GAC/g $VS_{foodwaste}$ (designated as LBR-1), 0.38 g GAC/g $VS_{foodwaste}$ (designated as LBR-2), and 0.51 g GAC/g $VS_{foodwaste}$ (designated as LBR-3). LBR-C was the control LBR to which the performance of other LBRs was compared. Figure 3.5 shows the packing of the simulated food waste in the food waste holding basket. The leachate was recirculated over the food waste at a LRR of 36.6 L/L_{bed}·day. In the leachate holding bed, the leachate was mixed by internal circulation at a rate of 19.4 L/L_{bed}·day. The pH of the leachate was maintained at 6.5 by using 1 M NaOH.

The liquid sampling was performed everyday up to day 6, and then it was performed every other day till the end of the 14 days batch cycle. TS and VS analyses were performed at the beginning and end of the batch cycle to evaluate the VS destruction efficiency. All LBR operations were performed at least in duplicates. The SCOD and VS were measured at least in triplicates while SCFA was analyzed at least in duplicates for all the samples.

Figure 3.5 Food waste holding basket packed with food waste.

3.3.5 Analytical method

TS and VS were analyzed by using the standard method (EPA, 2001). To analyze SCOD, the samples were filtered with a 0.45 µm pore size filter membrane in a vacuum filter. SCOD was analyzed by using a chemical oxygen demand (COD) reagent tube (SCP Science, Canada). The SCFA measurements were performed at the University of Waterloo (Waterloo, ON, Canada). The samples were first filtered using 0.45 µm filter, followed by 0.25 µm filter membrane in a vacuum filter. After that, the SCFA analysis was carried out using a gas chromatograph (HP 5890 Series II, Hewlett Packard, USA) with a flame ionization detector (FID) and a capillary column (30m×0.53mm×0.5µm PAG, Supelco, Bellefonte, PA). The temperature of the oven was set to hold 150°C for 2 min initially, then raise to 190 °C at a 4°C/min rate and maintained at 190°C for 3 min. The SCFA was primarily composed of acetate, propionate, and butyrate. Hence, total SCFA was determined by summing up the quantity of all three components of SCFA (acetate, propionate, and butyrate).

3.3.6 Calculation

The performance of LBR was assessed by five different parameters: (1) Leachate percolation rate, (2) VS destruction efficiency, (3) Hydrolysis yield, (4) Acidification (or SCFA/carboxylates) yield, and (5) SCFA:SCOD ratio.

The leachate percolation rate was estimated everyday based on the collected volume of leachate in a leachate holding bed in a fixed interval of one minute, immediately after the recirculation of leachate to the food waste holding basket (equation 3.1) (Han et al., 2015).

$$\text{Leachate percolation rate (ml/min)} = \frac{\text{volume of leachate collected in a leachate holding bed (ml)}}{\text{time (minute)}} \quad (3.1)$$

VS destruction efficiency was computed as the percentage of total VS removed in the LBR to the initial VS added to the LBR (equation 3.2). To calculate VS destruction efficiency, the VS of GAC is considered constant since it does not degrade during acidogenic fermentation (as discussed in Section 3.3.1). The separation of GAC from food waste was difficult at the end of fermentation, thus VS was analyzed for mixture of GAC and food waste remained in the LBR. Hence, in calculation of VS destruction efficiency, VS of food waste and inoculum added into LBR initially was considered.

$$\text{VS destruction efficiency (\%)} = \frac{VS_{(added)(initial)}(g) - VS_{(remained)(final)}(g)}{VS_{(added)(foodwaste+inoculum)}(g)} \quad (3.2)$$

Where,

$VS_{(added)(initial)}$ = VS of food waste (g) + VS of inoculum (g) + VS of GAC (g)

$VS_{(remained)(final)}$ = VS of remaining food waste and GAC mixture (g) + VS of leachate (g)

$VS_{(added)(foodwaste+inoculum)}$ = VS of food waste (g) + VS of inoculum (g)

Hydrolysis yield was calculated based on the mass of cumulative SCOD produced in the leachate to the initial VS added to the LBR (equation 3.4).

$$\text{SCOD (g SCOD)} = \text{concentration of SCOD in leachate (g SCOD/L)} \times \text{volume of leachate (L)} \quad (3.3)$$

$$\text{Hydrolysis yield (g SCOD/kg VS}_{\text{added}}\text{)} = \frac{\text{cumulative SCOD produced (g SCOD)}}{\text{VS}_{\text{added initially}}\text{(kg)}} \quad (3.4)$$

Where,

Cumulative SCOD produced (g SCOD) =

Final SCOD of leachate (g SCOD) − Initial SCOD of inoculum (g SCOD)

$VS_{added\ initially}$ (kg) = VS of food waste (kg) + VS of inoculum (kg)

Acidification yield was calculated as total SCFA produced in leachate to the initial VS added to the LBR (equation 3.5). Components of SCFA were expressed as COD equivalent using standard conversion factors given in (Lim et al., 2008). Total SCFA production was a sum of three main components of SCFA. i.e., acetate, propionate, butyrate.

$$\text{Acidification yield (g COD}_{\text{SCFA}}\text{/kg VS}_{\text{added}}\text{)} = \frac{\text{total SCFA produced (g COD}_{\text{SCFA}}\text{)}}{\text{VS}_{\text{added initially}}\text{(kg)}} \quad (3.5)$$

Where,

total SCFA produced (g COD_{SCFA}) =

final total SCFA of leachate(g COD_{SCFA}) − initial total SCFA of inoculum(g COD_{SCFA})

$VS_{added\ initially}$ (kg) = VS of food waste (kg) + VS of inoculum (kg)

SCFA:SCOD ratio (%) was calculated based on ratio of the acidification yield to the hydrolysis yield.

3.3.7 Statistical analysis

Single factor analysis of variance (ANOVA) test was performed to investigate the impact of different GAC loadings ($P \leq 0.05$) using Microsoft Excel software (2019).

3.4 Result and discussion

3.4.1 Leachate percolation rate

The leachate percolation rate was measured to investigate the impact of different GAC loadings in maintaining the permeability of the food waste bed. As shown in Figure 3.6, the leachate percolation rate was similar for all four LBRs until day 4. Thereafter, the leachate percolation rate decreased for LBRs with no or low GAC loadings (LBR-C and LBR-1), which could be due to reduction in permeability of food waste bed caused by the disintegration of food waste particles. In contrast, the leachate percolation rate gradually increased for LBR-2 and LBR-3, indicating that higher GAC loading of 0.38-0.51 g GAC/g $VS_{foodwaste}$ could better maintain the permeability of the food waste bed. An improvement in leachate percolation for LBR-C and LBR-1 was observed from day 10, however, it remained lower to those obtained for LBR-2 and LBR-3. The higher leachate percolation in LBR-2 and LBR-3 also resulted in higher hydrolysis and acidogenesis of food waste as discussed below.

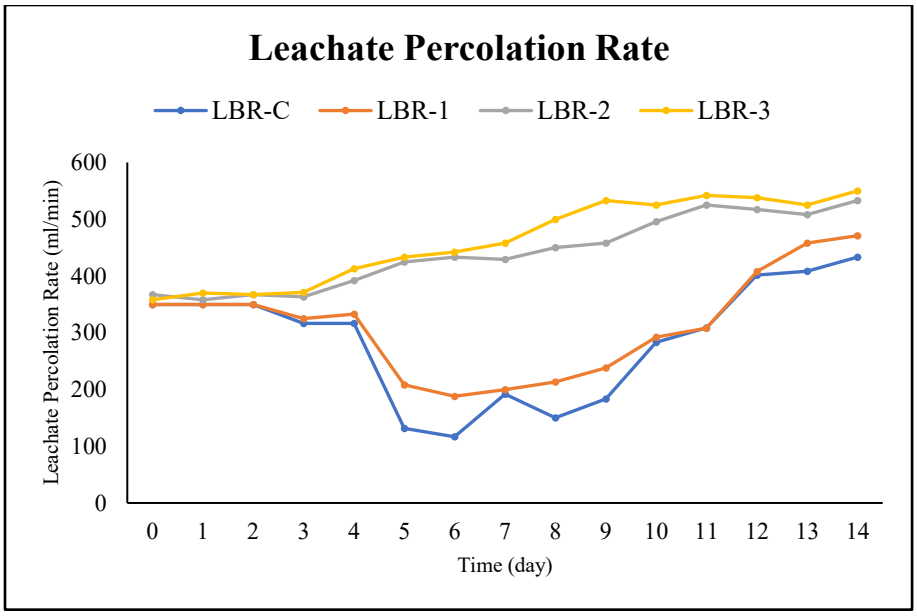


Figure 3.6 Leachate percolation rate in LBRs with different GAC loadings. (LBR-C: 0 g GAC/g $VS_{foodwaste}$, LBR-1: 0.25 g GAC/g $VS_{foodwaste}$, LBR-2: 0.38 g GAC/g $VS_{foodwaste}$, LBR-3: 0.51 g GAC/g $VS_{foodwaste}$).

3.4.2 Hydrolysis and substrate degradation

Hydrolysis of food waste in a LBR can be demonstrated by three different process performance parameters, namely, (1) cumulative SCOD production (g SCOD), (2) hydrolysis yield (g SCOD/kg VS_{added}), and (3) VS destruction efficiency (%). Figure 3.7 shows the cumulative SCOD production in LBRs with different GAC loadings. The cumulative SCOD produced on day 14 was 112 ± 1.9 g SCOD in LBR-3, followed by 111 ± 2.3 g SCOD in LBR-2, 88 ± 1.9 g SCOD in LBR-1, and 89 ± 0.6 g SCOD in LBR-C. The results indicated that solubilization (or hydrolysis) of particulate organic matter in food waste was improved at higher GAC loadings of 0.38-0.51 g GAC/g $VS_{foodwaste}$ (LBR-2 and LBR-3), which were statistically ($P \leq 0.05$) higher than the solubilization obtained for the control reactor (LBR-C, without GAC loading) and at lower GAC loading (LBR-1). The enhanced solubilization at higher GAC loadings could be attributed to a better distribution of hydrolytic enzymes and replenishment of the microenvironments (i.e., pH) due to

improved leachate percolation at higher GAC loadings (Figure 3.6). This was further corroborated by evaluating the hydrolysis yield and VS destruction efficiency.

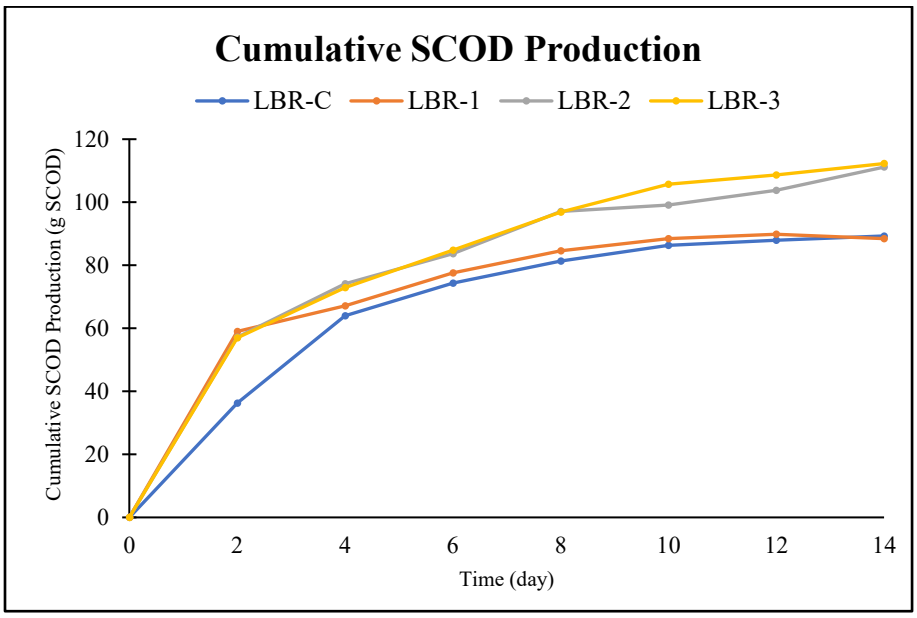


Figure 3.7 Cumulative SCOD production in LBRs with different GAC loadings. (LBR-C: 0 g GAC/g $VS_{foodwaste}$, LBR-1: 0.25 g GAC/g $VS_{foodwaste}$, LBR-2: 0.38 g GAC/g $VS_{foodwaste}$, LBR-3: 0.51 g GAC/g $VS_{foodwaste}$). (Each datapoint on graph represents average of six SCOD values).

The hydrolysis yield (g SCOD/ kg VS_{added}) is a ratio of cumulative SCOD produced to VS added to the LBR, implying the fractions of VS transformed into SCOD. The same information is provided by VS destruction efficiency, but it considers VS removed instead of SCOD production during acidogenic fermentation. The hydrolysis yield and VS destruction efficiency for different LBRs demonstrated a similar observation to cumulative SCOD production; that the solubilization of food waste improved at higher GAC loadings (Table 3.3). The highest hydrolysis yield of 620 ± 10.4 g SCOD/kg VS_{added} was obtained for LBR-3, which was almost 26-28% higher than that obtained for LBR-1 and LBR-C (485-491 g SCOD/kg VS_{added}). Concomitantly, increasing GAC loading from 0 to 0.51 g GAC/g $VS_{foodwaste}$ resulted in 23% higher VS destruction efficiency, supporting that

improved hydrolysis could be attained with higher GAC loading of 0.38-0.51 g GAC/g $VS_{foodwaste}$ (Table 3.3).

Table 3.3 Parameters in LBRs with different GAC loadings at the end of fermentation

Parameters	LBR-C	LBR-1	LBR-2	LBR-3
VS destruction efficiency (%)	57.6 ± 0.6	59.5 ± 1.4	69.4 ± 1.2	71.0 ± 1.4
Hydrolysis yield (g SCOD/kg VS_{added})	491 ± 3.2	485 ± 10.4	614 ± 12.7	620 ± 10.4
Cumulative SCOD production (g SCOD)	89 ± 0.6	88 ± 1.9	111 ± 2.3	112 ± 1.9
Acetate (g COD)	26 ± 0.6	27 ± 0.6	18 ± 1.7	22 ± 0.8
Propionate (g COD)	16 ± 0.9	16 ± 0.4	15 ± 1.0	18 ± 1.0
Butyrate (g COD)	26 ± 0.8	24 ± 0.6	49 ± 0.4	52 ± 1.6
Total SCFA production (g COD_{SCFA})	68 ± 2.0	67 ± 1.5	82 ± 1.2	92 ± 2.0
SCFA:SCOD (%)	76	76	73	82
Acidification yield (g COD_{SCFA}/ kg VS_{added})	375 ± 11.0	371 ± 8.3	451 ± 6.6	507 ± 11.0

Similar to cumulative SCOD production, the hydrolysis yield for LBR-3 and LBR-2 was statistically ($P \leq 0.05$) different than that obtained for LBR-1 and LBR-C. However, no statistical difference was found between the hydrolysis yields for LBR-3 and LBR-2, indicating that increasing the GAC loading from 0.38 to 0.51 g GAC/g $VS_{foodwaste}$ did not improve food waste solubilization at a fermentation time of 14 days. However, as shown in Table 3.3 and Table 3.4, LBR-3 achieved almost the same hydrolysis yield (602 g SCOD/kg VS_{added}) two days (day 12) prior to LBR-2 (day 14). A similar trend was also observed for LBR-1 and LBR-C. The hydrolysis yield was not statistically different

between LBR-1 and LBR-C if calculated based on fermentation time of 14 days, but LBR-1 produced the maximum hydrolysis yield of 497 ± 5.0 g SCOD/kg VS_{added} on day 12, while it took additional two days to achieve the almost similar hydrolysis yield in LBR-C (day 14). These results clearly indicate that the hydrolysis yield and cumulative SCOD could have been statistically ($P \leq 0.05$) different, if the fermentation time was 12 days instead of 14 days as shown in Table 3.4, thus GAC improved the hydrolysis rates and can shorten the fermentation time required to reach maximum yields.

Table 3.4 Parameters in LBRs with different GAC loadings on day 10 and day 12

Parameters	LBR-C	LBR-1	LBR-2	LBR-3
Hydrolysis yield[a] (g SCOD/kg VS_{added})	485 ± 3.3	497 ± 5.0	568 ± 11.7	602 ± 16.5
Cumulative SCOD production[a] (g SCOD)	88 ± 0.6	90 ± 0.9	103 ± 2.1	109 ± 3.0
Acetate[b] (g COD)	22 ± 2.0	24 ± 0.7	18 ± 1.2	19 ± 0.8
Propionate[b] (g COD)	14 ± 1.4	17 ± 1.6	18 ± 3.5	12 ± 0.5
Butyrate[b] (g COD)	30 ± 2.8	30 ± 0.7	56 ± 4.0	65 ± 1.7
Total SCFA production[b] (g COD_{SCFA})	66 ± 0.6	71 ± 2.2	92 ± 1.9	96 ± 1.7
SCFA:SCOD[b] (%)	77	80	93	91
Acidification yield[b] (g COD_{SCFA}/kg VS_{added})	364 ± 3.3	390 ± 12.1	507 ± 10.5	529 ± 9.4

[a]Hydrolysis yield and cumulative SCOD production at day 12
[b]Total SCFA production and composition of SCFA at day 10

Table 3.5 Cumulative SCOD production and its fraction at different GAC loadings

Time	Cumulative SCOD Production											
Day	LBR-C			LBR-1			LBR-2			LBR-3		
	g SCOD	g SCOD /L_{bed}	%	g SCOD	g SCOD /L_{bed}	%	g SCOD	g SCOD /L_{bed}	%	g SCOD	g SCOD /L_{bed}	%
0	0	0	0%	0	0	0%	0	0	0%	0	0	0%
2	36	17	40%	59	28	66%	57	27	51%	57	27	51%
4	64	30	72%	67	32	75%	74	35	67%	73	35	65%
6	74	35	83%	78	37	86%	84	40	75%	85	40	76%
8	81	39	91%	85	40	94%	97	46	87%	97	46	86%
10	86	41	97%	88	42	98%	99	47	89%	106	50	94%
12	88	42	99%	90	43	100%	103	49	93%	109	52	97%
14	89	43	100%	88	42	99%	111	53	100%	112	53	100%

The improvements in hydrolysis rate can be further seen by analyzing the cumulative SCOD production. For example, LBR-2 and LBR-3 achieved a cumulative SCOD of 97 g SCOD on day 8, which was 86-87% of the total SCOD generated (111 – 112 g SCOD), which was higher than that obtained in LBR-1 and LBR-C on day14 (Table 3.5). It implies that the higher GAC loading resulted in higher cumulative SCOD production along with a high rate of cumulative SCOD production (ΔSCOD/Δtime), thus shortening the fermentation time needed to reach maximum performance.

3.4.3 SCFA production

Three main SCFAs, acetate, propionate, and butyrate were considered in this study. Thus, the total SCFA was the sum of acetate, propionate, and butyrate as a COD equivalent (g COD). Similar to SCOD generation, total SCFA production increased with the fermentation time at all GAC loadings, demonstrating the growth of SCFA-generating acidogenic bacteria. As shown in Figure 3.8, total SCFA production differed among LBRs. The maximum SCFA production was achieved at the highest GAC loading (LBR-3). The total SCFA of 92 ± 2.0 g COD_{SCFA} for LBR-3, 82 ± 1.2 g COD_{SCFA} for LBR-2, 67 ± 1.5 g COD_{SCFA} for LBR-1, and 68 ± 2.0 g COD_{SCFA} for LBR-C were obtained. This finding clearly indicates that total SCFA production could be enhanced by increasing the GAC loading. Notably, between LBR-3 and LBR-2, the total SCFA production on day 14 were statistically ($P \leq 0.05$) different even if cumulative SCOD production was statistically indifferent (as discussed in section 3.4.2).

The positive impact of higher GAC loading on acidogenesis was further evident from the acidification yield (g COD_{SCFA}/kg VS_{added}) and SCFA:SCOD ratio (%). The acidification yield is the ratio of total SCFA produced to the VS added to the LBR initially.

The highest acidification yield of 507 g COD_{SCFA}/kg VS_{added} was achieved in LBR-3, followed by 451 g COD_{SCFA}/kg VS_{added} for LBR-2, 371 g COD_{SCFA}/kg VS_{added} for LBR-1, and 375 g COD_{SCFA}/kg VS_{added} for LBR-C, respectively. The acidification yield for LBR-3 was statistically higher to the rest of the LBRs. This result shows that acidification efficiency could be improved by almost 35% with GAC loading of 0.51 g GAC/g $VS_{foodwaste}$ (LBR-3) as compared to GAC loading of 0 g GAC/g $VS_{foodwaste}$ (LBR-C). In LBR-2 and LBR-3, the maximum acidification yield (507-529 g COD_{SCFA}/kg VS_{added}) was achieved on day 10 (Table 3.4), which was statistically ($P \leq 0.05$) greater than the maximum acidification yield obtained in LBR-C on day 14. This implies that higher GAC loading helped to achieve a high acidification yield in a shorter fermentation time of 10 days. The acidification yield obtained in this study was significantly higher than prior studies treating food waste at a comparable volumetric organic loading in LBR. Hussain et al. (2017) obtained a maximum acidification yield of 330 g COD_{SCFA}/kg VS_{added} at thermophilic temperature with LRR of 2.6 L/L_{bed}·day at volumetric organic loading of 46 g $VS/L_{reactor}$. Similarly, Xu et al. (2011) achieved a high acidification yield of 101 g COD_{SCFA}/kg VS_{added} at high volumetric organic loading of 60 g $VS/L_{reactor}$ over the fermentation time of 17 days in LBR by using wood chips as an exogenous material and daily dilution of leachate.

The positive impact of higher GAC loading was further supported by SCFA:SCOD ratio. SCFA:SCOD denotes the fractions (%) of SCOD as total SCFA. In this study, the maximum SCFA:SCOD was 82% in LBR-3 (Table 3.3). Whereas, SCFA:SCOD was 73-76% in LBR-2, LBR-1, and LBR-C. A remaining portion of SCOD may be constituted by other products such as other SCFA (isovalerate, n-valerate, iso-caproate, hexanoate), alcohol, LCFA, and soluble microbial product (Saha & Lee, 2020; Xiong et al., 2019b).

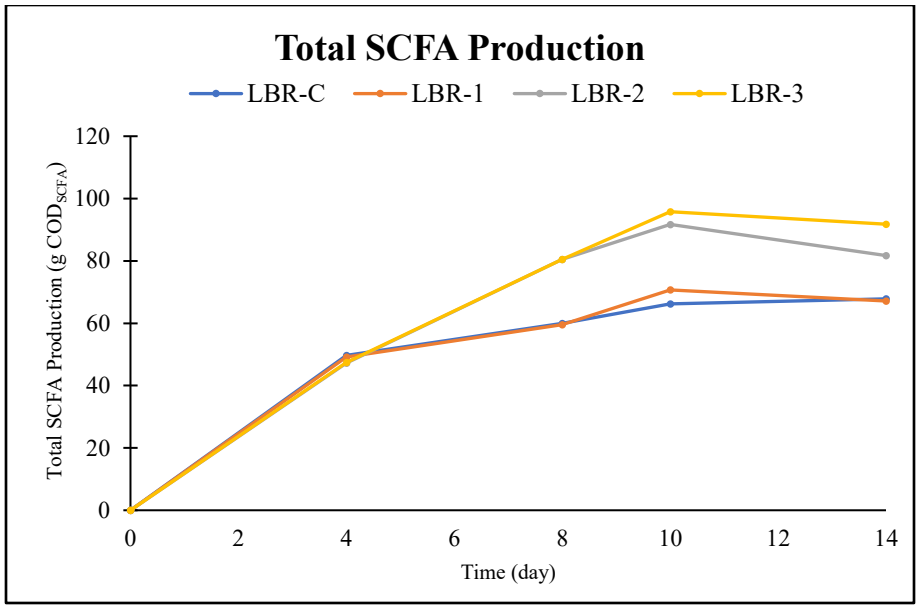


Figure 3.8 Total SCFA production in LBRs with different GAC loadings. (LBR-C: 0 g GAC/g $VS_{foodwaste}$, LBR-1: 0.25 g GAC/g $VS_{foodwaste}$, LBR-2: 0.38 g GAC/g $VS_{foodwaste}$, LBR-3: 0.51 g GAC/g $VS_{foodwaste}$). (Each datapoint on graph represents average of four SCFA values).

3.4.4 SCFA composition

It is critical to analyze the production of each component of SCFA at different GAC loadings since the composition of SCFA directly impacts the downstream processes to recover the SCFA from the leachate. The production of each SCFA component with time is illustrated in Figure 3.9, while Figure 3.10 interprets each SCFA component as a percentage of total SCFA. The SCFA composition was different among LBRs at high GAC loadings (0.38-0.51 g GAC/g $VS_{foodwaste}$) and LBRs at low or no GAC loadings (0-0.25 g GAC/g $VS_{foodwaste}$). Butyrate was the most dominant SCFA in LBR-2 and LBR-3, accounting for 57-60% of total SCFA production on day 14. In LBR-2 and LBR-3, acetate (22-24%) was the second most dominant SCFA. On other hand, butyrate composition (36-38%) was almost similar to the composition of acetate (38-40%) in LBR-C and LBR-1. This result indicated that GAC loading has an impact on SCFA composition with high butyrate production at high GAC loadings (0.38-0.51 g GAC/g $VS_{foodwaste}$). As compared

to acetate and butyrate, propionate (18-24% of total SCFA) was lower at all GAC loadings (0-0.51 g GAC/g $VS_{foodwaste}$). It means that acidogenic bacteria followed the acetate-butyrate metabolic pathway at nearly acidic-neutral (6-7) pH (Jiang et al., 2013; Zhang et al., 2005). The higher composition of butyrate at nearly neutral to acidic pH is consistent with the literature (Dahiya et al., 2015; Hussain et al., 2017). This higher composition of butyrate is more desirable as an end product since butyrate has wide variety of applications in different industries.

The SCFA composition changed with the fermentation time. For example, for all the LBRs, the butyrate was increased until day 10 and reached to 30-65 g COD. Correspondingly, butyrate was 42-68% of total SCFA on day 10. However, the butyrate composition decreased to 36-60% of total SCFA by day 14 in all LBRs. On other hand, acetate was increased from 19-34% on day 10 to 22-40% on day 14. This increase in acetate production along with a decline in the butyrate production after day 10 indicated that the butyrate was being converted into acetate. It agrees with a prior study working on AF of food waste where a decline in butyrate composition was accompanied by an increase in acetate composition at pH 6-7 (Duncan et al., 2002; Xiong et al., 2019b). Hence, a shorten fermentation time would be preferable if butyrate is the desired SCFA.

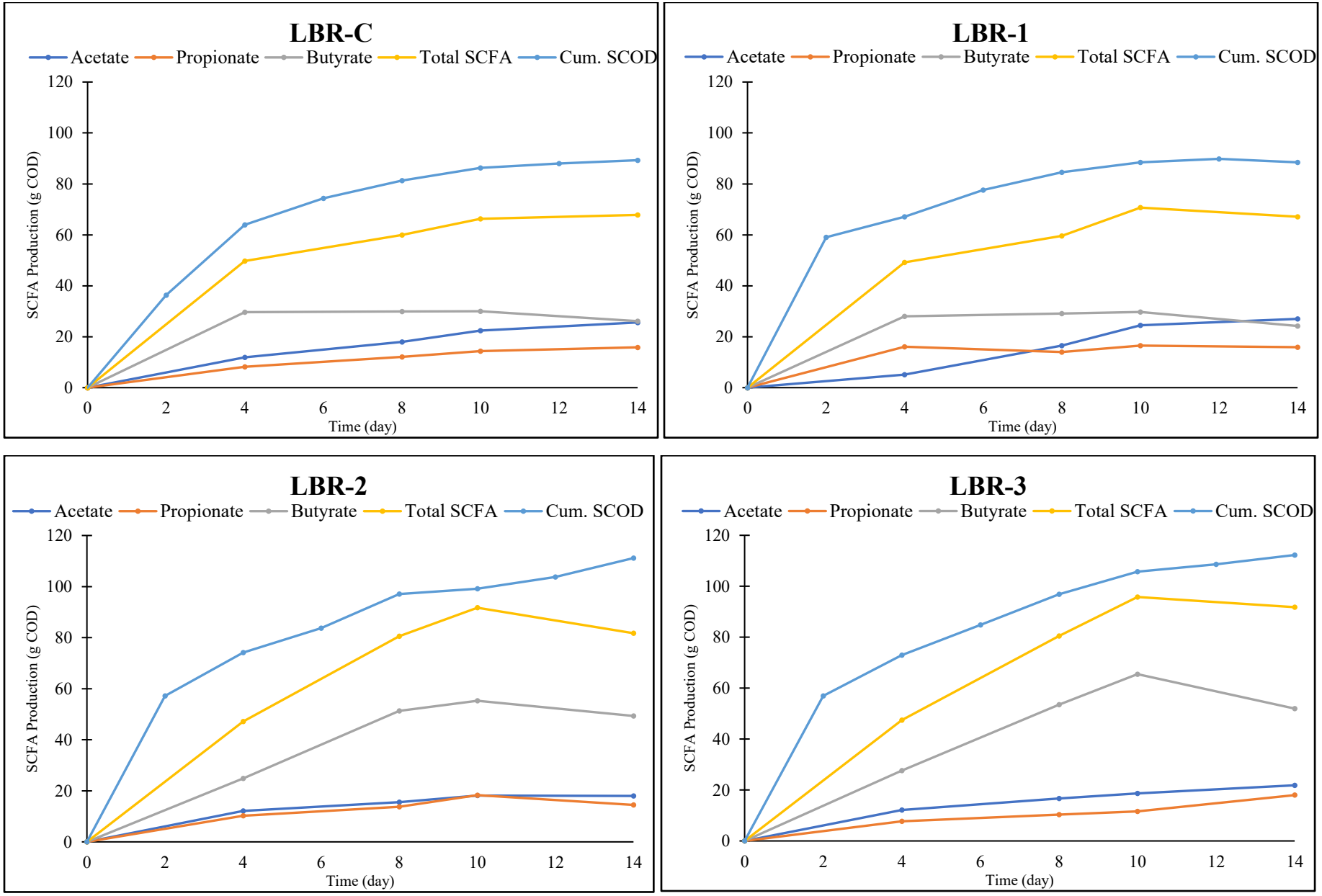


Figure 3.9 Production of each SCFA component in LBRs with different GAC loadings. (LBR-C: 0 g GAC/g $VS_{foodwaste}$, LBR-1: 0.25 g GAC/g $VS_{foodwaste}$, LBR-2: 0.38 g GAC/g $VS_{foodwaste}$, LBR-3: 0.51 g GAC/g $VS_{foodwaste}$). (Each datapoint on graph represents average of four SCFA values and six SCOD values). (The unit of cum. SCOD is g SCOD).

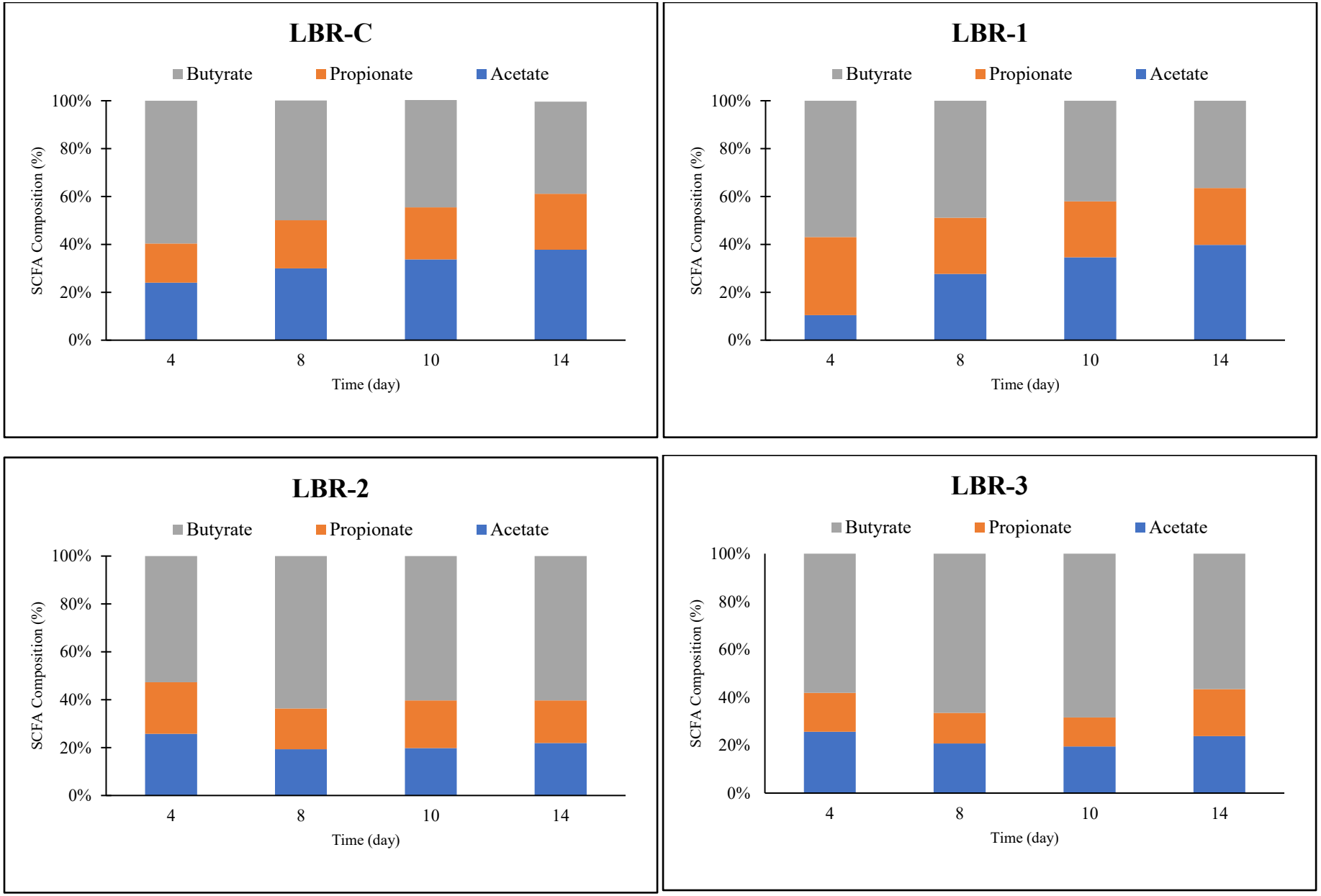
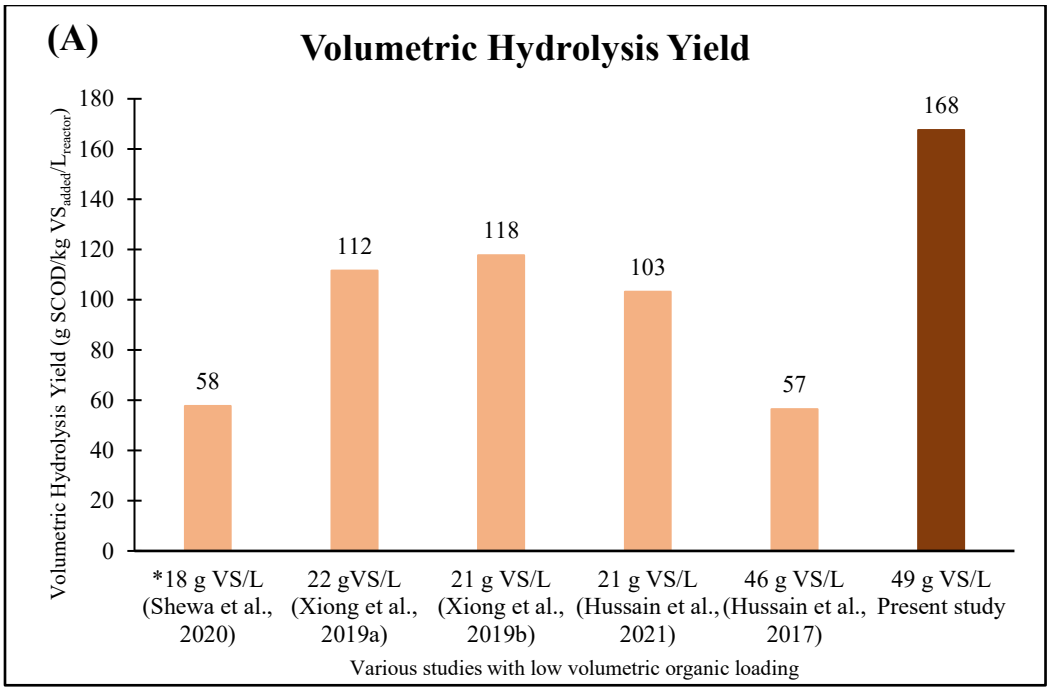


Figure 3.10 Fraction of each SCFA component in LBRs with different GAC loadings. (LBR-C: 0 g GAC/g $VS_{foodwaste}$, LBR-1: 0.25 g GAC/g $VS_{foodwaste}$, LBR-2: 0.38 g GAC/g $VS_{foodwaste}$, LBR-3: 0.51 g GAC/g $VS_{foodwaste}$).

3.4.5 Discussion

This study evaluated the performance of LBR operated with different GAC loadings for the production of carboxylates (SCFA) from food waste at high volumetric organic loading of 49 g VS/$L_{reactor}$. The volumetric organic loading in this study was 1.5-2.7 times higher to the organic loading in recent studies on LBR (Saha & Lee, 2020, Shewa et al., 2020; Xiong et al., 2019a; Xiong et al., 2019b; Hussain et al., 2021). The maximum hydrolysis yield of 620 g SCOD/kg VS_{added} and acidification yield of 507 g COD_{SCFA}/kg VS_{added} was obtained in LBR-3 with the GAC loading of 0.51 g GAC/g $VS_{foodwaste}$. This corresponded to a volumetric hydrolysis and acidification yields (i.e., per unit volume of LBR) of 168 g SCOD/kg $VS_{added}/L_{reactor}$ and 137 g COD_{SCFA}/kg $VS_{added}/L_{reactor}$, respectively, which were significantly higher than those reported for LBRs with low volumetric organic loading at mesophilic temperatures (Figure 3.11). Furthermore, the volumetric performance of LBR-3 was 2-3 times higher than LBR that was operated at thermophilic conditions at similar volumetric organic loading (Hussain et al., 2017).

These results demonstrated that with the addition of GAC, a stable LBR performance can be achieved at high volumetric organic loading. Not much gas production was observed. It could be due to several reasons: (1) RAS had very less methanogenic activity, and (2) at high organic loading, a high SCFA concentration caused acidification, resulting in inhibition of the methanogenesis (Yuan & Zhu, 2016; Wainaina et al., 2019). Future studies are required to ascertain the economic returns accrued by the improvement in this volumetric performance of the GAC supplemented-LBR for large-scale applications. Multiple factors such as product yields, energy cost, cost of GAC, etc., would have to be considered for such an economic analysis.



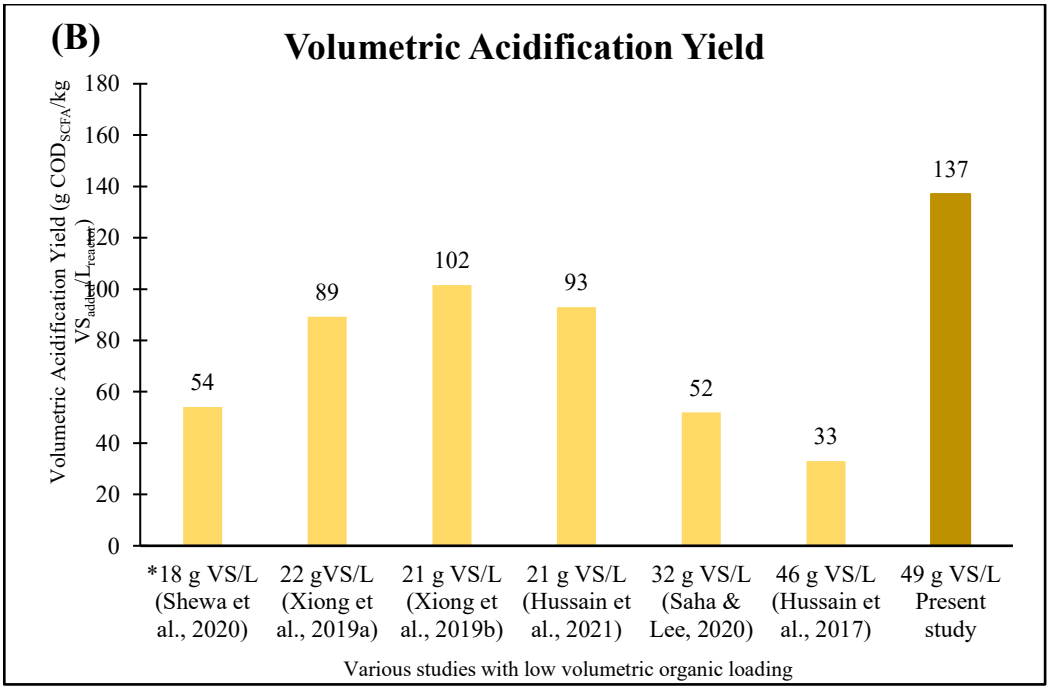
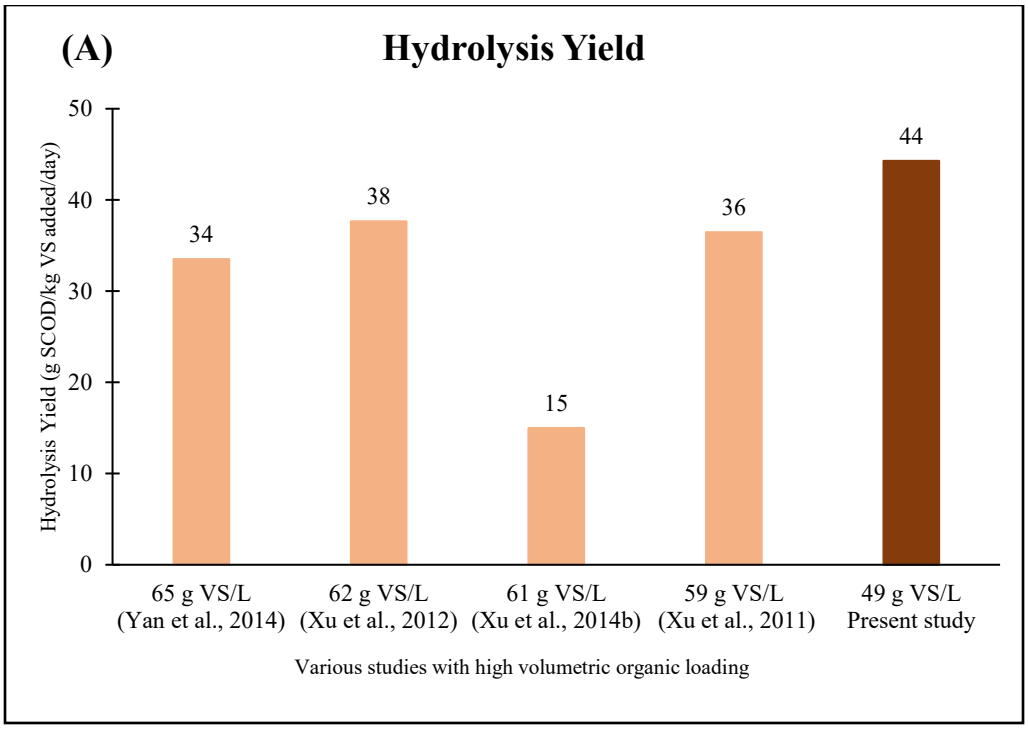


Figure 3.11 Comparison of product yields based on per unit volume of reactor (A) volumetric hydrolysis yield, and (B) volumetric acidification yield
Fermentation time of 14 days was maintained for all the research studies except the research study of Shewa et al. (2020). *The research study of Shewa et al. (2020) was performed at a fermentation time of 6 days.

The performance of LBR-3 was also compared to the LBRs operated at high volumetric organic loading. Some studies have operated LBR at relatively high volumetric organic loading (59-65 g VS/$L_{reactor}$) and longer fermentation time (16-17 days). These studies used extensive leachate dilution along with the addition of exogenous materials (i.e., wood chips, sawdust, etc.) to prevent the deterioration of process performance of LBR (Xu et al., 2014a; Xu et al., 2014b; Xu et al., 2012; Yan et al., 2014; Xu et al., 2011). The hydrolysis (240-640 g SCOD/kg VS_{added}) and acidification yields (82-390 g COD_{SCFA}/kg VS_{added}) for these studies were lower or comparable to that obtained for LBR-3. Notably, high LBR performance was obtained in this study without the dilution of leachate or addition of process water. To compare the performance of LBR-3 with LBRs operated at high volumetric organic loading, the hydrolysis and acidification yields calculated on per day basis was considered. On a per day basis, the hydrolysis and acidification yield of LBR-3 was estimated as 44 g SCOD/kg VS_{added}/day and 36 g COD_{SCFA}/kg VS_{added}/day, respectively, which were significantly higher than those reported by previous studies (Figure 3.12). The performance of LBR-3 could be further improved by optimizing other operational parameters such as start-up inoculum.



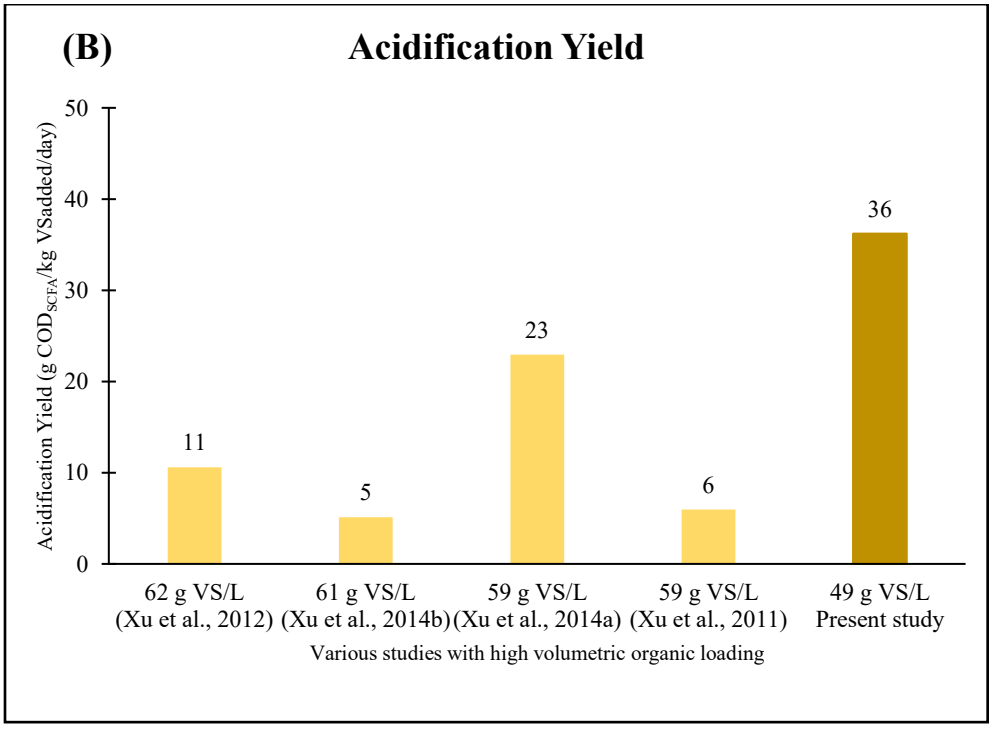


Figure 3.12 Comparison of product yields based on per day (A) hydrolysis yield, and (B) acidification yield

3.5 Conclusion

GAC loadings clearly impacted the hydrolysis and acidification yields of the LBRs. The higher GAC loading of 0.51 g GAC/g $VS_{foodwaste}$ (LBR-3) achieved the highest hydrolysis yield of 620 g SCOD/kg VS_{added}, which was statistically ($P \leq 0.05$) different than that obtained at low (0.25 g GAC/g $VS_{foodwaste}$) (LBR-1) or no GAC loading (LBR-C). The hydrolysis rate of LBR was gradually improved with increase in GAC loading. Furthermore, a high GAC loading of 0.51 g GAC/g $VS_{foodwaste}$ (LBR-3) resulted in 35% higher acidification yield than LBR-C (with no GAC loading). Butyrate formed the major fractions of SCFA at GAC loading of 0.38 g GAC/g $VS_{foodwaste}$ (LBR-2) and GAC loading of 0.51 g GAC/g $VS_{foodwaste}$ (LBR-3). Whereas similar composition of acetate and butyrate was obtained at GAC loading of 0-0.25 g GAC/g $VS_{foodwaste}$ (LBR-C and LBR-1). A decline in butyrate was observed after day 10 in all LBRs irrespective of GAC loading. Therefore, LBR can be operated at a shorter fermentation time of 10 days if butyrate is the desired SCFA.

Chapter 4. Carboxylates production from food waste in a LBR: Effect of inoculum type and sequential enrichment method

4.1 Abstract

The effects of two different inoculums (RAS and AD-sludge) and sequential enrichment procedure on hydrolysis and acidogenesis of food waste were evaluated for LBRs at high volumetric organic loading of 49 g VS/$L_{reactor}$. LBR inoculated with RAS achieved hydrolysis yield of 614 g SCOD/kg VS_{added} on day 14, which was significantly ($P \leq 0.05$) higher to that obtained for LBR inoculated with AD-sludge. RAS also resulted in a higher acidification yield of 451 g COD_{SCFA}/kg VS_{added}, which was 48 % higher than that obtained with AD sludge (304 g COD_{SCFA}/kg VS_{added}). Butyrate (60% of total SCFA) was the dominant SCFA with RAS, while LBR-AD had similar compositions for acetate (36% of total SCFA) and butyrate (37% of total SCFA). Enrichment of RAS further improved the hydrolysis and acidogenesis yields by 10-22% resulting in the final hydrolysis yield of 683 g SCOD/kg VS_{added} and acidification yield of 617 g COD_{SCFA}/kg VS_{added}.

4.2 Introduction

Carboxylates production from food waste using LBRs has been gaining attention as a sustainable and economical platform for stabilization of food waste and production of value-added bioproducts (Hussain et al., 2021; Xiong et al., 2019a; Arras et al., 2019; Zhou et al., 2018). Several operating parameters such as temperature, pH, ISR, LRR, and inoculum affect the hydrolysis and acidogenesis of food waste to produce carboxylates (Hussain et al., 2017; Xiong et al., 2019b; Hussain et al., 2017; Xu et al., 2014a; Xiong et al., 2019a). Among them, inoculum is an important parameter which has not received much attention. To our knowledge, only two studies have focused on the effect of inoculum type

on carboxylates production from food waste in LBR (Yan et al., 2014; Shewa et al., 2020). Hydrolytic and acidogenic bacteria are primary drivers of carboxylates production from food waste. Hence, variations in the population of these bacteria among different inoculums could result in distinct hydrolysis and acidification yields (Dahiya et al., 2015; Agler et al., 2011). Therefore, it is critical to provide a suitable inoculum to enhance carboxylates production from food waste and shorten the fermentation time. Microbial populations in an inoculum can differ based on its source, enrichment/acclimatization, storage conditions, etc. (Parra-Orobio et al., 2018; Arras et al., 2019). AD-sludge originating from an anaerobic environment has been widely utilized as an inoculum for carboxylates production from food waste in LBR (Table 4.1). On the other hand, several studies have used RAS originating from an aerobic environment as an inoculum in anaerobic bioreactors and demonstrated better hydrolysis performance. This is generally attributed to the higher hydrolytic activity of inoculums originating from aerobic environment. (Gao et al., 2014; Saad et al., 2019; Wang et al., 2014; Li et al., 2020). However, information on impact of RAS as an inoculum for carboxylates production from food waste in LBRs is sparse.

Another important factor is inoculum enrichment/acclimatization. Enriched inoculum is acclimatized to the operating conditions and has a relatively higher population of the desired bacteria. Therefore, enriched inoculum would help to reduce the fermentation time along with improvement in hydrolysis and acidification yields. Only two studies have used the enriched inoculum for hydrolysis and acidogenesis of food waste in LBRs. Both of these studies showed improvement in hydrolysis and acidification yields at low volumetric organic loading of 18-21 g VS/$L_{reactor}$ (Hussain et al., 2021; Shewa et al.,

2020). Therefore, enriched inoculum could improve LBR performance even at high volumetric organic loading, however, this needs to be experimentally evaluated.

This study evaluated the effects of inoculum type and enrichment/acclimatization on the hydrolysis and acidogenesis of food waste at high volumetric organic loading (49 g $VS/L_{reactor}$) in LBR. In the first phase of this study, the hydrolysis and acidogenesis yields were compared for LBRs seeded with two different inoculum – AD-sludge and RAS. In the second phase, LBR performance was further improved by the serial (or sequential) enrichment of the better-performing inoculum in phase one.

Table 4.1 Comparison of inoculum in acidogenic fermentation of food waste in LBR and CSTR

Substrate	Inoculum	Reactor Type	Volumetric Organic Loading (g VS/ $L_{reactor}$)	Fermentation time (day)	Temperature (˚C)	pH	ISR (%) (VS basis)	Hydrolysis yield (g SCOD/ kg VS_{added})	Acidification yield (g COD_{SCFA}/kg VS_{added})	Mode of Operation	Reference
[a]FW	AD-sludge	CSTR	81	27	25	5.5 - 6	-	-	-	Batch	(Greses et al., 2021)
[a]FW	Acclimated acidogenic culture	LBR	21.7	10	22	6	4	774	697	Batch	(Hussain et al., 2021)
[a]FW	AD-sludge + acclimated acidogenic culture	LBR	18.1	6	22	7	4	693	649	Batch	(Shewa et al., 2020)
[a]FW	AD-sludge	LBR	32.3	14	22	6	10	-	571	Batch	(Saha & Lee, 2020)
[a]FW	AD-sludge	LBR	22.7	14	22	6	10	837	669	Batch	(Xiong et al., 2019a)
[a]FW	AD-sludge	LBR	21.7	14	22	7	5	883	761.81	Batch	(Xiong et al., 2019b)
[a]FW	Enriched AD-sludge	CSTR	-	30	37	-	-	-	-	Semi-continuous	(Lukitawesa et al., 2019)

[a]FW + [c]CEPT Sludge	AD-sludge	CSTR	99.1	20	35	6.5 - 7	-	760	-	Batch	(Chakraborty et al., 2018)
[a]FW	Enriched AD-sludge	CSTR	-	-	37	6.9	-	-	-	Batch	(Chang et al., 2018)
[a]FW	AD-sludge	LBR	46	14	50	7	4	565	330	Batch	(Hussain et al., 2017)
[a]FW	AD-sludge from digester treating food waste at mesophilic temperature	CSTR	-	-	37	7.7-8	-	-	-	batch	(Zamanzadeh et al., 2016)
[a]FW	Cow manure	LBR	65.16	18	-	6	-	570	-	Batch	(Yan et al., 2014)
[a]FW	AD-sludge	LBR	61.31	17	35	6	-	240.16	82.08	Batch	(Xu et al., 2014a)
[a]FW	AD-sludge	LBR	59.80	17	35	6	-	-	390	Batch	(Xu et al., 2014b)
[a]FW	Granular sludge from UASB system treating high	LBR	-	24	37	5	-	-	-	-	(Browne et al., 2013)

	COD effluent										
MSW	Acclimated sewage sludge	LBR	-	31	22	-	-	-	-	-	(Uke & Stentiford, 2013)
[a]FW	Waste Activated Sludge	CSTR	-	20	30	6	-	482	-	Batch	(Wang et al., 2014)
[a]FW	AD-sludge	LBR	62.43	17	35	6	6.9	640	180	Batch	(Xu et al., 2012)
[a]FW	AD-sludge	LBR	59.80	17	-	6	-	620	101	Batch	(Xu et al., 2011)
[a]FW	AD-sludge	CSTR	72[b]	8	35	5.5	-	-	350	Semi-continuous	(Lim et al., 2008)
[a]FW	Acclimated mesophilic acidogenic culture	CSTR	-	13	35	5.5	-	-	-	Batch	(Shin et al., 2004)

[a]FW = Food Waste

[b]Volumetric organic loading (g VS/$L_{reactor}$) = Organic loading rate (g VS/$L_{reactor}$/day) × hydraulic retention time (day)

[c]CEPT Sludge = Chemically enhanced primary treatment sludge

4.3. Material and methods

4.3.1 Characteristics of substrate, GAC, and inoculum

Due to the COVID-19 pandemic, restaurants, and cafeterias were shut down. Consequently, real food waste was not available, thus simulated food waste was used in this study. The simulated food waste consisted of 40% carrot, 35% potato, 15% bread, and 10% pet food on a wet weight basis. This composition of the simulated food waste was similar to the real food waste used in the previous research studies (Xiong et al., 2019a; Xiong et al., 2019b; Hussain et al., 2017). The pet food was used to increase the VS content of the simulated food waste. Pet food is high in VS content and contains carbohydrates, proteins, and lipids (Fernández et al., 2005; Xu & Li, 2012). Each component of the simulated food waste was stored at -10˚C to prevent any degradation. The individual components were defrosted as per the required quantity at room temperature (22˚C) for two hours before the experiment and then immediately chopped to an average particle size of ~1 cm by using a mesh chopper (Starfrit, Canada). Required quantity of pretreated GAC pellets (as described in Section 3.2.3 of Chapter 3) were loaded into the food waste holding basket as an exogenous material. GAC pellets (CNZ, USA) with a length of approximately 7-25 mm and a diameter of 4 mm were used. Two separate inoculums, RAS and AD-sludge were tested for AF of food waste. The RAS and AD-sludge were collected from the Robert O. Pickard Environmental Centre (ROPEC) wastewater treatment facility (Ottawa, Ontario, Canada) and stored at 4˚C before used in the experiments. AD-sludge was heated at 75˚C for 15 minutes prior to the experiment to kill the methanogens. The characteristics of simulated food waste, GAC pellets, and inoculums are described in Table 4.2.

Table 4.2 Characteristics of simulated food waste, GAC pellets, and inoculums

Parameters	Simulated Food Waste	GAC Pellets	RAS	AD-sludge	Biomass from LBR-0RAS	Biomass from LBR-1RAS	Supernatant of RAS
TS (%)	36.4 ± 0.5	99.4 ± 0.06	0.69 ± 0.03	1.02 ± 0.01	22.9 ± 0.2	19.7 ± 0.4	0.161 ± 0.04
VS (%)	34.8 ± 0.4	73.0 ± 1.0	0.46 ± 0.01	0.60 ± 0.01	19.4 ± 0.3	17.3 ± 0.3	0.106 ± 0.01
TS (g/kg)	364 ± 5	994 ± 0.6	6.9 ± 0.3	10.2 ± 0.1	229 ± 2	197 ± 4	1.61 ± 0.4
VS (g/kg)	348 ± 4	730 ± 10	4.6 ± 0.1	6.08 ± 0.1	194 ± 3	173 ± 3	1.06 ± 0.1
VS/TS (%)	96	73	67	60	85	88	66
SCOD (g/L)	-	-	1.4 ± 0.2	2.8 ± 0.3	-	-	-

4.3.2 LBR configuration

The LBR used in this study was made with acrylic material in a cylindrical shape with a total volume of 3.7 L. The LBR had a diameter of 110 mm and a total height of 390 mm. The LBR was comprised of headspace with the gas collection and sprinkling system, food waste holding basket with perforated base, and funnel-shaped leachate holding bed. The headspace with 0.4 L volume was equipped with a detachable cover having a gas collection port and sprinkler nozzle. The gas was collected in the gas collection bag (MODEL 22052, Restek, Canada) connected to a gas collection port. The pump (MODEL 300308P, Burcam) was used to recirculate the leachate from the leachate holding bed over the food waste in a food waste holding basket through a shower head with 3 mm pore size. The perforated base of the 1.2 L food waste holding basket had a 3 mm mesh size to prevent food waste from entering into the leachate holding bed. Leachate was collected in the 2.1 L leachate holding bed. The sample was collected from the sampling port in the leachate holding bed. The pH probe was inserted into the retaining wall of the leachate holding bed to monitor the pH by a pH controller (MODEL MC122, Milwaukee, USA). To maintain the pH of leachate at the desired level, a pH controller was coupled with a dosing pump (MODEL 7553-80, Cole Parmer, USA) to inject the neutralizing agent. Leachate was mixed in the leachate holding bed by using the pump (MODEL 07559-00, Cole Parmer, USA). The recirculation and mixing of leachate both were performed at specific intervals using a timer (MODEL XT-4, ChronTrol, USA). The joints of the LBR had O-ring to maintain anaerobic conditions inside the LBR. The schematic diagram and set-up of LBR are shown in Figure 4.1 and Figure 4.2 respectively.

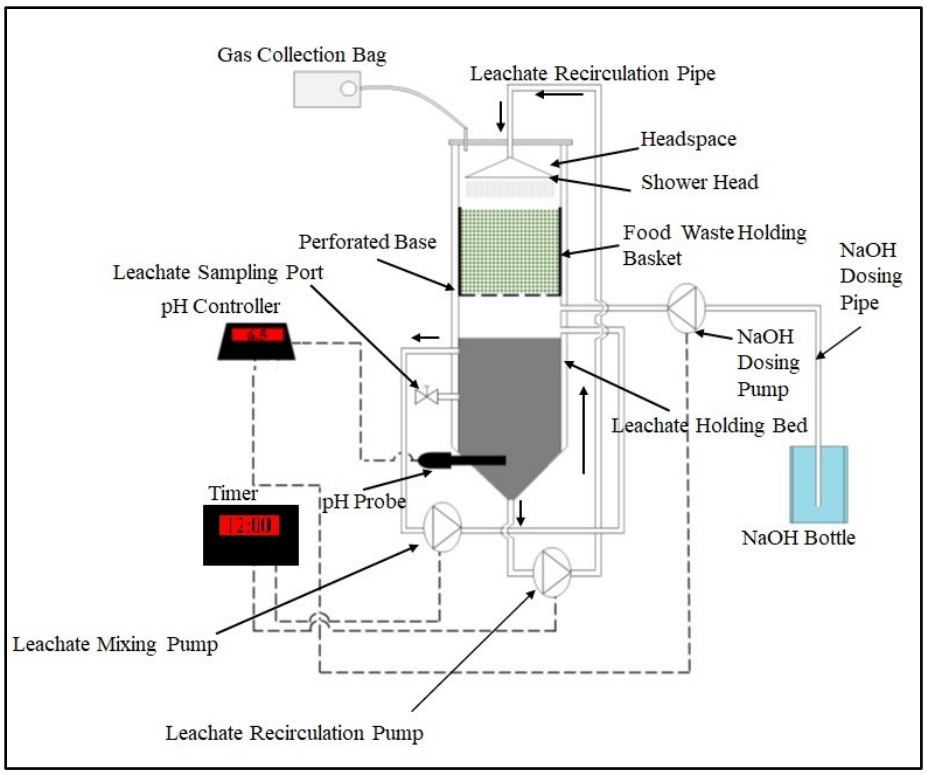


Figure 4.1 Schematic diagram of LBR.

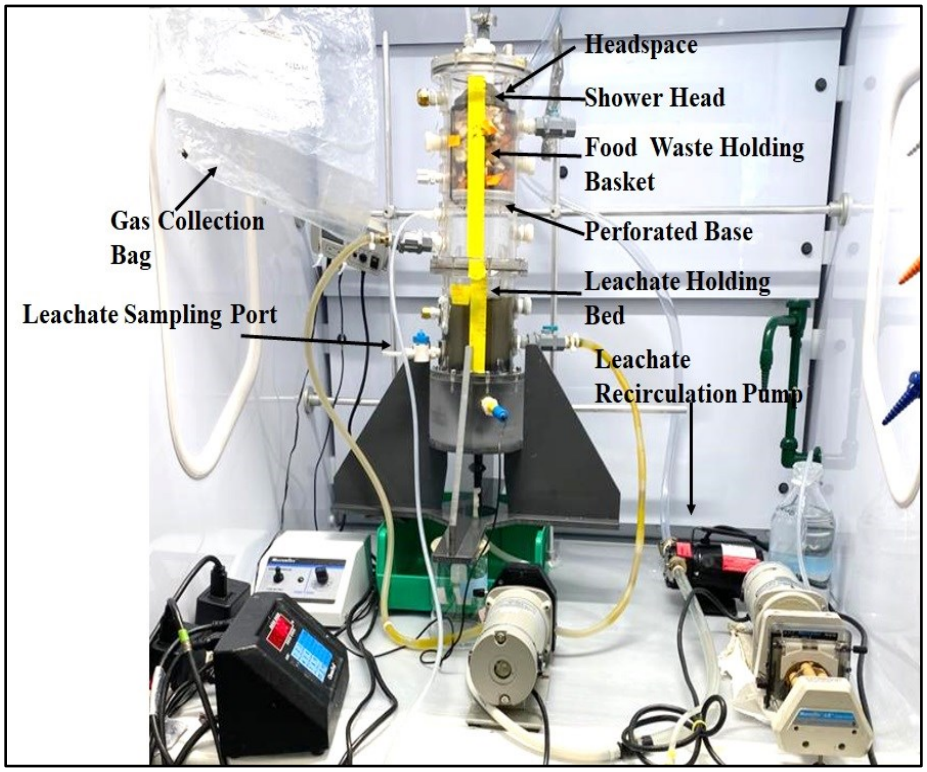


Figure 4.2 LBR set-up of LBR used in this study.

4.3.3 Inoculation of LBR

The impact of two different inoculum (RAS and AD-sludge) and sequential enrichment of inoculum on hydrolysis and acidogenesis of the simulated food waste were studied as illustrated in Figure 4.3. In the first phase of the study, heat-treated AD-sludge was used as an inoculum in the LBR (hereby referred to as LBR-AD). The LBR-AD had a GAC loading of 0.38 g GAC/g $VS_{foodwaste}$. The performance of LBR-AD was compared to the performance of LBR-2 (in chapter 3 of this thesis). LBR-2 was operated at the same conditions as LBR-AD but was inoculated with RAS (Figure 4.3A). Furthermore, the effect of sequential enrichment of inoculum on hydrolysis and acidogenesis of the simulated food waste was investigated by collecting leaching biomass from LBR-3 of chapter-3 (a LBR-3 was inoculated with RAS and operated with GAC loading of 0.51 g GAC/g $VS_{foodwaste}$). The leachate from LBR-3 was centrifuged using a centrifuge (Sorvall legend RT+, Thermo Fisher Scientific) at 8000 rpm for 20 minutes to obtain the biomass. After that, the biomass was mixed with supernatant of the RAS and used as a first-level enriched inoculum in LBR-1RAS (Figure 4.3B). Similarly, biomass from LBR-1RAS was mixed with supernatant of the RAS and used as a second-level enriched inoculum in LBR-2RAS (Figure 4.3B). In LBR-1RAS and LBR-2RAS, GAC loading was 0.51 g GAC/g $VS_{foodwaste}$. The process performance of the LBR-1RAS and LBR-2RAS was compared with the process performance of LBR-3 of chapter-3. For better understanding, LBR-2 of chapter-3 will be referred to as LBR-RAS, while LBR-3 of chapter-3 will be referred to as LBR-0RAS in this study. ISR was maintained at 4-4.9% in all the LBRs. The Characteristics of all the inoculums are given in Table 4.2.

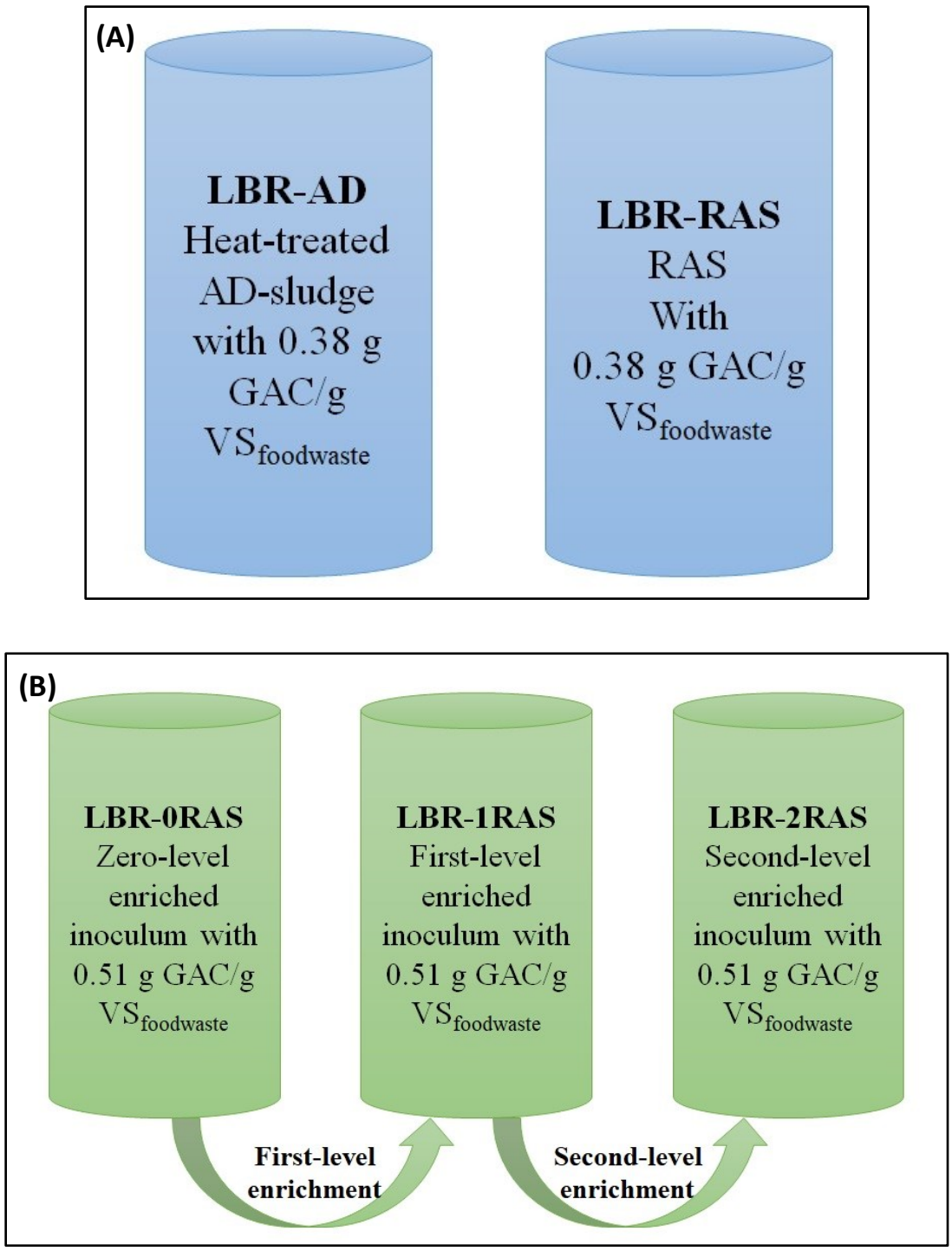


Figure 4.3 Inoculation of LBR (A) impact of two different inoculum (i.e., AD-sludge and RAS), and (B) impact of sequential enrichment of inoculum

4.3.4 LBR operation

A LBRs were operated with volumetric organic loading of 49 g VS/$L_{reactor}$ for 14 days (batch cycle) at room temperature (22˚C). LBRs were loaded with simulated food waste of 500 g (wet basis). Pretreated GAC was mixed with the simulated food waste in a food waste holding basket. The inoculation of LBR and GAC loading were performed as discussed in section 4.3.3. The leachate was recirculated over the food waste at a LRR of 36.6 L/L_{bed}·day and slowly percolated down in the leachate holding bed. To mix the leachate in the leachate holding bed, leachate was circulated internally at a rate of 19.4 L/L_{bed}·day. The pH of the leachate was controlled at 6.5 by using 1 M NaOH.

Leachate samples were collected daily until day 6, thereafter sampling was performed every other day. Analysis of TS and VS was performed at the beginning and end of the batch cycle to calculate VS destruction efficiency. For first and second level enrichment (Figure 4.3B), the leachate from previous LBR run was centrifuged using a centrifuge (Sorvall legend RT+, Thermo Fisher Scientific) at 8000 rpm for 20 min to collect the biomass. The collected biomass was then used to inoculate the next LBR run (serial enrichment). LBR tests were performed in duplicates. All the sample analyses for SCOD and VS were performed in triplicates, while SCFA analysis was performed at least in duplicates. Average values were considered for evaluating the process performance parameters. All the above explained operating conditions were kept the same in each batch cycle unless indicated.

4.3.5 Analytical method

The standard procedure was used to perform the analysis of TS and VS (EPA, 2001). The SCOD was analyzed using a reagent tube (SCP Science, Canada) after filtering the sample with 0.45 µm pore size filter membrane in a vacuum filter. For the analysis of SCFA, the sample was first filtered by 0.45 µm pore size filter membrane and subsequently with 0.25 µm pore size filter membrane in a vacuum filter. The SCFA was analyzed at the University of Waterloo (Waterloo, ON, Canada) using a gas chromatograph (HP 5890 Series II, Hewlett Packard, USA) with a flame ionization detector (FID) and a capillary column (30m×0.53mm×0.5µm PAG, Supelco, Bellefonte, PA). At the beginning, the temperature of oven was set at 150°C for 2 min, following that raised to 190 °C at a rate of 4°C/min, and maintained at 190°C for 3 min. SCFA comprised three main SCFA (i.e., acetate, propionate, and butyrate). Therefore, the total SCFA was the total of quantity of all three main components of SCFA.

4.3.6 Calculation

The Performance of LBR was assessed based on four performance parameters: 1) VS destruction efficiency, 2) Hydrolysis yield, 3) Acidification (or SCFA/carboxylates) yield, and 4) SCFA:SCOD ratio.

The VS destruction efficiency was calculated by subtracting the VS remaining (leachate, food waste, and GAC) in the LBR at the end of 14 days batch cycle from VS added (inoculum, food waste, and GAC) in LBR at the beginning of the batch cycle (equation 4.1). The VS of GAC was considered constant in the calculation of VS destruction efficiency since GAC is composed of complex compounds that is resistant to microbial and chemical degradation (Altamirano-Corona et al., 2021). After digestion of

food waste, it was difficult to separate food waste and GAC. Therefore, VS of mixture of GAC and food waste remained was analyzed and the VS destruction efficiency was calculated based on VS added through food waste and inoculum at day 0 in the LBR. VS destruction efficiency indicates the degradation efficiency of the system.

$$\text{VS destruction efficiency (\%)} = \frac{VS_{(added)(initial)}(g) - VS_{(remained)(final)}(g)}{VS_{(added)(foodwaste+inoculum)}(g)} \quad (4.1)$$

Where,

$VS_{(added)(initial)}$ = VS of food waste (g) + VS of inoculum (g) + VS of GAC (g)

$VS_{(remained)(final)}$ = VS of remained food waste and GAC mixture (g) + VS of leachate (g)

$VS_{(added)(foodwaste+inoculum)}$ = VS of food waste (g) + VS of inoculum (g)

Hydrolysis yield was estimated based on the mass of cumulative SCOD produced to total VS added in LBR (equation 4.3).

$$\text{SCOD (g SCOD)} = \text{Concentration of SCOD in leachate (g SCOD/L)} \times \text{volume of leachate (L)} \quad (4.2)$$

$$\text{Hydrolysis yield (g SCOD/kg } VS_{added}) = \frac{\text{cumulative SCOD produced (g SCOD)}}{VS_{\text{added initially}}(kg)} \quad (4.3)$$

Where,

Cumulative SCOD produced (g SCOD) =

Final SCOD of leachate (g SCOD) – Initial SCOD of inoculum (g SCOD)

$VS_{added\ initially}$ (kg) = VS of food waste (kg) + VS of inoculum (kg)

Total SCFA production was evaluated by a sum of all three main components of SCFA, (i.e., acetate, propionate, butyrate), and it was expressed as a COD equivalent using standard conversion factors given in (Lim et al., 2008). Acidification yield was calculated as the ratio of the mass of total SCFA produced to the mass of total VS added initially in LBR (equation 4.4)

$$\text{Acidification yield (g COD}_{SCFA}\text{/kg VS}_{added}) = \frac{\text{total SCFA produced (g COD}_{SCFA})}{\text{VS}_{\text{added initially}}(\text{kg})} \quad (4.4)$$

Where,

$$\text{total SCFA produced (g COD}_{SCFA}) = \text{final totalSCFA of leachate(g COD}_{SCFA}) - \text{initial total SCFA of inoculum(g COD}_{SCFA})$$

$VS_{added\ initially}$ (kg) = VS of food waste (kg) + VS of inoculum (kg)

SCFA:SCOD ratio (%) was the ratio of the acidification yield to the hydrolysis yield. SCFA:SCOD shows the proportion of SCOD as SCFA.

4.3.7 Biomass sampling, DNA extraction, and 16S rRNA gene sequencing

Centrifuged biomass from the LBR leachate and the GAC pellets from the food waste holding basket were collected at the end of the batch cycle for community analysis. Microbial community analysis was performed at Metagenom Bio Life Sciences (Waterloo, ON, Canada). Firstly, Sox DNA Isolation Kit (Metagenom Bio Inc.) was used to extract genomic DNA from centrifuged biomass and GAC pellets. PCR was carried out in triplicates for 25µl of each sample, as suggested by the supplier. Each reaction solution 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. Denaturation of DNA was conducted at 95^0C for 5 minutes, then subjected to 35 cycles of 95^0C for 30 seconds, 30^0C for 30 seconds, and 72^0C for 50 seconds, followed by 10 minutes extension at 72^0C. The triplicates PCR products were pooled, then 2% TAE agarose gel was used to resolve it. PCR products in an equal amount of correct amplicons were pooled, after that purified with gel and quantified with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific INC.).

MiSeq Reagent Kit v2 (2×250 cycles) was used to sequence library DNA. For taxonomic analysis, FASTQ files were generated. DADA2 v1.6 was used to process demultiplexed sequences, which was handled using QIIME 2 v.2018.2. Amplified sequence variant was formed after chimera filtering for further analysis. A naive Bayesian classifier developed in QIIME 2 and trained against SILVA release 132 clustered at 99% identity was used to assign taxonomy to representative sequences. A confidence level of 0.7 was used to accept assignments.

4.3.8 SEM analysis

The SEM analysis was performed for the GAC pellets at the end of the fermentation at Carleton Nano Imaging Facility (Carleton University, Ottawa, ON, Canada). Firstly, the GAC pellets were suspended in 2.5% glutaraldehyde solution for two hours. After that, the GAC pellets were dehydrated using a series of ethanol solutions (30% to 80% concentration). The dehydrated GAC pellets were dried for 12 hours in a vacuum desiccator, followed by sputter-coating with gold. The SEM images were captured using vapour pressure scanning electron microscope (Vega – II XMU VPSEM, USA) at the scale of 5 μm.

4.3.9 Statistical analysis

Single factor analysis of variance (ANOVA) analysis was used to verify the impact of different inoculum ($P \leq 0.05$) using Microsoft Excel software (2019).

4.4 Result and discussion

4.4.1 Hydrolysis and substrate degradation

4.4.1.1 Impact of type of inoculum (AD-sludge vs RAS)

Hydrolysis of food waste can be assessed by cumulative SCOD production. The cumulative SCOD production in LBR-RAS and LBR-AD is depicted in Figure 4.4. As shown in Figure 4.4, the cumulative SCOD production was almost similar in LBR-AD and LBR-RAS until day 2, following which the SCOD production differed between the inoculums. The cumulative SCOD produced on day 14 in LBR-RAS was 111 ± 2.3 g SCOD, which was 28% higher than that was obtained in LBR-AD (87 ± 1.2 g SCOD). This finding clearly indicated that higher hydrolysis of food waste was achieved with RAS in comparison to AD-sludge as an inoculum. It could be attributed to better hydrolytic activity in RAS (Mshandete et al., 2005; Saad et al., 2019). Similar observations were obtained for hydrolysis yield and VS destruction efficiency.

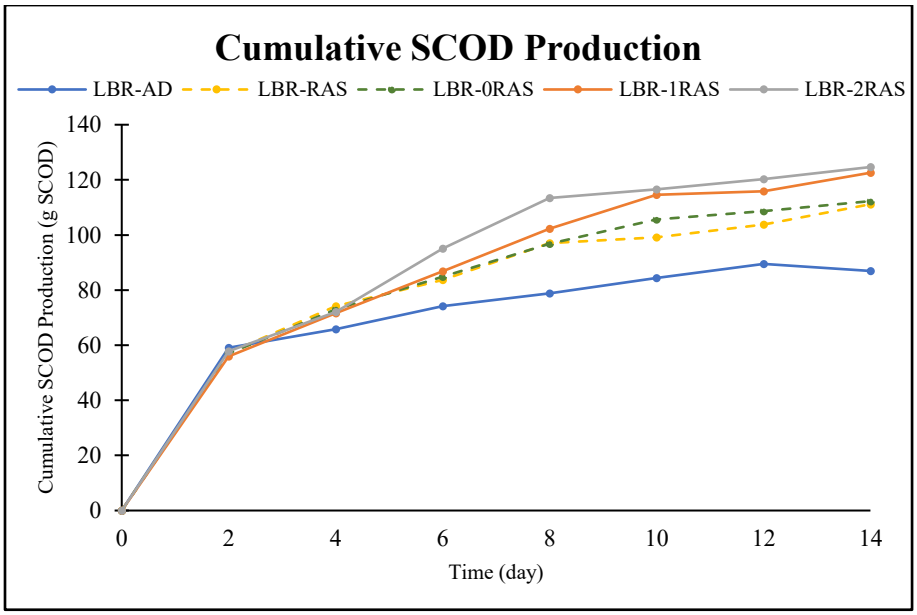


Figure 4.4 Cumulative SCOD Production in LBRs with different inoculum. (Each datapoint on graph represents an average of six SCOD values).

Table 4.3 summarizes the hydrolysis yield and VS destruction efficiency at the end of the fermentation batch cycle. The highest hydrolysis yield was 614 ± 12.7 g SCOD/kg VS_{added} for LBR-RAS, which was statistically higher ($P \leq 0.05$) than that obtained for LBR-AD (480 ± 6.6 g SCOD/kg VS_{added}). Correspondingly, the VS destruction efficiency was 13% higher for LBR-RAS (69.4 ± 1.2 %) as compared to LBR-AD (61.2 ± 1.9 %). Inoculum also impacted the hydrolysis rate. For example, in LBR-RAS, the cumulative SCOD produced on day 8 was 97 g (87% of the maximum cumulative SCOD produced in LBR-RAS), which was higher than the maximum cumulative SCOD achieved on day 12 in LBR-AD (Table 4.4), indicating the hydrolysis rate was improved with RAS as an inoculum. Overall, these results demonstrated that faster and higher hydrolysis of food waste can be achieved using RAS as an inoculum.

Table 4.3 Parameter in LBRs with different inoculums at the end of fermentation

Parameters	LBR-AD	LBR-RAS	LBR-0RAS	LBR-1RAS	LBR-2RAS
Vs destruction efficiency (%)	61.2 ± 1.9	69.4 ± 1.2	71.0 ± 1.4	75.6 ± 0.2	76.7 ± 0.9
Hydrolysis yield (g SCOD/kg VS_{added})	480 ± 6.6	614 ± 12.7	620 ± 10.4	672 ± 5.5	683 ± 13.6
Cumulative SCOD production (g SCOD)	87 ± 1.2	111 ± 2.3	112 ± 1.9	123 ± 1.0	125 ± 2.5
Acetate (g COD)	20 ± 0.6	18 ± 1.7	22 ± 0.8	31 ± 0.3	31 ± 0.1
Propionate (g COD)	15 ± 0.6	15 ± 1.0	18 ± 1.0	20 ± 0.8	19 ± 0.5
Butyrate (g COD)	20 ± 0.3	49 ± 0.4	52 ± 1.6	56 ± 0.9	63 ± 0.7
Total SCFA Production (g COD_{SCFA})	55 ± 1.0	82 ± 1.2	92 ± 2.0	107 ± 0.4	113 ± 1.3
SCFA:SCOD (%)	63	73	82	87	90
Acidification yield (g COD_{SCFA}/ kg VS_{added})	304 ± 5.5	451 ± 6.6	507 ± 11.0	585 ± 2.2	617 ± 7.1

Table 4.4 Cumulative SCOD production and its fraction with different inoculum

	Cumulative SCOD Production														
Day	LBR-AD			LBR-RAS			LBR-0RAS			LBR-1RAS			LBR-2RAS		
	g SCOD	g SCOD/ L_{bed}	%	g SCOD	g SCOD /L_{bed}	%	g SCOD	g SCOD/ L_{bed}	%	g SCOD	g SCOD/ L_{bed}	%	g SCOD	g SCOD/ L_{bed}	%
0	0	0	0%	0	0	0%	0	0	0%	0	0	0%	0	0	0%
2	59	28	66%	57	27	51%	57	27	51%	56	27	46%	58	28	46%
4	66	31	73%	74	35	67%	73	35	65%	72	34	58%	72	34	58%
6	74	35	83%	84	40	75%	85	40	76%	87	41	71%	95	45	76%
8	79	38	88%	97	46	87%	97	46	86%	102	49	83%	113	54	91%
10	84	40	94%	99	47	89%	106	50	94%	115	55	93%	116	55	93%
12	89	43	100%	103	49	93%	109	52	97%	116	55	94%	120	57	97%
14	87	41	97%	111	53	100%	112	53	100%	123	58	100%	125	59	100%

4.4.1.2 Impact of sequential enrichment

As RAS performed better than AD-sludge, RAS was selected as the inoculum for enrichment studies. Sequential enrichment of RAS further improved the hydrolysis of food waste. The cumulative SCOD production was 125 ± 2.5 g SCOD in LBR-2RAS and 123 ± 1.0 g SCOD in LBR-1RAS on day 14, which were statistically ($P \leq 0.05$) higher than LBR-0RAS (112 ± 1.9 g SCOD). Consequently, improved hydrolysis yield of 672-683 g SCOD/kg VS_{added} and VS destruction efficiency of 75.6-76.7% were achieved in LBR-1RAS and LBR-2RAS (Table 4.3). The improved performance with sequential enrichment of inoculum could be due to the stimulation of desired microbial populations and acclimatization to the reactor operating conditions, resulting in better hydrolysis yields (Chang et al., 2018; Shewa et al., 2020).

The enriched inoculum also improved the hydrolysis rate. LBR-0RAS produced a cumulative SCOD of 112 g SCOD on day 14, while almost similar cumulative SCOD was obtained 4-6 days earlier with LBR-1RAS and LBR-2RAS (Table 4.4), demonstrating that inoculum enrichment led to faster solubilization of food waste. Interestingly, there was no statistical difference for hydrolysis yield and VS degradation efficiency between LBR-2RAS and LBR-1RAS. However, the hydrolysis rate ($\Delta SCOD/\Delta time$) was higher for LBR-2RAS as compared to LBR-1RAS. For example, LBR-2RAS achieved 91% of the maximum cumulative SCOD by day 8. The corresponding percentage was 83% with LBR-1RAS (Table 4.4), indicating that a high level of enrichment improved the rate of hydrolysis of the food waste. Therefore, inoculum enrichment could shorten the fermentation time in future studies.

4.4.2 SCFA production

4.4.2.1 Impact of type of inoculum (AD-sludge vs RAS)

Total SCFA production (sum of three main SCFA components such as acetate, propionate, and butyrate in COD equivalent) with different inoculum is demonstrated in Figure 4.5. Total SCFA production stabilized on day 6 in LBR-AD, while it consistently increased until day 10 in LBR-RAS. There was a significant difference ($P \leq 0.05$) in maximum total SCFA production between LBR-RAS and LBR-AD. The total SCFA production was 55 ± 1.0 g COD_{SCFA} in LBR-AD and 82 ± 1.2 g COD_{SCFA} in LBR-RAS. This result indicated that total SCFA production was faster initially with LBR-AD, however, the total SCFA production was higher with LBR-RAS. It could be due to several reasons: 1) AD-sludge is enriched with acidogenic bacteria resulting in faster production of SCFA (Wang et al., 2013; Wang et al., 2014), however poor hydrolysis with AD-sludge may have limited SCFA production, and 2) RAS had better hydrolytic activity, which may have provided more hydrolysates for the acidogenic bacteria, resulting in higher total SCFA production for LBR-RAS. Notably, in LBR-AD, total SCFA production started declining after day 6, despite a slight increment in cumulative SCOD production, which could be due to production of other fermentative products (i.e, alcohol).

Table 4.3 represents the acidification yield and SCFA:SCOD obtained in LBRs with different inoculums. The acidification yield of 451 ± 6.6 g COD_{SCFA}/kg VS_{added} was obtained in LBR-RAS on day 14, which was statistically ($P \leq 0.05$) higher than that obtained in LBR-AD (304 ± 5.5 g COD_{SCFA}/kg VS_{added}). This finding suggested that the acidogenesis of food waste could be enhanced by 48% by using RAS rather than AD-sludge as inoculum. Consequently, SCFA:SCOD was 73% in LBR-RAS, whereas it was

63% in LBR-AD. Remaining SCOD may comprise of other products such as alcohol, and soluble microbial product (Saha & Lee, 2020; Xiong et al., 2019b).

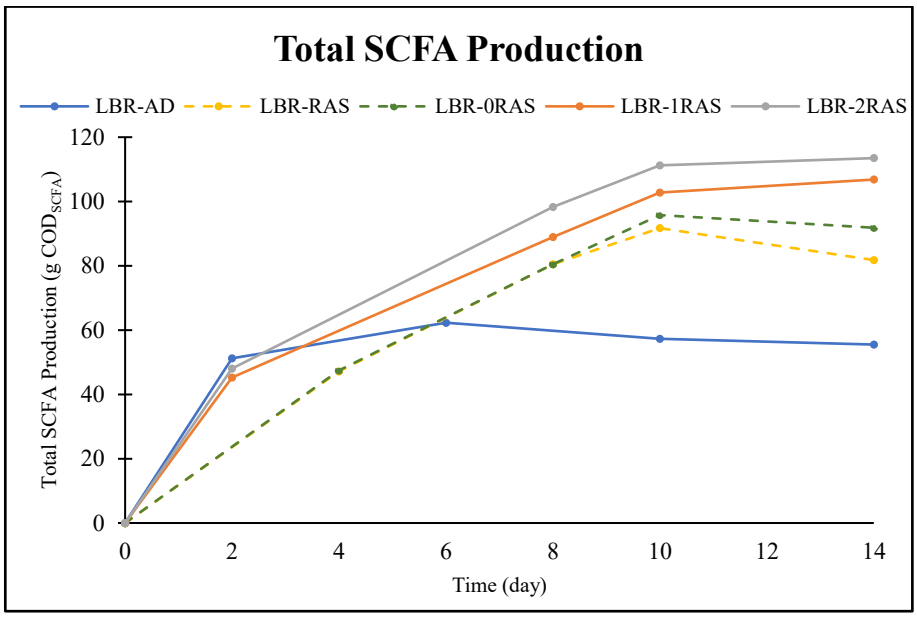


Figure 4.5 Total SCFA Production in LBRs with different inoculums. (Each datapoint on graph represents an average of four SCFA values).

4.4.2.2 Impact of sequential enrichment

Total SCFA production was further improved by using enriched RAS inoculum. The total SCFA production was 113 ± 1.3 g COD_{SCFA} for LBR-2RAS, followed by 107 ± 0.4 g COD_{SCFA} for LBR-1RAS and 92 ± 2.0 g COD_{SCFA} for LBR-0RAS. Notably, LBR-1RAS and LBR-2RAS achieved statistically ($P \leq 0.05$) different total SCFA production.

The positive impact of enriched inoculum on the acidogenesis of food waste was further supported by the acidification yield. The acidification yields of 585-617 g COD_{SCFA}/kg VS_{added} were obtained in LBR-1RAS and LBR-2RAS, which were statistically ($P \leq 0.05$) higher than that obtained for LBR-0RAS (507 g COD_{SCFA}/kg VS_{added}). SCFA:SCOD ratio was also improved with enrichment. For example, SCFA:SCOD ratio was 82% for LBR-0RAS, 87% for LBR-1RAS, and 90% for LBR-

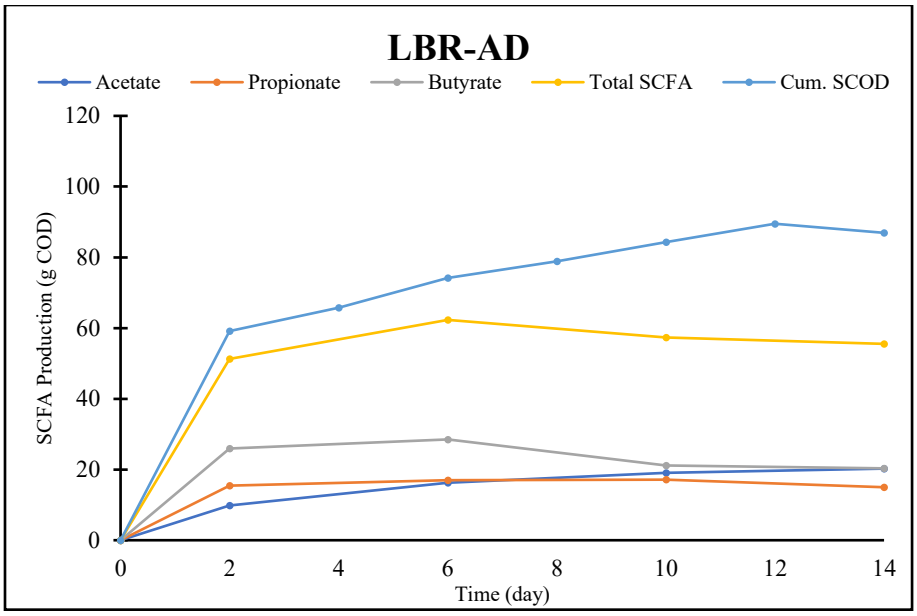
2RAS. Overall, the results demonstrated that acidogenesis of food waste in LBR can be significantly improved with enrichment/acclimatization of the inoculum.

4.4.3 SCFA composition

4.4.3.1 Impact of type of inoculum (AD-sludge vs RAS)

Figure 4.6 depicts the production of each SCFA component over the 14 days of fermentation with AD-sludge and RAS, whereas Figure 4.7 shows the production of each SCFA component as a percentage of total SCFA. The production of each SCFA varied with the inoculum. In LBR-RAS, butyrate production (49 g COD; 60% of total SCFA) was the highest, followed by acetate (18 g COD; 22% of total SCFA) and propionate (15 g COD; 18% of total SCFA). Whereas the production of butyrate (37% of total SCFA) was lower and similar to acetate production (36% of total SCFA) in LBR-AD. This variation in SCFA composition among LBR-RAS and LBR-AD could be because of the different microbial compositions of inoculum from different origins. Overall, in both LBRs, acetate and butyrate were higher than the propionate, as the pH was maintained between 6-7, leading to acetate-butyrate type fermentation (Hussain et al., 2017; Xiong et al., 2019b).

Notably, decline in butyrate was accompanied by an increase in acetate in both LBR-RAS and LBR-AD. LBR-AD produced the highest butyrate of 28 g COD (46% of total SCFA) on day 6. After that, butyrate was transformed into acetate and had similar composition as acetate on day 14 in LBR-AD. Similarly, LBR-RAS produced maximum butyrate of 56 g COD on day 10 and started transforming the butyrate into acetate.



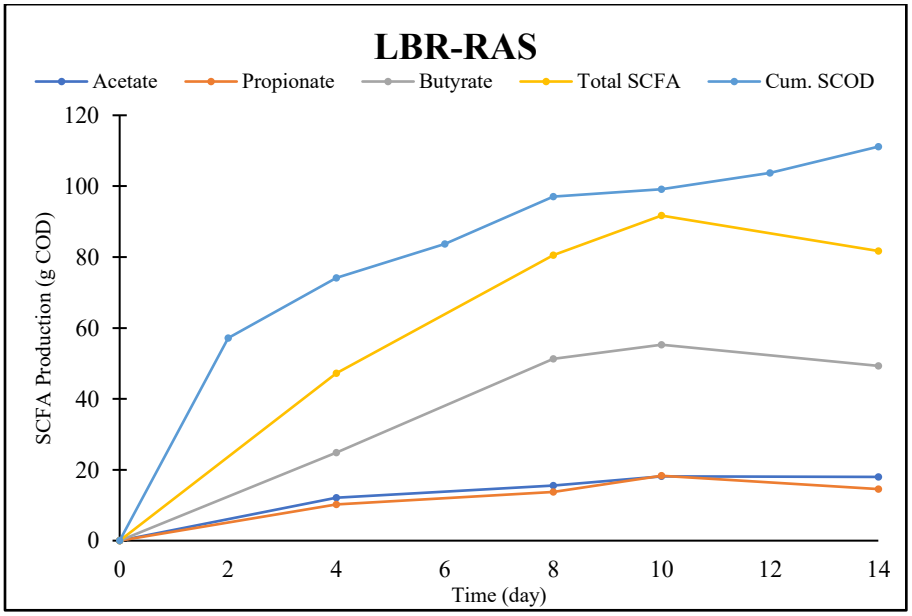
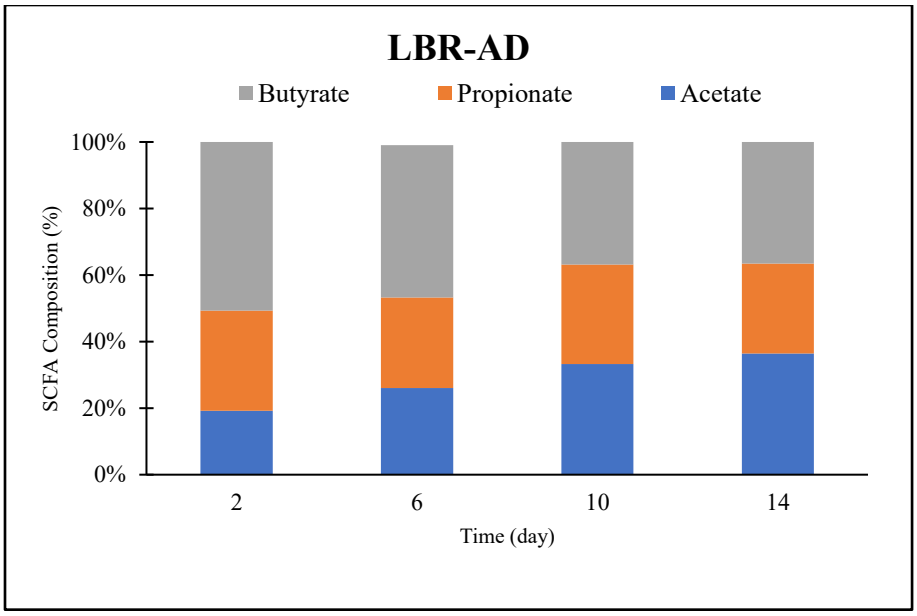


Figure 4.6 Production of each SCFA component in LBRs with AD-sludge and RAS (Each datapoint on graph represents an average of four SCFA values and six SCOD values). (The unit of cum. SCOD is g SCOD)



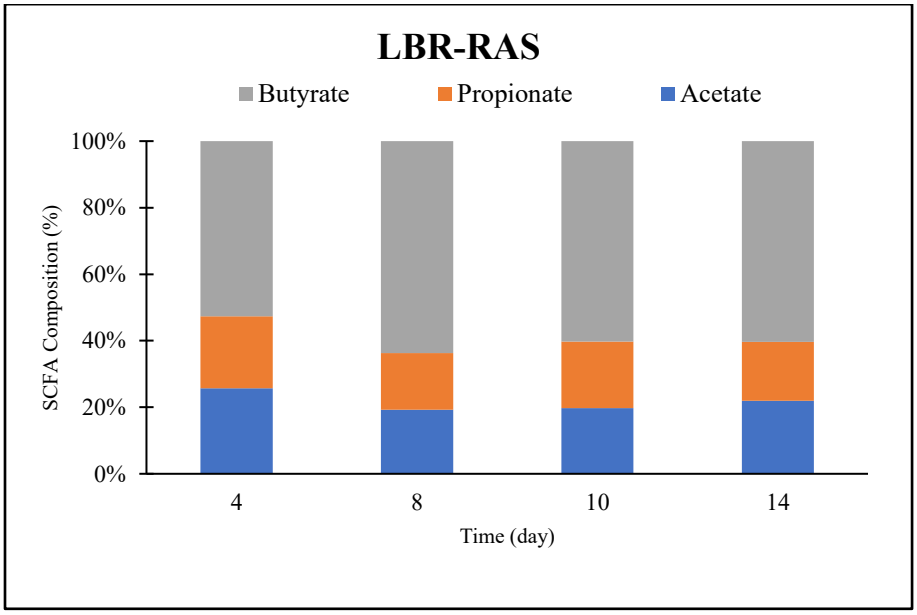
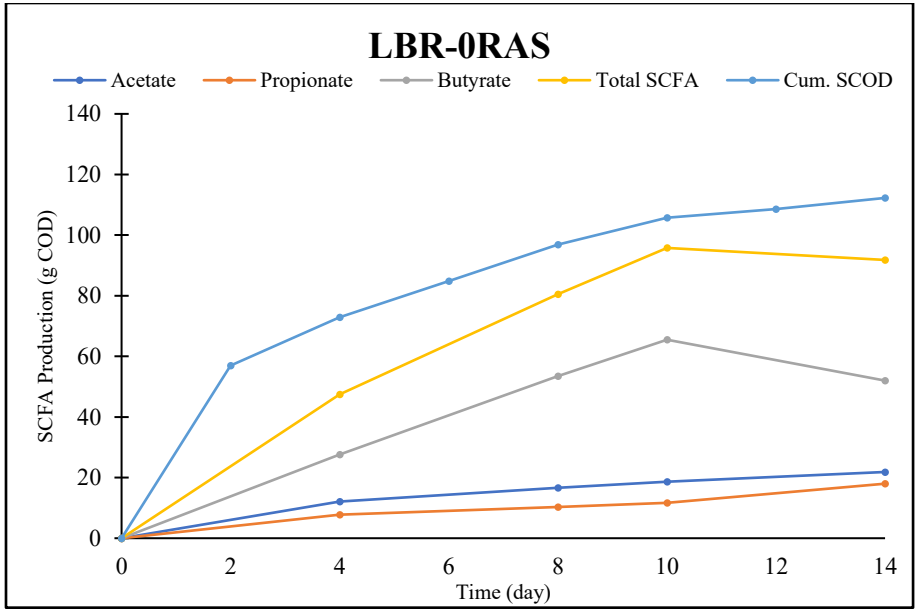
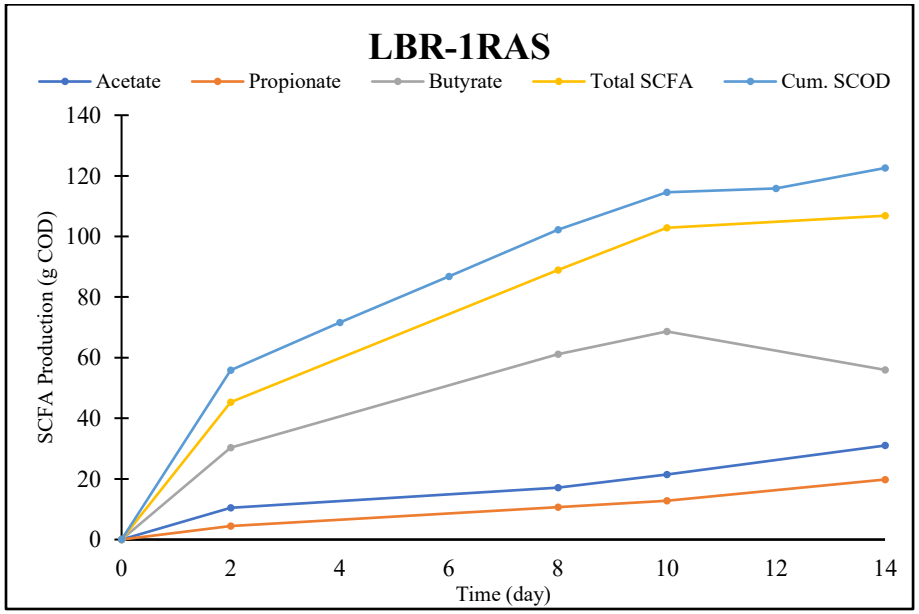


Figure 4.7 Fraction of each SCFA component in LBRs with AD-sludge and RAS.

4.4.3.2 Impact of sequential enrichment

SCFA composition was similar in LBR-0RAS, LBR-1RAS, and LBR-2RAS (Figure 4.9). Acetate and butyrate constituted 80-83% of total SCFA, with butyrate (52-57% of total SCFA) as a dominant SCFA component on day 14. Acetate was the second most dominant SCFA accounting for 24-29%, followed by propionate with 17-20%. Higher production of butyrate and acetate indicated that the butyrate-acetate fermentation pathway is followed at 6-7 pH (Jiang et al., 2013; Hussain et al., 2017; Xiong et al., 2019b; Shewa et al., 2020). Similar SCFA composition with all the levels of inoculum demonstrated that the level of enrichment does not affect the SCFA composition. This agrees with the previous study that worked on AF of food waste using enriched inoculum (Shewa et al., 2020). The butyrate was transformed into acetate after day 10 in all LBRs. This result means the highest butyrate production was obtained on day 10 in all LBRS. Hence, a fermentation time of 10 days is sufficient to achieve high butyrate production.





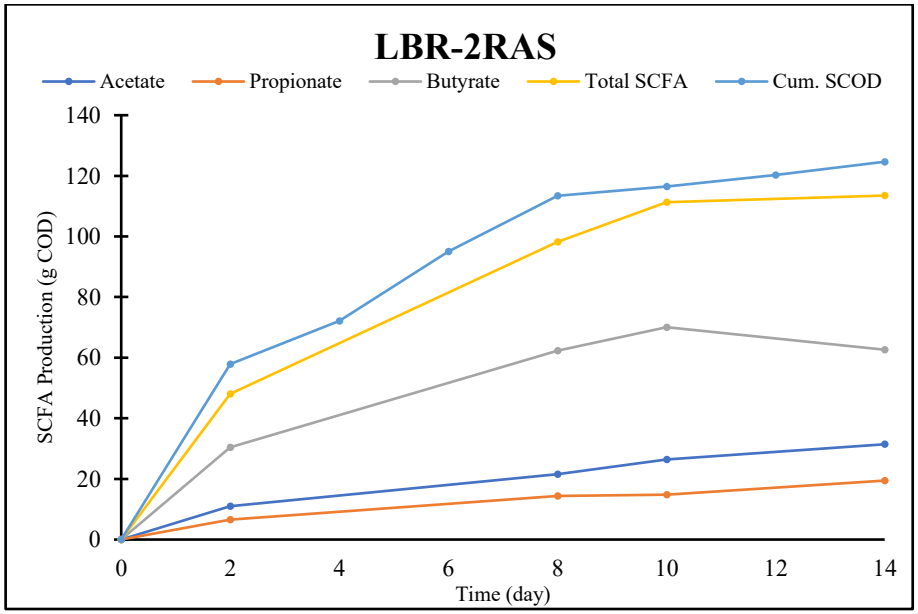
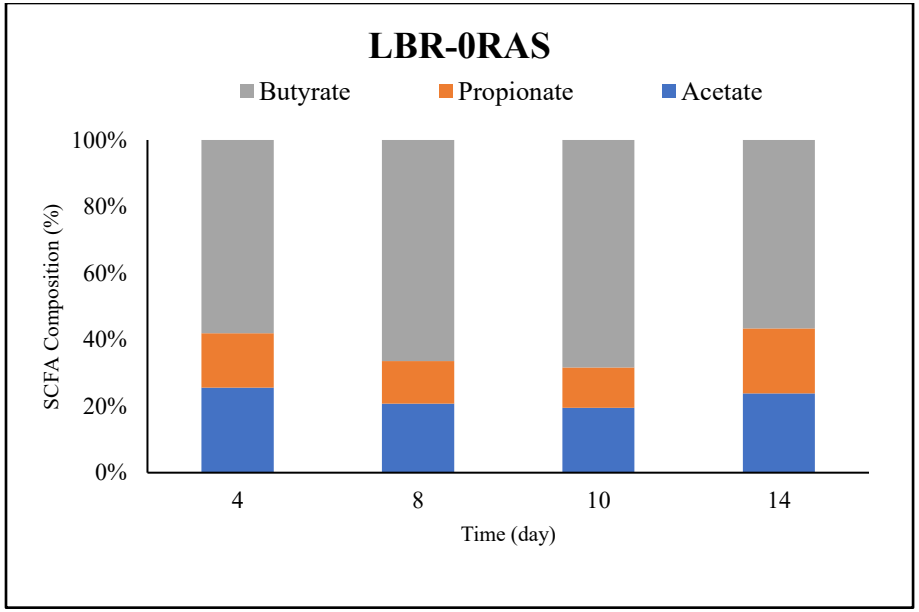
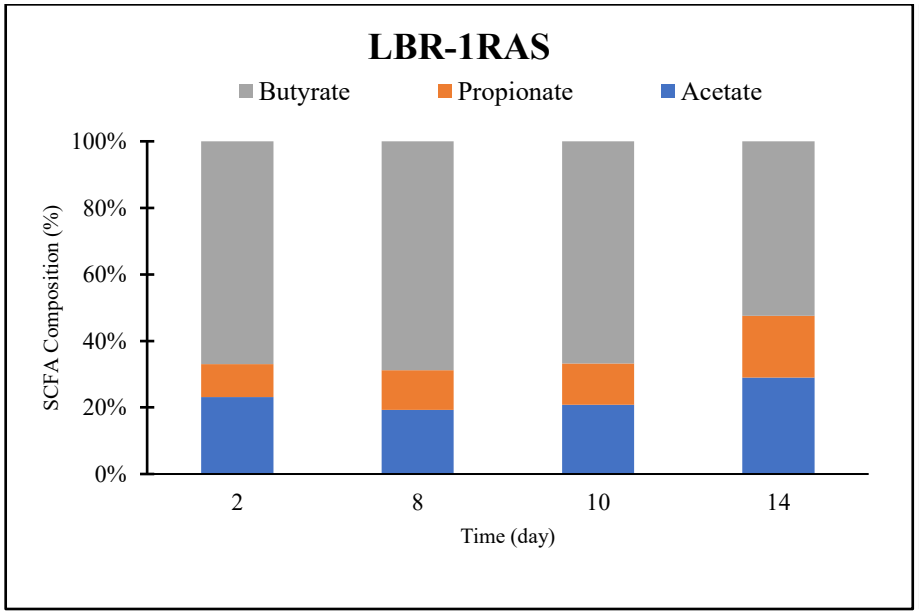


Figure 4.8 Production of each SCFA component in LBRs with enriched inoculum. (Each datapoint on graph represents an average of four SCFA values and six SCOD values). (The unit of cum. SCOD is g SCOD)





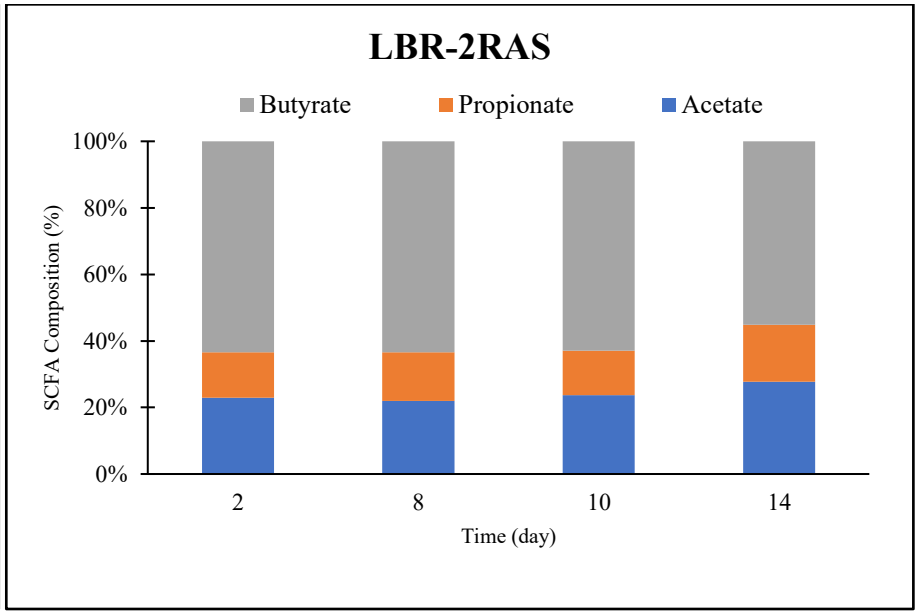


Figure 4.9 Fraction of each SCFA component in LBRs with enriched inoculum.

4.4.4 Microbial community composition with different GAC loading and inoculums

Figure 4.10 illustrates the microbial community in the LBR leachate with different inoculums (RAS, AD-sludge, enriched RAS) and GAC loadings (0, 0.38, 0.51 g GAC/g $VS_{foodwaste}$) at the phylum and genus level. The microbial community composition varied with inoculum and GAC loading, as was observed for hydrolysis and acidogenesis yields. At the phylum level (Figure 4.10A), *Bacteroidota* was the most abundant (67 %) in LBR-C (0 g GAC/g $VS_{foodwaste}$ with RAS as inoculum), followed by *Firmicutes* (15 %) and *Actinobacteria* (1.5 %). *Bacteroidota* is a phylum of hydrolyzing bacteria that hydrolyze carbohydrates and protein to produce acetate and propionate (Besten et al., 2013; Atasoy et al., 2019; Yin et al., 2016). *Firmicute* plays an important role in the fermentation of food waste components (carbohydrate, protein, and lipid) to acetate and butyrate (Levén et al., 2007; Atasoy et al., 2019; Yin et al., 2016). *Actinobacteria* hydrolyze the carbohydrate and fibers and ferment these compounds into SCFAs (acetate, propionate, and butyrate) (Mawang et al., 2021).

In LBR-RAS with GAC loading of 0.38 g GAC/g $VS_{foodwaste}$, *Bacteroidota* (27%) became less abundant, but *Firmicutes* (24%) and *Actinobacteria* (33%) were higher than LBR-C. It indicated that SCFA producing bacteria became more abundant when GAC was loaded with RAS as an inoculum, resulting in improved hydrolysis and acidogenesis of food waste. LBR-AD with the similar GAC loading of 0.38 g GAC/g $VS_{foodwaste}$ and AD-sludge as an inoculum showed higher abundancy of *Bacteroidota* (49%) and lower abundancy of *Actinobacteria* (4.6%). These results indicated that RAS contained a more diversified microbial community with both hydrolytic and acidogenic bacteria than AD-sludge, supporting the higher hydrolysis and acidogenesis achieved in LBR-RAS.

Further at higher GAC loading of 0.51 g GAC/g $VS_{foodwaste}$ with RAS in LBR-0RAS, the microbial community was also diverse similar to LBR-RAS (GAC loading of 0.38 g GAC/g $VS_{foodwaste}$; RAS), thus achieving almost similar performance in terms of hydrolysis and acidogenesis yields. Interestingly, at the same GAC loading of 0.51 g GAC/g $VS_{foodwaste}$ but inoculation with enriched RAS in LBR-2RAS, fermentative *firmicute* became more dominant, as compared to LBR-0RAS (0.51 g GAC/g $VS_{foodwaste}$ with RAS). Consequently, the highest hydrolysis and acidification yields were obtained in LBR-2RAS.

The microbial community in leachate at the genus level (Figure 4.10B) also varied with inoculum and GAC loading. In LBR-C, the microbial community was dominated by *Bacteroides* (64.4%), with a comparatively lower abundancy of *Enterococcus* (4.3%) and *Prevotella* (3.0%). *Bacteroides* belong to *Bacteroidota* phylum, thus it is a hydrolyzing bacteria that hydrolyzes the dietary fibers (hemicellulose, pectin, etc.), and produces acetate and propionate (Wan et al., 2013; Zamanzadeh et al., 2016; Thomas et al., 2011). *Enterococcus* ferments the carbohydrate and lignocellulose, and produce butyrate and acetate (Eder et al., 2020; Yin & Wang, 2019; Esquivel-Elizondo et al., 2017; Saini et al., 2015). *Prevotella* can hydrolyze polysaccharides like cellulose and xylan, and produce acetate and propionate during the fermentation (Zhou et al., 2018).

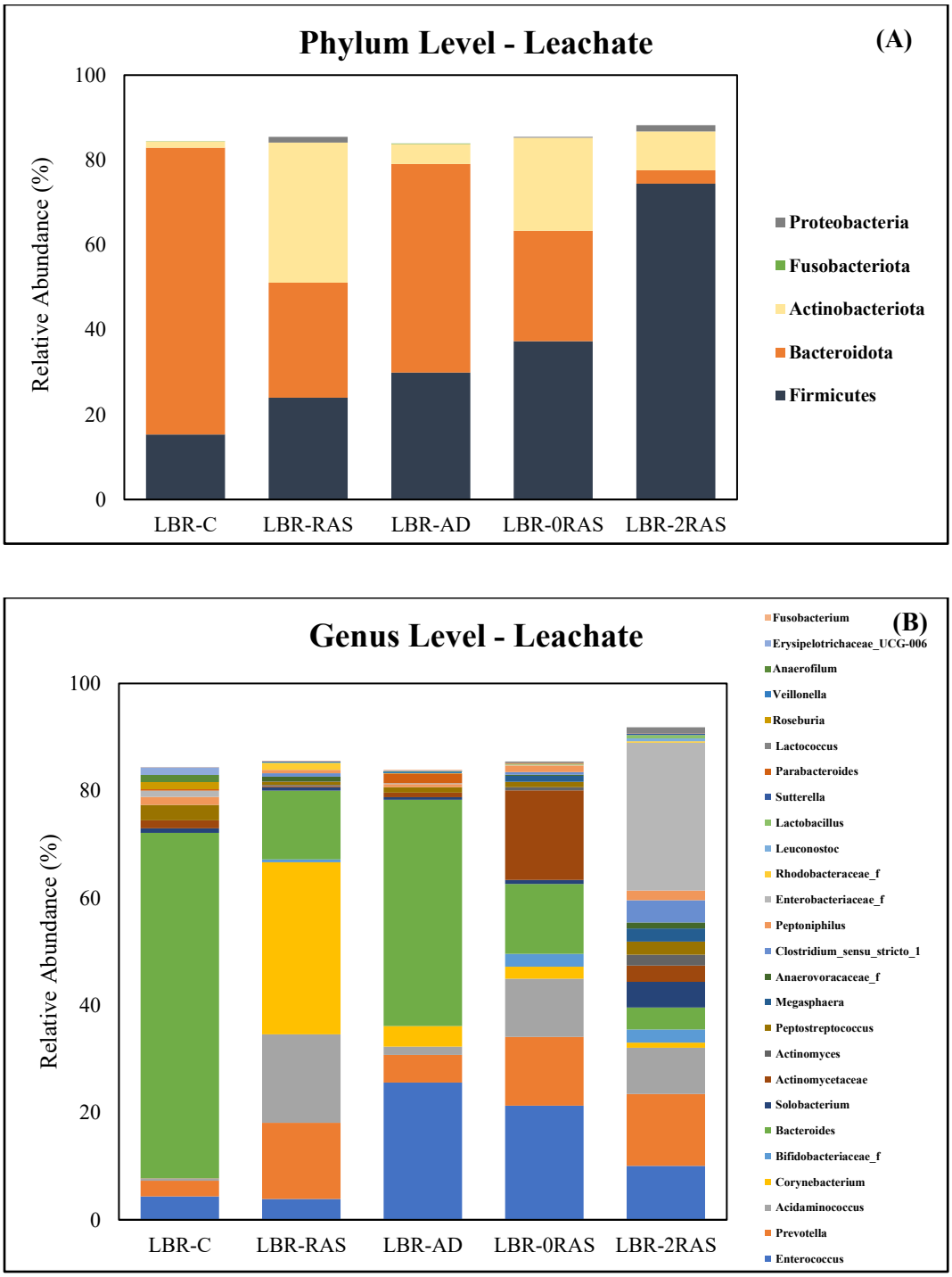


Figure 4.10 Microbial community composition in leachate at A) Phylum level, and B) Genus level.

When GAC loading of 0.38 g GAC/g $VS_{foodwaste}$ was applied in LBR-RAS, the microbial community became diverse with *Corynebacterium* (32.0%), *Acidaminococcus* (16.5%), *Prevotella* (14.2%), *Bacteroides* (12.8%), *Enterococcus* (3.9%), and *Clostridium*

(0.7%). *Corynebacterium* is a genus from the *Actinobacteria* phylum, capable to convert the carbohydrate into acetate and butyrate (Seon et al., 2014; Yasuda et al., 2007). *Acidaminococcus* is fermenting bacteria belonging to the *Firmicute* phylum and can produce propionate and butyrate (Louis & Flint, 2017). *Clostridium* is known for fermenting organic compounds into acetate, butyrate, and hydrogen (Cabrol et al., 2017; Xiong et al., 2019b). It clearly demonstrated that microbial community in LBR-RAS was more dominated with fermenting bacteria than LBR-C, thus significant SCFA production was achieved in LBR-RAS with GAC loading (0.38 g GAC/g $VS_{foodwaste}$). At the same GAC loading (0.38 g GAC/g $VS_{foodwaste}$) with AD-sludge as an inoculum in LBR-AD, *Bacteroides* (42.2%) was higher, however, *Prevotella* (5.2%), *Acidaminococcus* (1.5%), and *Corynebacterium* (3.8%) were lower than LBR-RAS. This means that RAS had a more balanced microbial community that can hydrolyze and ferment the compounds (carbohydrate, protein, and lipid) of food waste and produce the SCFA. Therefore, RAS in LBR-RAS could have achieved higher SCFA production than LBR-AD.

With RAS as an inoculum at higher GAC loading of 0.51 g GAC/ g $VS_{foodwaste}$ in LBR-0RAS, *Prevotella* (12.8%), *Acidaminococcus* (10.8%), *Enterococcus* (21.3%), *Bacteroides* (13%), *Corynebacterium* (2.2%) were still abundant along with *Actinomycetaceae* (16.7%) and *Bifidobacterium* (2.4%). *Actinomycetaceae* ferments the carbohydrate and lignocellulose into SCFA such as butyrate and acetate (Esquivel-Elizondo et al., 2017; Saini et al., 2015). *Bifidobacterium* is a genus of *Actinobacteria*, capable of hydrolyzing carbohydrates and fibers to SCFA. It indicated that at high GAC loading (LBR-0RAS), the microbial community composition was more or less similar to

low GAC loading (LBR-RAS), hence LBR-0RAS achieved similar hydrolysis yield and slightly higher SCFA production than LBR-RAS.

At similar GAC loading as LBR-0RAS but with enriched inoculum in LBR-2RAS, the microbial community became most dominant with fermentative bacteria such as *Acidaminococcus, Bifidobacterium, solobacterium, Actinomycetaceae, Enterococcus, Enterobacteriaceae*, and *Clostridium* along with *Bacteroides* and *Prevotella. Enterobacteriaceae* produces SCFA such as acetate, propionate, and butyrate (Shewa et al., 2020). *Solobacteria* is SCFA producing bacteria that ferment carbohydrate, protein, and lipid into acetate, butyrate, and hydrogen (Feng et al., 2018; Lukitawesa et al., 2020). This presence of fermentative bacteria could be the reason behind higher hydrolysis and acidification yields in LBR-2RAS than LBR-0RAS.

GAC from all the LBRs (LBR-RAS, LBR-AD, LBR-0RAS, LBR-2RAS) showed microbial attachment as confirmed with microbial community composition and (Figure 4.11) and SEM imaging (Figure 4.12), which indicates that GAC served as a media for microbial immobilization during the fermentation of food waste. At both phylum (Figure 4.11 A) and genus (Figure 4.11 B) levels, the microbial community of the GAC was similar to the leachate for each LBR. This could be due to the contact of GAC and leachate through leachate recirculation. However, some bacteria were more abundant in GAC not in the leachate. For example, *Bacteroides* were dominant in the GAC in almost all the LBRs irrespective of abundancy in leachate. It might be due to *Bacteroides* can secrete extracellular polysaccharide (EPS) which helps in microbial attachment (Chatzidaki-Livanis et al., 2008; Xiong et al., 2019b). Further studies need to be performed to ascertain

the mechanisms leadings to the preferential attachment of certain microbes to GAC and its impact on food waste fermentation in GAC supplemented LBRs.

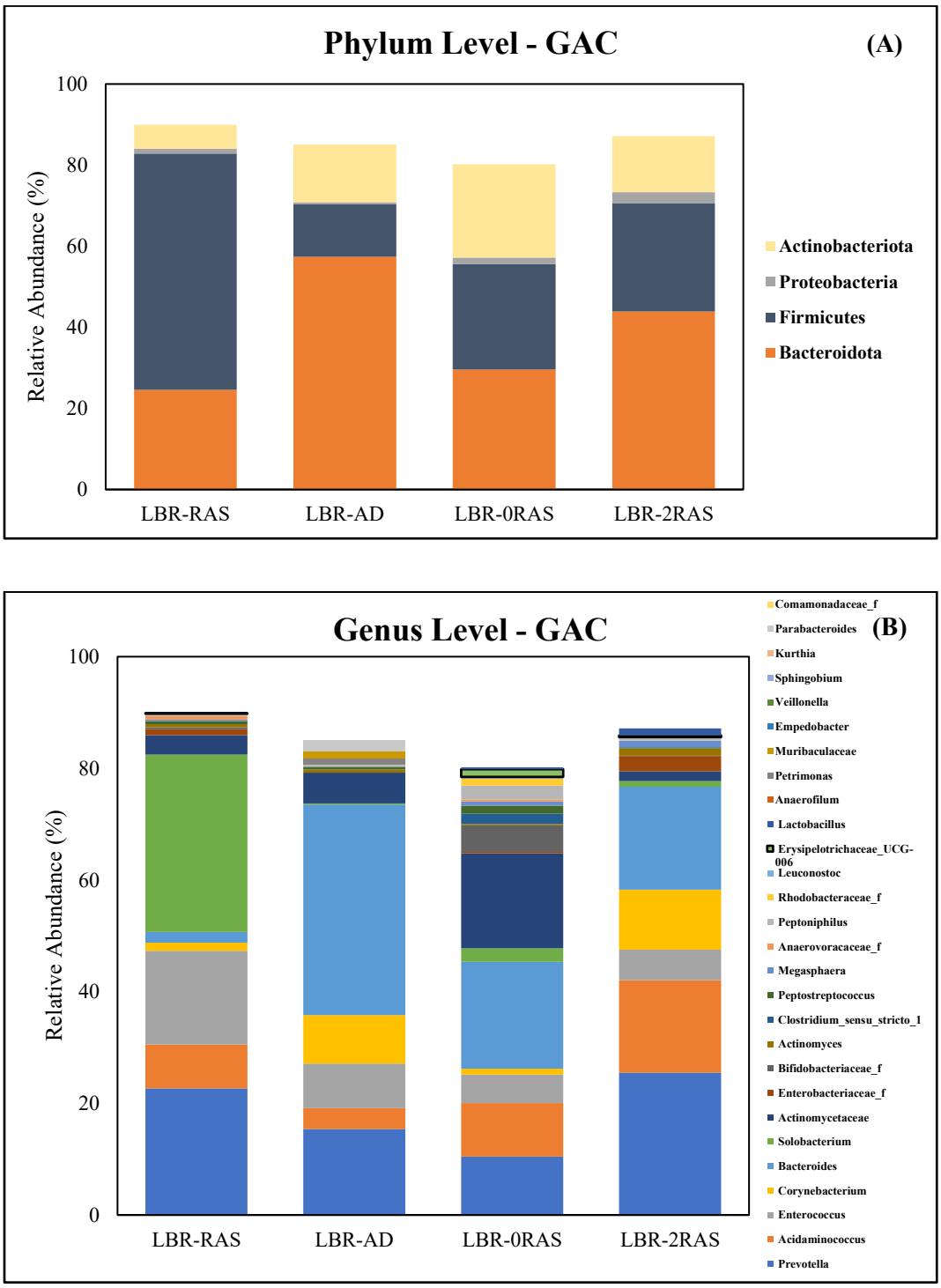


Figure 4.11 Microbial community composition in GAC at A) Phylum level, and B) Genus level.

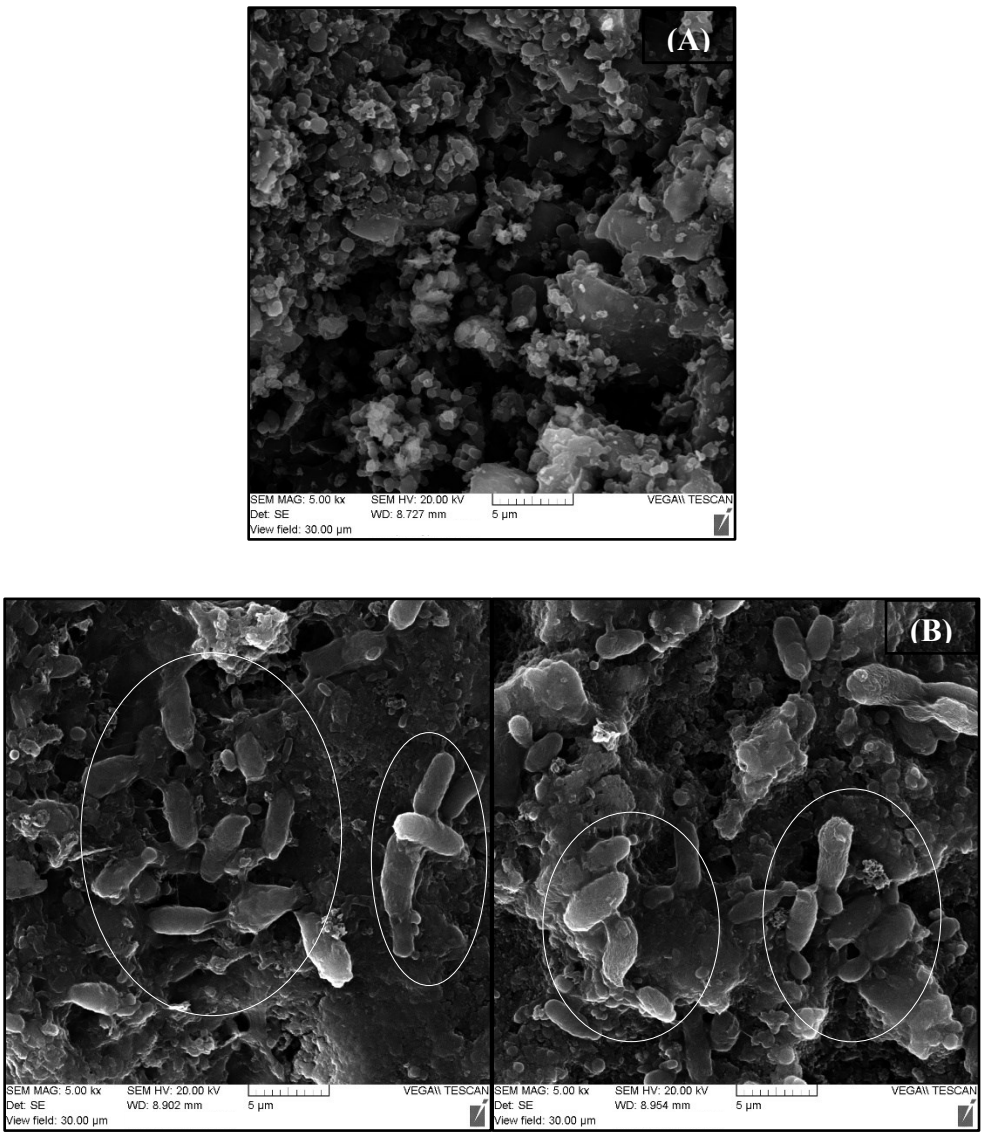


Figure 4.12 SEM image of A) fresh GAC without bacteria, and B) GAC with bacteria. (circle shows the bacteria on GAC).

4.4.5 Discussion

Inoculum is a critical parameter that impacts hydrolysis and acidogenesis of food waste to produce carboxylates (SCFA) in LBRs. Conventionally, AD-sludge has been used as an inoculum for the production of carboxylates from food waste (Saha & Lee, 2020; Xiong et al., 2019a; Xiong et al., 2019b). However, AD-sludge contains methanogens which leads to the consumption of SCFA for methane production. To inhibit methanogenesis, AD-

sludge requires thermal (heat shock) and chemical pre-treatments (Cai & Wang, 2016; Shanmugam et al., 2016; Shewa et al., 2020). For example, some studies have added 2-bromoethanosulfophate (BES) into the AD-sludge to avoid methanogenesis (Yin et al., 2016; Lukitawesa et al., 2019). All this inoculum pre-treatment adds cost and complexity to the bioreactor (LBR) operation. Moreover, studies have used fresh AD-sludge for every LBR operation, thus adding transportation costs to the facility. It was estimated that pre-treatment and transportation of AD-sludge results in operational costs of up to \$51.4/ton VS_{added}, which is almost 61-75% of the total operating cost (Shewa et al., 2020). Therefore, SCFA production from food waste requires low-cost inoculation methods.

The use of RAS establishes a low-cost inoculation method for acidogenic fermentation of food waste in LBRs. First, the RAS has low population of methanogens, thus eliminating pre-treatment costs for inhibition of methanogens, and secondly, the use of enriched RAS inoculum minimizes the transportation costs while enhancing the hydrolysis and acidogenesis of food waste. Sequential enrichment of RAS resulted in a hydrolysis yield of 683 g SCOD/kg VS_{added}, which was 10% higher than that obtained for fresh RAS inoculum. Consequently, 22% increase in acidification yield was obtained with inoculum enrichment (617 g COD_{SCFA}/kg VS_{added}) in comparison to fresh inoculum (507 g COD_{SCFA}/kg VS_{added}). The volumetric hydrolysis and acidification yields with sequential enrichment were deduced to be 185 g SCOD/kg $VS_{added}/L_{reactor}$ and 167 g COD_{SCFA}/kg $VS_{added}/L_{reactor}$, respectively. This volumetric performance of LBR at high volumetric organic loading of 49 g $VS/L_{reactor}$ was up to 2 times higher than that reported for LBRs at low volumetric organic loading (Saha & Lee, 2020, Shewa et al., 2020; Xiong et al., 2019a; Xiong et al., 2019b; Hussain et al., 2021). These findings demonstrated that the use of

enriched RAS along with higher GAC loading can significantly improve carboxylates production from food waste at high volumetric organic loading in LBRs. However, the long-term stability of RAS for SCFA production has to be investigated in a future study. Furthermore, leachate with concentrated SCFA would improve the cost-effectiveness of the downstream process. Bio-based carboxylates have extensive application in different industries such as, petrochemical, pharmaceutical, cosmetics, and so on. It has a high economic value of 800-2500 USD/ton of SCFA in the international market (Zacharof & Lovitt, 2013; Bastidas-Oyanedel et al., 2015; Bruni et al., 2021). Overall, the production of carboxylates from food waste using enriched inoculum is worthwhile and sustainable. However, the use of enriched inoculum has a centrifugation cost of 12.65$/ton VS_{added}. To avoid this cost, a sequential batch reactor (SBR) can be used since some amount of settled biomass of leachate is left during the idle period in SBR to inoculate the subsequent cycle. Therefore, the potential of SBR to produce SCFA from food waste can be investigated in a future study.

Moreover, Future research should be undertaken to improve the production of a particular component of SCFA such as butyrate to improve economic returns. There are various approaches to improve the production of butyrate such as cathodic electro-fermentation (Zhang et al., 2021), the addition of transitional metal such as copper, nickel (Fu et al., 2019), supply of redox mediators such as methyl viologen (Choi et al., 2012), and so on. The application of enriched inoculum is not limited to only carboxylates production from food waste, but it can be applied in all biological approaches to produce carboxylates from the organic feedstock.

4.5 Conclusion

The hydrolysis and acidogenesis of food waste was significantly different between LBR inoculated with RAS and AD-sludge. RAS as an inoculum achieved 28% higher hydrolysis yield than AD-sludge. Also, RAS resulted in 48% higher acidification yield than AD-sludge (LBR-AD). Moreover, RAS achieved higher composition of butyrate. Enrichment of RAS further improved the hydrolysis and acidogenesis yields. LBR inoculated with the second-level enriched RAS achieved the highest hydrolysis yield of 683 g SCOD/kg VS_{added} and acidification yield of 617 g COD_{SCFA}/kg VS_{added}, which were 10-22 % higher than LBR inoculated with fresh RAS. Enrichment of inoculum did not impact the SCFA composition. Butyrate dominates the SCFA mixture at all the levels of enrichment.

Chapter 5. Conclusions and recommendations

This research study was conducted to improve the hydrolysis and acidogenesis of food waste in LBR at high volumetric organic loading (49 g VS/$L_{reactor}$) using various GAC loadings (0, 0.25, 0.38, and 0.51 g GAC/g $VS_{foodwaste}$) and inoculum (RAS, AD-sludge, enriched inoculum) with a fermentation time of 14 days. Optimization of GAC loading and inoculum resulted in a high hydrolysis and acidification yield of 683 g SCOD/kg VS_{added}, and 617 g COD_{SCFA}/kg VS_{added}, respectively, which is amongst the highest reported in the literature. The LBR performance also indicated potential findings for future work to further improve hydrolysis and acidogenesis of food waste in LBR at high volumetric organic loading while shortening the fermentation time.

1. At higher GAC loading of 0.38-0.51 g GAC/g $VS_{foodwaste}$, the hydrolysis yield was 25-28% higher than lower (0.25 g GAC/g $VS_{foodwaste}$) or no GAC loadings. The highest hydrolysis yield of 620 g SCOD/kg VS_{added} was obtained at GAC loading of 0.51 g GAC/g $VS_{foodwaste}$, but it was not statistically ($P \leq 0.05$) different than that obtained at GAC loading of 0.38 g GAC/g $VS_{foodwaste}$.
2. As the GAC loading was increased, the hydrolysis became faster (hydrolysis rate). The highest hydrolysis rate was achieved with 0.51g GAC/g $VS_{foodwaste}$ (LBR-3), followed by 0.38 (LBR-2), 0.25 (LBR-1), 0 (LBR-C) g GAC/g $VS_{foodwaste}$. It suggests that fermentation time can be reduced with high GAC loading (0.38-0.51 g GAC/g $VS_{foodwaste}$) in a future study.
3. The highest acidification yield of 507 g COD_{SCFA}/kg VS_{added} was obtained with GAC loading of 0.51 g GAC/g $VS_{foodwaste}$ (LBR-3), which was statistically higher than GAC loading of 0.38 (LBR-2) and 0.25 (LBR-1) g GAC/g $VS_{foodwaste}$. This

acidification yield was 35% higher to that obtained in LBR-C that had no GAC loading.

4. The GAC loading also impacted the SCFA composition. An increase in GAC loading resulted in higher butyrate composition. Butyrate was the dominant product at the GAC loading of 0.38-0.51 g GAC/g $VS_{foodwaste}$, comprising 57-60% of total SCFA. However, acetate and butyrate had almost the same composition at lower or no GAC loading. This implies higher GAC loadings of 0.38-0.51 g GAC/g $VS_{foodwaste}$ can be preferred if butyrate is the targeted product.
5. The RAS as an inoculum resulted in a higher hydrolysis yield than AD-sludge. The hydrolysis yield with RAS was 614 g SCOD/kg VS_{added}, which was 28% higher than that obtained with AD-sludge. RAS also resulted in 48% higher acidification yield than AD-sludge.
6. A clear impact of inoculum on SCFA composition was observed. With AD-sludge, composition of acetate and butyrate were similar. Whereas butyrate was the dominant product with RAS by constituting 60% of total SCFA, suggesting the applicability of RAS as an inoculum to obtain high butyrate production in a future study.
7. The sequential enrichment of RAS further improved the hydrolysis yield by 10% in comparison to fresh RAS inoculum. The high hydrolysis yield also resulted in high acidification yields with enriched RAS. The acidification yield increased by 22% when compared to fresh RAS inoculum.

8. In all the LBRs inoculated with RAS and enriched inoculum, a decline in butyrate was observed after day 10. Therefore, a fermentation time of 10 days can be investigated if butyrate is the desired product.

The use of GAC and enriched RAS inoculum significantly improved the performance of LBR at high volumetric organic loading of 49 g VS/$L_{reactor}$. Future studies need to be performed to evaluate the economic returns for LBR operating at high volumetric organic loading, based on different factors including cost of GAC, energy requirement, and product yields. The reutilization of GAC can be compared with fresh GAC to evaluate its impact on hydrolysis and acidogenesis of food waste in a future study. Moreover, GAC loading as a support media in a leachate holding bed can be tested to improve the hydrolysis and acidification yields. Future studies can also explore the impact of high leachate recirculation rate and ISR at high volumetric loading, which are expected to shorten the fermentation time.

To ensure economic viability and environmental sustainability, LBR operation in semi-continuous mode might result into higher SCFA production at high volumetric organic loading rate. However, high SCFA concentration in fermented broth can also inhibit the hydrolysis and acidogenesis of food waste. Therefore, future research should be undertaken to optimize the volumetric organic loading rate to improve hydrolysis and acidogenesis of food waste for a semi-continuous operation of LBR. Besides increasing SCFA production, it is also important to develop low-carbon and energy-efficient technologies for purification and separation of individual SCFA component from the fermented broth in a future

study. Moreover, Future research should be undertaken to improve the production of a particular component of SCFA such as butyrate to improve economic returns.

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Appendix A

Supplementary Table and Figure

Table A.1 Characteristics of individual component of simulated food waste

Parameter	Carrot	Potato	Bread	Pet food
TS (%)	12.5±0.5	24.5±0.7	79.8±0.1	97.0±0.01
VS (%)	11.7±0.5	21.0±1.78	77.4±1.5	93.6±0.1
TS (g/kg)	125.4±5.0	245.1±6.9	798.3±1	970.5±0.1
VS (g/kg)	117.3 ±5.1	210.3±17.8	774.5±15.4	936.1±1.2
VS/TS (%)	94	86	97	96

Leachate Percolation in LBRs with different GAC loading:

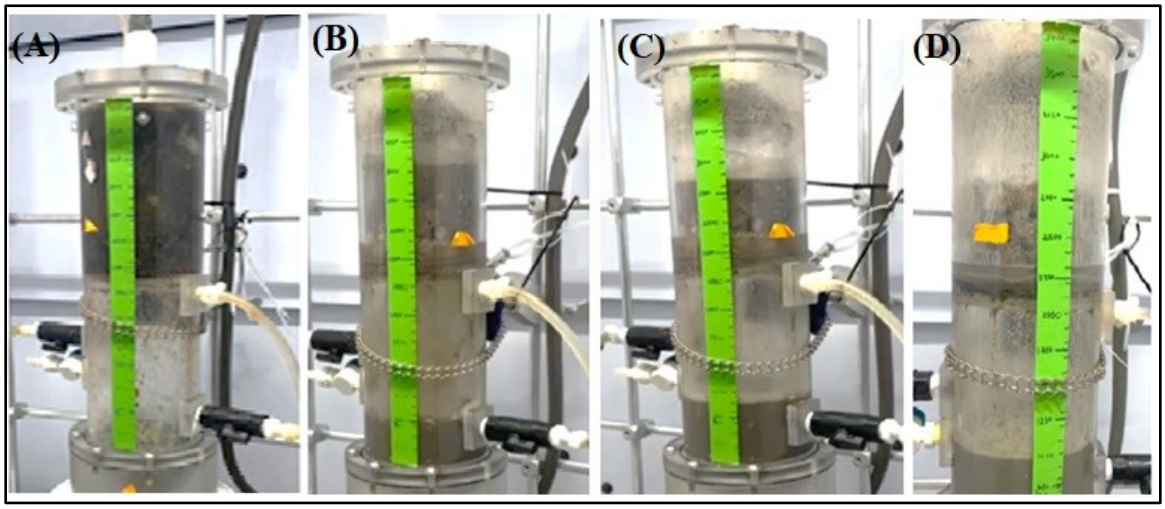

Figure A.1 Leachate percolation in LBR at different GAC loadings; (A) 0 g GAC/g $VS_{foodwaste,}$ (B) 0.25 g GAC/g $VS_{foodwaste}$, (C) 0.38 g GAC/g $VS_{foodwaste}$, and (D) 0.51 g GAC/g $VS_{food\ waste}$

Food waste residue with GAC at the end of fermentation at high GAC loading of 0.38-0.51 g GAC/g $VS_{foodwaste}$:

Figure A.2 Food waste residue with GAC at the end of fermentation