Improving leach bed reactor design for medium-chain fatty acid production from food waste at room-temperature.

by

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Author’s declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Food waste treatment is an urgent problem which if addressed can make substantial reductions in global greenhouse gas emissions while recovering energy, nutrients, and valuable biomolecules. While anaerobic digestion has become more common in recent years, digesters are expensive to operate, and renewable natural gas is too costly to compete with fossil natural gas in many markets. Acidogenic or dark fermentation is a potential treatment method for food waste which generates hydrogen and fatty acids as products, which are both more valuable on a molar basis than natural gas. Acidogenic fermentation can be performed in leach bed reactors which use less water, less energy and less space than stirred tank reactors.

This thesis addresses several questions related to how leach bed reactors performing acidogenic fermentation operate under relatively extreme conditions. The goal of this is to provide insights which will reduce the risk of building pilot scale fermenters which are next step in commercializing this technology. Clogging is a common problem cited by authors studying leach bed reactors and will certainly be a challenge as the scale of reactors increases. A study of clogged reactors revealed evidence that clogged reactors encourage different bacterial cultures than unclogged reactors and that in unclogged reactors hydrogen production is favoured over acid production. Further, if a small disturbance to the container of food waste in a leach bed reactor is made once per day clogging can be prevented, greatly increasing biogas production.

For many applications such as the generation of bioplastic and the biorefining of commodity fatty acids the production of acids over biogas is preferred and the generation of medium-chain fatty acids over short-chain fatty acids is ideal. Medium-chain fatty acids can be generated in acidogenic fermenters with low concentrations of ethanol present, and low temperatures (10-25°C) have been shown to improve the ratio of medium chain to short chain fatty acids. Low-temperature chain elongation was tested in a leach bed reactor and although a good medium-chain to short-chain fatty acid ratio was obtained, acid yield was not competitive with similar mesophilic reactors. These studies suggest that a pilot-scale acidogenic fermentation could effectively produce hydrogen at low temperature and a high organic loading rate if a small disturbance to the food waste container was incorporated, but short- and medium-chain fatty acid production is strongly affected by many inhibitory factors which must be considered during reactor design.

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Contents

List of Abbreviations

μ	Cell Growth Rate
AD	Anaerobic Digestion
AF	Acidogenic Fermentation
ATP	Adenosine Triphosphate
AWWA	American Water Works Association
CoA	Co-Enzyme A
COD	Chemical Oxygen Demand
CODeq	Chemical Oxygen Demand Equivalent
CSTR	Continuous Stirred Tank Reactor
GC-FID	Gas Chromatography with Flame Intensity Detector
GC-TCD	Gas Chromatographer with Thermal Conductivity Detector
HRT	Hydraulic Residence Time
IPCC	International Panel on Climate Change
ISR	Inoculum Substrate Ratio
LBR	Leach Bed Reactor
MCFA	Medium-Chain Fatty Acid
MSW-OF	Municipal Solid Waste Organic Fraction
OD600	Optical Density 600nm
OLR	Organic Loading Rate
PHA	Polyhydroxy Alkynoate
sCOD	Soluble Chemical Oxygen Demand
SLR	Solids Loading Rate
SRT	Solids Residence Time
tCOD	Total Chemical Oxygen Demand
TS	Total Solids
TSS	Total Suspended Solids
USD	United States Dollars
VFA	Volatile Fatty Acid
VS	Volatile Solids
VSS	Volatile Suspended Solids

Chapter 1
Introduction

1.1 Motivation

Food waste is a resource that is currently under-valued and under-utilized. Circular economy principles tell us that each product should become the feedstock to a new product at the end of its life. This principle is based on the value that has been invested in the materials and complexity of a product. This value should not be thrown away if it can be used economically. Food waste is a particularly valuable raw material, it is the ready-made fuel that runs all animals, all bacteria, archaea, and fungi in the natural world. These biological refineries are the most efficient way to generate untold numbers of biomolecules. However, food waste is not an efficient fuel to feed into an industrial process. It is not homogeneous and even when fed to animals it takes many mechano-chemical and bacterial processes to be nutritious to the animal. Additionally, food waste is heavy, bulky, rapidly-degrading and can carry many species of pathogenic and otherwise undesirable organisms.

That is what motivates this thesis. The fact that food waste is valuable, but is not usable as a fuel for many industrial bioprocesses. The thesis addresses improvements to the process of acidogenic fermentation (AF) in specific types of bioreactors to better turn food waste into a fuel that can be integrated into the circular economy.

The thesis focuses on the generation of fatty acids as fuel for the bioplastic-generating bacteria Pseudomonas putida, with acknowledgement and discussion that the same fuel could be used by many other microbiological applications. These applications include, but are not limited to methanogenic anaerobic digestion, biological wastewater treatment, biohydrogen production and the generation of medium-chain fatty acids which are added to animal feeds and food products as a natural preservative.

1.2 Scope and Objectives

This thesis focuses on the treatment of food waste, distinguished from agricultural waste by its heterogeneous nature, high water content, and low cellulose content. The motivation is to improve scalable processes for the biorefinery of commodity chemicals. Therefore, this thesis focuses on low-cost additives and changes to bioreactor design/operation rather than engineering novel biochemical pathways. Specific scoping parameters are described below:

Previous work has established leach bed reactors (LBRs) as an efficient and low—cost choice for acidogenic fermentation. This thesis moves forward on the premise that LBRs are worth investigating for this purpose and focuses on refinement of this design rather than comparison of types of reactors.

This thesis confined studies to batch and semi-batch processes. While many continuous processes are competitive to produce short- and medium-chain fatty acids, the rapid prototyping of a continuous system was not feasible. The findings in thesis can be applied to continuous systems and its relevance to continuous system design are addressed in the discussion of the results.

The objective of this thesis is to provide useful insights to the bioreactor design of leach bed reactors used for acidogenic fermentation when scaled-up from the bench-top to pilot scale operations. The thesis provides specific insights into the causes and potential treatments to clogging in leach bed reactors and to provide insights into the how to tailor reactor conditions to generate desired products. The resulting insights are directly applicable to biogas engineers and industrial microbiologists in the field of bioplastic generation – so that a pilot fermenter to produce fatty acids as precursors to polyhydroxy-alkenoates can be built with minimal risk.

1.3 Thesis Outline

This thesis has five chapters, the second of which is a literature review focused on familiarizing the reader with the background and landscape of the industry with specific attention to studies that try to optimize bioreactor performance in leach bed reactors of acidogenic fermenters fed with food waste or other low-cost feedstocks. Chapter three of the thesis describes an original study investigating the metabolic behavior associated with reactor clogging and an iterative design process that tests solutions to rector clogging. The study compared the experimental results to a model developed by the author based on the Monod model of cell growth for the purpose of identifying the metabolic processes that are most affected by clogging. Chapter four described a study performed with an established bioreactor design, testing the effect of ethanol addition on medium-chain fatty acid yield in a room-temperature reactor with a high circulation rate. The findings of this thesis are concluded in Chapter 5 and references are provided.

Chapter 2
Background and Literature Review

2.1 Food Waste in Canada

Global food systems are under threat by climate change, political insecurity, and changing diets. The International Panel on Climate Change (IPCC) annual report six (AR6) finds that food security is already under stress from climate change and that the current global food system is not adjusting in a sustainable way to address the threat of food insecurity (Pörtner, 2022). Reducing food waste and retaining the value of food is a potential route to make the global food system more efficient, more circular, and less extractive. The circular economy is proposed as an alternative to the modern extractive economy where a resource is extracted from some pool, such as oil from a well or nutrients from a field and then used and disposed of, either as an externality or as a cost to the producer. The circular economy proposes that waste should be an input to the cycle, for example nutrients are used from a field to grow food, food is processed and consumed generating energy for humans and additional biomass, this biomass can be used to generate energy and nutrients, which can then be returned to the field to grow food. In this simple example, the inputs are the outputs. By describing the system as a circular economy it is understood that these cycles are going to be much larger and interconnected. The use and reuse of resources will create long chains of use, over multiple products and industries, but the core principle remains the same. An ideal circular economy will strive for the material inputs to match the material outputs and the energy consumed by the cycle will be minimised.

In Canada in 2019 35.5 million tonnes of food waste was produced across all sectors (Gooch, et al., 2019). The same report found that more than 60% of this waste is generated by necessary processing of agricultural products to food products, the irresponsible disposal of organic waste streams in landfills causes 13% of Canada's methane emissions (Environment and Climate Change Canada, 2020). Treating this waste effectively and cheaply with minimal net energy use is an urgent priority for preventing further climate change.

Currently in Canada, a significant amount of food waste is valorized through use as animal feed and 13.9% of food waste is valorized to generate methane. Other options for diversion from landfill include field application and composting, these applications are focused on retaining the nitrogen, phosphorus, and other nutrients in the food system, while anaerobic digestion and acidogenic fermentation can both capture the chemical energy in food waste in addition to producing nutrient rich digestate which can be used as fertilizer (Canadian Council for Ministers of the Environment [CCME] ,

2018). Food waste is produced in every part of the supply chain, from field crops being left in the field due to poor harvesting conditions, to trimmings and losses during processing, to the unused food thrown away in homes. All the food waste can be divided into preventable and non-preventable waste. Preventable waste can be prevented though economic and social measures while the non-preventable waste can be the target for bioenergy and biorefinery applications. As this application of non-preventable waste does not create an incentive to process food less efficiently and generate more waste.

Food waste treatment methods that are alternatives to landfill, compost, and anaerobic digestion have been proposed, though none have reached commercial use in Canada. Pyrolysis and hydrothermal processing, acidogenic fermentation, and biorefinery are among the most cited "next generation" organic waste treatment processes (Ding, et al., 2021; Lin, Xu, Ge, & Li, 2018; Wainaina, et al., 2020). Pyrolysis and hydrothermal processing are processes where food waste is heated to temperatures between 400 and 900 °C to covert the carbon compounds into hydrocarbons which can be used as a fuel or as a precursor for chemical production which traditionally rely on petroleum. The processes generate volatile organic-compound-rich wastewaters that require further treatment, and the process requires the heating of large amounts of material. Therefore, it is not yet a popular solution for treating wet organic waste (Su, et al., 2022; Leng & Zhou, 2018).

While developing methods to treat this waste effectively, using an embodied energy model can be useful in approximating the total impact of a cradle-to-cradle material cycle. The complex issue of food waste can be simplified when viewed though the lens of embodied energy. An apple has an embodied energy of approximately 179 kJ depending on growing conditions (Ekinci, Demircan, Atasay, Karamursel, & Sarica, 2020). If that apple is thrown away and landfilled, it then consumes energy, and more than 179 kJ is lost. If the apple is used for anaerobic digestion it can generate 0.8 L of methane which has an embodied energy of 33 kJ and the nutrient rich digestate can be used in land application to replace energy intensive fertilizers reducing the energy lost in the cycle to less than 150 kJ (Tulun & Bilgin, 2018). The most valuable and energy intensive products that biological processes can produce are polymers and specialized organic compounds, this has led to many scientists proposing biorefinery, i.e., turning waste into these complex compounds as the best use of food waste as a resource (Ramos-Suarez, Zhang, & Outram, 2021).

Biorefinery refers to the purification of biological compounds from bulk biomass for use in the chemical, pharmaceutical, and energy industries. This can be accomplished through chemical means such as liquid-liquid extraction, physical means such as ultrafiltration, and biological means using enzymes or microbial catalysts. A challenge to the adoption of biorefinery from food waste are the high purification

costs and the heterogeneous feedstocks which must be incorporated (Lokesh, Ladu, & Summerton, 2018) (Pandeeti, Veeraiah, & Routha, 2019). The process of acidogenic fermentation is a natural stage of anoxic decomposition which converts organic waste into simple metabolites, acetic acid, propionic acid, butyric acid (referred to together as volatile fatty acids or VFAs), ammonia, ethanol, hydrogen, and carbon dioxide. Acidogenic reactors turn heterogeneous waste streams into uniform mixtures rich in the nutrients that bacteria require. Currently, they are used to improve the efficiency of methane generation from food waste and can feed microbial factories with a uniform feedstock (Ramos-Suarez, Zhang, & Outram, 2021).

2.2 Fundamentals of Acidogenic Fermentation

Acidogenic fermentation refers to the anaerobic pathways which generate short-chain fatty acids, specifically acetic acid, propionic acid, butyric acids, and valeric acids. These compounds are generated from simple sugars, fats, and proteins in many bacteria when growing in an oxygen poor environment (Ramos-Suarez, Zhang, & Outram, 2021). The process generates fatty acids to regenerate co-enzyme A (CoA) when oxygen is not available as the terminal electron acceptor, as shown in Figure 1. CoA oxidizes pyruvate to acetyl-CoA or butyryl-CoA generating energy for the cell in the form of NADPH and hydrogen. The CoA is then regenerated by replacement with active phosphate. Finally, the phosphate group is removed in the generation of ATP molecule, creating acetate or butyrate.

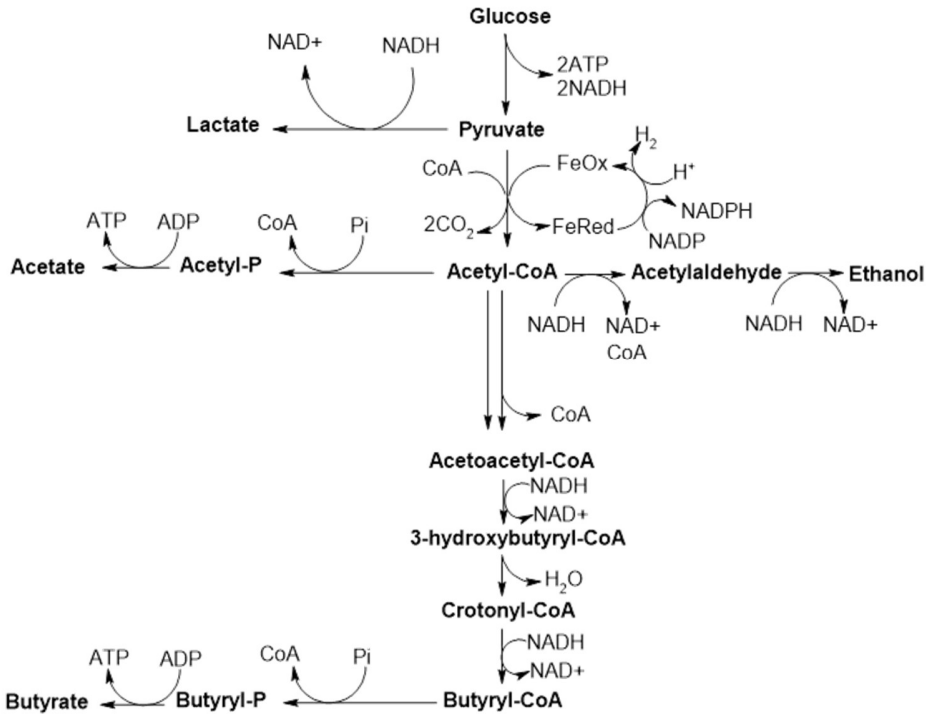

Figure 1: Metabolic pathway to common products of anaerobic fermentation adapted from the work of Jones and Woods (Jones & Woods, 1986).

Other potential anaerobic pathways exist which generate different terminal molecules depending on the cells favoured by the biological conditions. In yeast cells, ethanol fermentation is favored, in acidogenic fermentation at neutral and acidic pH, significant ethanol coproduction has been observed. In controlled food systems with high salt concentrations, lactic acid is formed; however less energy is recovered in the production of lactate than ethanol or acetate, so it is not observed in high concentrations in mixed cultures. Acetic acid can be consumed as a carbon source by several anaerobic organisms including methanogenic archaea which are responsible for the third stage of digestion in anerobic digestion systems. However, these are not the only organisms capable of using volatile fatty acids (VFAs) as a feedstock. VFAs have been proposed as a feed for many biotechnological applications where the price of refined feedstock makes the process economically infeasible (Ramos-Suarez, Zhang, & Outram, 2021).

The efficiency and yield of acidogenic fermentation is highly dependent on the availability of simple molecules to convert to acetyl-CoA. The source of these simple molecules can be sugar processing waste or refined sugars, but more often they are the product of hydrolysis of more complex feedstocks (Ramos-Suarez, Zhang, & Outram, 2021). Food waste, source-separated organics, animal manure, and

slaughterhouse waste can be hydrolysed organically by environmental anaerobic bacteria under the right conditions. Harder feedstocks such as municipal solid waste organic fraction (MSW-OF), straw, animal bedding, and some fruit peels require chemical or physical pre-treatment to complete hydrolysis (Ramos-Suarez, Zhang, & Outram, 2021).

The acid conversion yield varies widely with the feedstock used, the pH, temperature, and hydrolysis yield. The optimal pH for acid yield is 7, however there are more factors that must be considered when designing an acidogenic fermenter (Xiong, Hussain, Lee, & Lee, 2019). Methanogens also are most productive at pH 7 and degrade any acid produced if a methanogenic culture is established, therefore, a pH of 6 or lower inhibits methanogens but allows acidogenesis to proceed (Ramos-Suarez, Zhang, & Outram, 2021). At pHs lower than 5.5, fatty acids are converted to their protonated form which is inhibitory to hydrolytic enzymes and bacteria (Hussain, Filiatrault, & Guiot, 2017). The lower pH also forces the acids out of solution which has benefits for industrial uses as product recovery is improved without the addition of chemicals (Ge, Usak, Spirito, & Angenent, 2015). Acidogenesis can also occur at higher pH, which improves the hydrolysis efficiency however bacterial growth is inhibited (Zhang, Chen, & Zhou, 2009).

A benefit of acidogenic fermentation over its alternatives for the treatment of waste is the wide range of temperatures that it can be performed at. Most studies find that mesophilic conditions (between 30-40°C) generate the highest yields of VFAs, that thermophilic conditions (50-70°C) improve the hydrolysis of feedstock and can generate the highest concentrations of VFAs, and that psychrophilic conditions (10-25°C) produce lower concentrations but consume less energy in operations (Ramos-Suarez, Zhang, & Outram, 2021).

Lower fermentation temperatures can also improve the conditions for medium-chain fatty acid formation. In a 2015 study Ramió-Pujol et al. compared the fatty acid production of a pure culture of Clostridium carboxidivorans P7 at 25°C and 37°C (Ramió-Pujol, Ganigué, Bañeras, & Colprim, 2015). The study found that although the observed growth rate was 25% slower in the low temperature fermentation, the lower temperature fermentation produced much higher concentrations of ethanol and of caproic acid. The study achieved 9.02 mM of caproic acid at 25°C from modified ATCC 1754 medium, while the 37°C fermentation produced only 0.419 mM of caproic acid. This suggests that when optimizing mixed culture acidogenesis to produce medium-chain fatty acids, low temperature fermentation should be considered.

2.3 Applications of Acidogenic Fermentation

Acidogenic fermentation (AF) transforms diverse feedstocks to uniform fatty acids, this offers potential for many applications related to organic waste treatment. The most studied wastes can be categorized loosely into food waste, agricultural waste, wastewater solids, and fat rich industrial waste. Each of these products contribute significantly to landfill gas production. Treatment with acidogenic fermentation reduces the production of harmful gasses and captures the gasses that are produced. Acidogenic fermentation also recovers more of the embodied energy from these high-energy waste products. Composting and anaerobic digestion also safely convert the biological oxygen demand from these feedstocks to environmentally safe forms. However the products that are produced are equivalent to products that are produced with much less energy, 605 kJ/kg CH_4 and 4106 kJ/kg compost compared to the approximately 5000 kJ/kg used to generate food. AF converts the high embodied energy waste to equivalents of high embodied energy industrial chemicals such as acids and polymers (Budsburg, Morales-Vera, Crawford, Bura, & Gustafson, 2020).

While a uniform product is generated by AF, it is produced in digestate that has low concentrations and many impurities, therefore any application must either justify the purification of products or be able to use the acid in digestate. Possible applications of VFAs include feed for anaerobic digestion, feed for biological nutrient removal, purification for refined food additives and industrial esters, and feeding biological factories for bioplastics or other biotech products (Ramos-Suarez, Zhang, & Outram, 2021). The use in anaerobic digestion (AD) is well established with 2-stage-digestion systems being used for lignocellulosic “yard waste”, food waste, and wastewater solids. When used for anaerobic digestion (AD) and biological waste treatment, the product does not need to be purified or concentrated. Additionally, when used in AD, methanogens do not need to be inhibited in the primary fermenter allowing for fast acid production without chemical additives. The purification of fossil-free acetic acid, along with the food additive butyric acid and other purified products is attractive because these acids are more valuable than energy products such as methane and hydrogen. Purified acetic acid from fossil sources costs approximately 1.40 USD/kg, butyric acid 1.48 USD/kg - that is 8.38 USD per 100 mols and 13.02 USD per 100 mols respectively compared to 0.81 USD per 100 mols carbon of fossil methane, (ChemAnalyst, 2020). One mole of acetic acid in anaerobic digestion produces one mole of methane, while one mole of butyric acid produces 2 moles of methane. Clearly, efficient recovery of these products would recover more of the cost of treatment than AD. While purification processes are currently too expensive for biological fatty acids to compete with fossil-based fatty acids, Ramos-Suarez et al. identify this as the most promising path for development in their 2021 review (Ramos-Suarez, Zhang, & Outram, 2021).

Industrial microbiology continues to expand the range of products that can be produced without synthetic chemistry (Pandeeti, Veeraiah, & Routha, 2019). These advancements in microbiology have created pathways to new medicines, foods, materials, and fuels. Many of these advancements are made prohibitively expensive by the requirement of refined uniform feedstock derived from sugar and oil crops (Lokesh, Ladu, & Summerton, 2018). AF provides a pathway that generates a uniform feedstock for bacteria - mixed volatile fatty acids (VFAs). While some concentration and solids removal will be required for any process, the energy intensive separation of fatty acids from water and other acids is not required for such applications. The most popular of the industrial microbiology applications for AF is the production of polyhydroxy alkynoates or PHAs (Ramos-Suarez, Zhang, & Outram, 2021). This class of bioplastics have superior properties to the corn-based poly-lactic acid along with better biodegradability (Chen, 2010). The plastic has had only limited industrial adoption due to its high cost, which is caused by the need for refined valerate and refined sugars fed to pure bacterial cultures. One of the many bacteria (*Pseudomonas putida*), that produce PHAs, also readily store fatty acids in polymer complexes, making them a perfect candidate for feeding with mixed fatty acids from AF (Saratale, et al., 2021). Economically competitive yields of PHA have been generated in the laboratory from synthetic fatty acid mixes, without any impurities from fermentation. More research into the purification and concentration steps to achieve competitive yields of PHA from AF digestate is required to scale this process.

2.4 Challenges in Acidogenic Fermentation

Improvements in acidogenic fermentation can be made to increase hydrogen yield, increase methane yield, and to generate a consistent and tunable product for bioproduct generation. A selection of representative AF performances has been summarized in Table 1 to summarize the state of the industry. Across all applications, lowering the cost, solids retention time, hydraulic retention time, and selective fatty acid generation are all pathways to improvement. Bonk et al. performed an economic analysis of AF for fatty acid formation in Abu Dhabi. They found that if the cost of operation could be maintained under $35 USD per tonne food waste the process would likely be financially viable (Bonk, Bastidas-Oyanedel, & Schmidt, 2015). This study was performed in 2015 and as the price of fossil-based acids has increased so has the hypothetical profit margins for biological alternatives. The authors also noted that the cost of purification is the most expensive stage, stating that a method of purifying VFAs from fermentation broth would need to cost less than $14.96 USD per m^3 of broth. Their findings suggest that pre-treatment of food waste for improved VFA yield was likely to improve financial viability. The study proposes a 2-stage treatment where leachate is generated in a leach bed reactor and fatty acids are formed in a

continuous stirred tank reactor (CSTR), this reduces the solids in the CSTR, reducing the energy required for stirring.

Attaining a low solids residence time is desirable for the industrial use of acidogenic fermentation. Fermentation times in literature range from 2 days to 22 days. The reactor design and substrate are the strongest determining factors of the solids residence time. This issue is complicated by the inhibitory nature of fatty acids on bacterial growth and hydrolysis (Zhou, Yan, & Wong, 2018). The shortest retention times are reported for continuous stirred tank reactors (CSTR) and in reactors fed with dairy/sugar processing wastewater (Ramos-Suarez, Zhang, & Outram, 2021). Specifically, a CSTR for acidogenic fermentation used by Pant et al. fed with blended food waste sieved to 2.4 mL in 3% volatile solids (VS) solution produced 414 mg CODeq of VFA[1] / g VS achieved a short residence time of 3 days. This was made possible as the blending and dilution of waste in addition to relatively low pH (4.5-5.5) – all factor which improve hydrolysis. The concentration of VFAs also remained low in their semi-continuous system with concentration peaking at 12.4 g CODeq VFA/L (Pant, et al., 2013). In their study of dairy and sugar processing wastewaters, Yu and Fang optimized the process for high acidification yield instead of high concentration. They found the at the effluent concentration of 1.142 g CODeq of VFA/L. higher concentrations could only be achieved at the cost of yield (Yu & Fang, 2001). For product recovery to be economical, high yield and high concentrations must be achieved in addition to short retention times.

Typical retention times for batch-style stirred reactors fed with food waste or anaerobic sludge are 4-9 days. Leach bed reactors typically have a longer retention time, from 6 to 20 days. While both reactor geometries consistently produce similar yields from 300 to 400 mg CODeq VFA per g VS added, high solids reactors, including leach bed reactors, typically produced higher concentrations of products. Han and Shin found that in a leach bed reactor with continuous removal of leachate at mesophilic temperatures could process a 1 kg batch of food waste in 6 days (Han & Shin, 2004). This reactor mode favoured hydrogen generation and produced relatively high VFA yields of 309 mg CODeq VFA per g VS FW added, though at low concentrations. In contrast Hussain et al. used a batch style, thermophilic fermenter with a high circulation rate, finding that the VFA production did not stop until the 12th day of operation and the operation achieved VFA yields of 340 mg CODeq VFA per g VS FW, with a fraction of water used (Hussain, Filiatrault, & Guiot, 2017). These studies show that high VFA concentrations are often in conflict with low hydraulic residence times. While the low retention times and high yields have been

[1] Total volatile fatty acid concentration is the sum of all fatty acid concentrations in solution. To sum the concentrations of fatty acids in a comparable and biological relevant manner the concentrations of individual fatty acids have been converted to their equivalent chemical oxygen demand (CODeq) before the sum is taken.

demonstrated in CSTRs, improved reactor designs are required to reduce operating costs while maintaining high yields, high concentrations, and high organic loading rates (OLRs).

Pre-treatment has the potential to improve VFA yields while reducing the required treatment time. This has been demonstrated using a variety of chemical and physical pre-treatment techniques. Elbeshbishy et al. tested heat treatment (70**°**C for 30 mins), ultrasonication (79 kJ/g TS), acid treatment (pH 3), alkaline treatment (pH 11) and combination thereof. These tests were performed on food waste and in 200 mL shaken bottles at 35°C. The authors found ultrasonication to be the most effective individual treatment, likely because it increases solubility by 25% without degrading the substrate. The authors found that most effective combination for VFA production was acid treatment paired with ultrasonication as this increased solubility by a similar amount but increased the amount of soluble carbohydrate by 15% over ultrasound alone (Elbeshbishy, Hafez, Dhar, & Nakhla, 2011). The method of pre-treatment depends upon which recalcitrant substrates are present in whatever feedstock is being used for fermentation. Therefore, optimization of each fermentation process must include consideration of available pre-treatment methods and the compounds which they target.

Table 1: Summary of the performance of acidogenic fermentation. Yield is expressed in mg CODeq of total VFA generated per g VS added to reactor; total VFA is a sum of products including all fatty acids with molecular weights between acetic acid and capric acid. CODeq is the equivalent chemical oxygen demand of all fatty acids in the mixture.

	VFA Yield (mg CODeq/g VS added)	Concentration (gCODeq/L)	pH	Pretreatment	Temperature (**°**C)
Pant 2013	414	12.4	4.5-5.5	Blending	35
Yu 2001	878	1.142	5.5	Washwater	35
Han 2004	309	2.988	5.5-6.5	None	35
Hussain 2017	340	49	7	Shredded	50
Elbeshbishy, 2011	367	16.90	5.5	Acid and ultrasonication	35

When used as pre-treatment for methanogenic anaerobic digestion, acidogenic fermentation is performed efficiently at low concentrations and low residence times. While fermentation for product recovery has the potential to recover more value from food waste, the processes must be improved. Specifically, that improvement can come in the form of improved product recovery paired with an increase in fermentation concentration, while maintaining low residence time and high yield. Previous studies have explored different reactor geometries, fermentation conditions and pre-treatment techniques but more work is required to demonstrate economic viability in a commercial process.

2.5 Performance in Leach Bed Reactors

Leach bed reactors (LBRs) have demonstrated advantages for high-solids, anaerobic treatment of organic waste such as food waste and manure. LBRs can produce leachates with high concentrations of VFA without large solids handling costs, large volumes of water, and without high mixing costs. Most LBR studies have been conducted at trickle flow rates with leaching flow rates of less than 1.5 L/h, at mesophilic temperatures, use between 1 and 2 parts water to one part substrate, and 0.1 to 1 part inoculum culture. The studies use a wide range of organic wastes including food waste, municipal organic waste, animal bedding, manure, agricultural waste and wastewater sludge.

Several authors have identified hydraulic clogging of the LBR as significant barrier to commercialization and scale-up of this technology (Hanif, Loetscher, & Sharvelle, 2021) (Xiong, Hussain, Lee, & Lee, 2019). While the problem can be addressed by mixing bulking agents such as ash and wood chips in the treated waste, these interventions significantly increase the cost of treatment, decreasing the market advantages of the technology (Xu, Karthikeyan, Selvam, & Wong, 2014) (Yang, Wang, Luo, & Zeng, 2019). Xu et al. used wood chips as a bulking agent in their leach bed reactor, generating 82.08 g/kg FW with 69.4% solids removal. The flow rate of 0.3 L/hr was very low despite the use of bulking agents and the batch took 16 days to reach equilibrium (Xu, Karthikeyan, Selvam, & Wong, 2014). Xiong et al. operated a leach bed reactor with food waste for acidogenesis with a recirculation rate of 4.4 L/hr. This generated much higher VFA yields, 479 g/kg food waste. Since higher VFA yields have been associated with higher recirculation rates, more work is needed to understand the metabolic effect clogging has on mixed microbial cultures in LBRs and to identify the necessary conditions for high-yield/fast-rate fermentation.

Clogging can be prevented through mechanical changes to reactor design which do not increase the process cost or energy used significantly. For example, higher VFA yield and higher VS removal was observed in a leach bed reactor fed with pig manure which used a ceramsite filter to prevent clogging by Yang et al.. This was a methanogenic reactor which continuously removed fatty acids, however over the

course of operation it removed 87% of volatile solids and generated 190 g VFAs per kg of pig manure (Yang, Wang, Luo, & Zeng, 2019). The flow rate in this trial was also very slow <0.625 L/hr, but it demonstrated that better efficiencies can be achieved in a reactor with a low flow rate if clogging is addressed and hydrolysis is not suppressed.

2.6 Chain Elongation of Fatty Acids

Medium-chain length VFAs are a more desirable bioproduct than short-chain VFAs because they are more energy dense for use as a biofuel, they require less energy to isolate because of their low solubility and they are needed to make high quality PHAs (Weimer, Nerdahl, & Brandl, 2015). The reverse beta-oxidation pathway converts acetate, propionate, and butyrate to longer chain fatty acids. The reverse beta-oxidation pathway needs a bioavailable two-carbon molecule which can be converted to acetyl-CoA spontaneously. Ethanol is a low-cost substrate which is readily converted to acetyl-CoA and has been demonstrated to be effective at various concentrations for the chain elongation of fatty acids. The pathway for ethanol chain elongation is shown in Equations 1-5. These equations have been simplified and their free energies of reaction have been calculated, as shown in Equations 6-8. Each addition of an ethanol to a fatty acid chain is thermodynamically favourable, however previous studies have shown that the limit of caproate production falls between 10 and 25 g/L (Roghair, et al., 2018; Ge, Usak, Spirito, & Angenent, 2015). This is due to the inhibitory effect of caproate on hydrolysis and cell growth (Grootscholten, Kinsky dal Borgo, Hamelers, & Buisman, 2013).

$$1\ ethanol + 1\ acetate^- + 2\ CoASH \rightarrow 2\ acetyl\ CoA, reducing\ 2\ NAD^+ \quad (1)$$

$$2\ acetyl\ CoA \rightarrow 1\ acetoacetyl\ CoA + 1\ CoASH \quad (2)$$

$$1\ acetoacetyl\ CoA \rightarrow 1\ crotonyl\ CoA + 1\ H_2O, reducing\ 1\ NADP \quad (3)$$

$$1\ crotonyl\ CoA \rightarrow 1\ butyrl\ CoA + \frac{2}{5} H_2, oxidizing\ \frac{7}{5}\ NADH \quad (4)$$

$$1\ butyrl\ CoA + 1\ acetate \rightarrow 1\ acetyl\ CoA + 1\ butyrate \quad (5)$$

$$1\ ethanol + 1\ acetate^- \rightarrow 1\ butyrate^- + 1\ H_2O, \Delta G^{\circ\prime} = -38.6 \frac{kJ}{mol} \quad (6)$$

$$1\ ethanol + 1\ propionate^- \rightarrow 1\ valerate^- + 1\ H_2O, \Delta G^{\circ\prime} = -38.6 \frac{kJ}{mol} \quad (7)$$

$$1\ ethanol + 1\ butyrate^- \rightarrow 1\ caproate^- + 1\ H_2O, \ \Delta G^{\circ\prime} = -38.6 \frac{kJ}{mol} \quad (8)$$

The performance of chain elongation can be measured by several comparable metrics and the products tuned by several operation parameters. The concentrations (in g/L) and yields (in g CODeq/g VS

added) of the six-, eight-, and ten-carbon fatty acid chains - caproate, caprylate, and caprate, are common measures of performance. Other relevant measures include the total yield of fatty acids and the ratio between short-chain fatty acids (VFAs) and medium-chain fatty acids (MCFAs). While the distinction between the shortest medium-chain and longest short-chain fatty acids is not well codified, for the purpose of this review short-chain fatty acids are acetate, propionate, and butyrate while medium-chain fatty acids are valerate, caproate, caprylate, and caprate along with any fatty acids which fall with those molecular weight ranges. This definition is adopted to distinguish the primary products of acidogenesis from the secondary products of reverse beta-oxidation.

The operational parameters that have the most effect on acidogenic fermentation with chain elongation for the production of medium-chain fatty acids are the concentrations of ethanol added, the temperatures of fermentation, the bacterial cultures, the pH, the substrate concentrations, and the product concentrations. Previous authors have demonstrated high yields of MCFAs under several conditions, yielding insights into the optimization process of this fermentation mode.

Lin et al. found that 9 g/L ethanol in mesophilic anaerobic digestion in rumen culture could convert most propionic acid to caproic acid and in the same media 18 g/L lactic acid converted most propionic acid to valeric acid via the same pathway. These yielded short to medium ratios of 2:1 and 0.7:1 respectively, but both showed reduced product yield overall on a mass per unit VS basis (Lin, Feng, Cheng, & Wang, 2021). The work demonstrated that lactic acid can out-perform ethanol as a chain extender but at greater chemical cost.

Ge at al. fed a 30°C anaerobic digestor with 44 g/L ethanol and 65 g COD/L mixed corn sugars at pH 5.5, for the purpose of continuous extraction of medium-chain VFAs. They found that caproic acid concentrations of 0.9 g/L could be achieved without continuous extraction, but inhibition of the culture was observed. In contrast, continuous extraction produced 10g of caproic acid per liter of volume added. They also found that lower concentrations of medium-chain VFAs inhibited hydrolytic activity (Ge, Usak, Spirito, & Angenent, 2015). The authors suggest that hydrolysis of complex feedstocks takes more than 15 days under these conditions due to the high concentrations of protonated caproic acid.

The cost of added chemicals can often make waste treatment and biorefinery processes costly. While pure ethanol is inexpensive and biologically generated, lower concentrations of ethanol and water can be generated though fermentation of certain waste streams as a part of treatment. This co-fermentation of ethanol and VFAs to generate MCFAs has been demonstrated in bench-scale systems. Andersen et al. produced caproic acid in concentrations up to 8.1 g/L and caprylic acid and up to 3.2 g/L in continuous operation fed with a mixture of distillation effluent (stillage) and grain fermentation beer. The reactor was

fed continuously to maintain an ethanol concentration of 6 g/L. The reactor maintained a total VFA concentrations between 6 and 8 g carbon/L, approximately 11 g/L, showing a medium-chain to short-chain ratio of between 1:1 and 1.5:1 (Andersen, et al., 2017). Similarly, den Boer et al. used hydrolysed food waste as the substrate for VFA production in conditions encouraging ethanol production. Ethanol concentration was maintained at an equilibrium concentration of 1 g/L and valeric acid was continuously produced by chain elongation. This was conducted at pH 6.5 and 37°C in a 250 L, bubble-mixed wet fermenter. The system yielded mostly butyrate and valerate with the medium to short-chain ratio being 1.6:1 (den Boer, et al., 2016). The work of both Den Boer et al. and Andersen et al. demonstrates that co-fermentation of ethanol and MCFAs may have potential as a pathway for biorefinery.

Table 2: Physical properties and microbial toxicity of fatty acids produced by acidogenesis and chain elongation

Properties	Octanol - water partitioning coefficient pKow	Vapour Pressure (atm-m^3/mole)	Boiling Point (°C)	Bacterial Toxicity Appears (g/L)	
Acetic Acid	0.17	1.00E-07	117.9	5 to 40	(Lasko, Schwerdel, Bailey, & Sauer, 1997)
Propionic Acid	-0.33	4.45E-07	141.1	5 to 25	(Lewis & Yang, 1992)
Butyric Acid	-0.79	5.35E-07	163.7	3.5 to 40	(Sun, et al., 1998)
Valeric Acid	-1.39	4.72E-07	185.4	1 to 2	(Kovanda, et al., 2019)
Caproic Acid	-1.92	7.58E-07	205.2	7 to 10	(Hismiogullari, et al., 2008)
Enanthic Acid	-2.42	1.04E-05	222.2	0.5 to 2.6	(Jang, Jeon, Baek, Lee, & Park, 2014)
Caprylic Acid	-3.05	1.33E-05	239	2 to 5	(Hismiogullari, et al., 2008)

Nzeteu et al. achieved 10 g/L caproate production in a LBR fed with food waste. They operated at mesophilic temperatures with a small leachate volume - approximately 2 kg of substrate to 1 kg water. Reactor operated at typical a recirculation rate of 0.3 L/hr, pH 7, and 37°C. The high concentration achieved is attributed to augmentation with enriched cultures of reverse beta-oxidizing bacteria such as Clostridium kluyveri (Nzeteu, Trego, Abram, & O'Flaherty, 2018).

Grootscholten et al. used LBR of MSWOF (mostly garden waste) dosed with ethanol to generate medium-chain fatty acids. They achieved a caproate concentration of 2.8 g/L, heptanoate concentration of 1.6 g/L, and a caprylate concentration of 0.6 g/L. The reactor was operated with 2 kg of MSWOF and 2 L of water at 30°C and pH 5.4 with a high circulation rate of 7 L/h. The ethanol concentration in the reactor was maintained between 1 and 1.5 g/L (Grootscholten, Steinbusch, Hamelers, & Buisman, 2013).

The work of these authors shows that low concentrations of ethanol are effective at converting volatile fatty acids to medium-chain fatty acids in leach bed and other high-solids reactors. The highest yields were achieved though continuous product removal and the highest concentrations were achieved though high solids mesophilic digestion at pH 7. A pH above the PKA of the most toxic products must be maintained or production will be limited to approximately 10 g/L of caproic acid and 5 g/L of caprylic acid as show in Table 2. The highest yields of medium-chain fatty acids have been produced at approximately 40°C with increased toxicity of medium-chain fatty acids being observed above this temperature. Despite this limitation very little work has been done to assess the effect that lower fermentation temperature has on chain elongation. It has been repeatedly observed that the toxicity of medium-chain fatty acids has a significant effect on the concentration of fatty acids generated in solution as well as the hydrolysis rate of more recalcitrant feedstocks such as food waste. To scale the process of chain elongation up from the bench scale and make this a commercial pathway for generating bioproducts more work must be done to reduce process energy while maintaining high product concentrations.

2.7 Summary

Food waste diversion costs municipalities and business money as landfilling costs are frequently low and the process uncomplicated. By generating more valuable products from food waste the cost treatment can be reduced and more waste can be diverted. The current generation of technology generates energy and fertilizer from food waste, mostly though anaerobic digestion and aerobic composting. Biorefinery refers to processes that refine specific chemicals from organic feedstock, such as food waste. Biorefinery has potential to generate more value from food waste than the current technologies but is not technologically ready to be used at a large scale.

Acidogenic fermentation, fatty-acid-chain-elongation, and bioplastic synthesis are promising biorefinery processes that generate hydrogen gas, fatty acids of various lengths from two-carbon acetate to ten-carbon caprate, as well as the bioplastic polyhydroxy alkenoate (PHA). Acidogenic fermentation anaerobically converts food waste to acetic acid, propionic acid, butyric acid, carbon dioxide and hydrogen. Well performing reactors fed with FW in the laboratory have produced between 40 and 60 gCODeq/L of VFA from food waste with VFA yields between 0.5 and 0.8 gCODeq produced per g of

volatile solids added (Ramos-Suarez, Zhang, & Outram, 2021). When optimized for hydrogen production acidogenic fermentation of FW has produced 50 to 70 cm^3/g VS from FW (Łukajtis, et al., 2018). Certain bacteria perform chain-elongation using the reverse beta oxidation pathway to add carbon to the end of fatty acid chains when they are deprived of oxygen. This converts the acetic acid, propionic acid, butyric acid of acidogenic fermentation to valeric acid, caproic acid, enanthic acid, caprylic acid, capric acid, and other medium chain fatty acids. Well preforming reactors fed with FW in the laboratory have produced between 15 and 25 gCOD eq/L of caproate and elongated nearly half of the short-chain fatty acids (Andersen, et al., 2017; Grootscholten, Steinbusch, Hamelers, & Buisman, 2013; Nzeteu, Trego, Abram, & O'Flaherty, 2018). Polyhydroxy alkenoates are plastics generated inside bacteria as a method of energy storage under aerobic conditions when carbon is over available, but bacterial growth is limited by other factors. In a complete integrated system Amulya et al. generated 0.17g COD PHA/g COD in the VFA rich broth generated from FW (Amulya, Srinivas Jukuri, & Venkata Monhan, 2015). This efficiency could be improved with a higher concentration and higher purity of VFAs in the broth, this is necessary for the economic viability of this pathway (Rodriguez-Pereza, Serrano, Pantión, & Alonso-Fariñasc, 2018).

The acidogenic fermentation of food waste must be improved before it is widely adapted as a mature technology. Previous authors have identified specific challenges to high yield and high concentration fatty acid production which must be addressed. Slow product formation remains a barrier to adoption of acidogenic fermenters at scale. While a stirred tank anaerobic digestor can fully decompose food waste to digestate and biogas in three to five days, many of the acidogenic fermenters tested in the lab required seven to 28 days to complete fermentation (Ramos-Suarez, Zhang, & Outram, 2021). This much slower solids loading rate is related to low hydrolysis rates and slow rates of fatty acid formation. Several pathways of pre-treatment of FW using chemical, thermal, and physical methods have shown promise in performing hydrolysis of FW faster than enzymatic hydrolysis alone. Finally, product inhibition is a complex challenge when improving high concentration fermentation. Most fatty acids have a pKa of approximately 5.5. if pH falls below this level the acids are converted to their protonated form which are much more toxic. When fatty acids are generated in higher concentrations and form more complex feedstocks the production of valerate is increased, which is more toxic than acetic acid and propionic acid, slowing fermentation. While hydrogen gas is not very soluble in water it has been found that when high concentrations of hydrogen gas are present in the headspace of a fermenter product inhibition is still observed (Das, Calay, Chowdhury, Nath, & Eregno, 2020).

Leach-bed reactors use a container above a reservoir, recirculating aqueous leachate though a bed of substrate. Carrying high concentrations of product away from the biofilm surface and allowing reactors to be operated with much less dilution than stirred tank or up-flow sludge blanket reactors. Leach- bed

reactors have specific problems with operation as a constant flow rate though the food waste or substrate container, but the container can become “clogged” slowing the flow rate and changing the fermentation conditions. The performance of clogged reactors in the literature is highly variable and more work is required to understand what is changed chemical and biologically when a reactor appears clogged.

Acidogenic fermentation and specifically leach bed reactors for AF as part of a complete treatment system generating refined fatty acids, hydrogen gas, or PHAs has been thoroughly investigated in the laboratory. As a result of this work the technology is at technology readiness level (TRL) six as subsystems have been tested at pilot-scale but a complete and integrated pilot-scale system has not demonstrated commercial viability. There are high risks associated with building such as system due to the challenges discussed in this review. To limit the risk of the transition to technology readiness level seven, a better understanding of acidogenic fermentation in leach bed reactors is desired, specifically at the limits of operation because ideal lab conditions can not be maintained in a larger commercial treatment system.

Chapter 3
Preventing Leach Bed Reactor Clogging

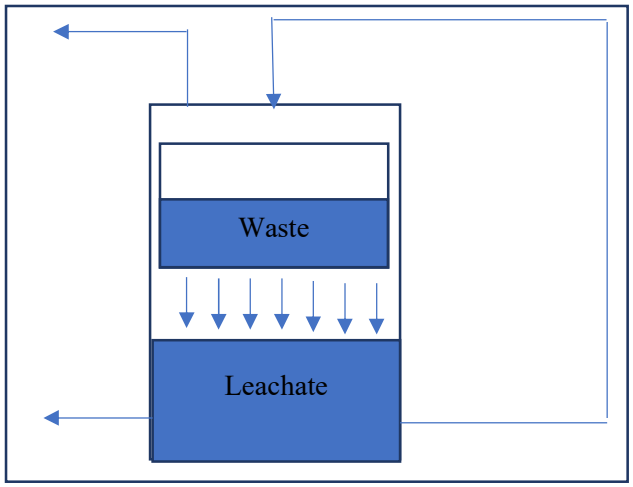

Figure 2: Basic configuration of an anaerobic leach bed reactor for anerobic digestion or fermentation

3.1 Introduction

A slow infiltration rate in a clogged leach bed reactor (LBR) (Figure 2) is correlated with slower hydrolysis, and slower product formation (Saha & Lee, 2020). There are many factors affecting the metabolism in clogged systems which might impede fermentation. Perhaps the most obvious is that a physical blockage might cause slower transport of products out of the container holding the food waste (FW), in this case fermentation is not affected but products are not observed in the leachate as they are trapped in the container. Similarly, when the clogged mesh acts as a filter the suspended solids including unhydrolyzed FW and cell biomass will be retained in the container and, hydrolysed FW will be transported away from the container to the leachate where cell density is low. This process can slow fermentation by creating low concentrations of available substrate in the container and low concentrations of cells outside of the container. Alternatively, the process may also be slowed by the rapid accumulation of inhibitory factors in the FW container. These factors might include a biofilm on the substrate which slows the transport of hydrolysates from the inner cells to the leachate, accumulation of toxic concentrations of fatty acids, or accumulation of hydrogen and carbon dioxide gasses trapped in the matrix of the FW at much higher concentrations than are possible in aqueous solution. The inhibition of

substrate consumption by biomass accumulation was first described mathematically by Contois in 1959 (Contois, 1959). Contois developed a model to describe systems that are inhibited by excess cell density. This built on the Monod model in which higher cell density only increases the rate of substrate consumption. For this reason, Xu et al. suggest that Contois kinetics better represent hydrolysis in leach bed reactors than Monod kinetics (Xu, Karthikeyan, Selvam, & Wong, 2012).

The inhibition caused by fatty acids, particularly medium-chain fatty acids has been observed by Andersen et al. and Grootsholten et al. as well as other authors who have achieved much higher fatty acid yields from fermentative systems which incorporate continuous product removal (Andersen, et al., 2017) (Grootscholten, Steinbusch, Hamelers, & Buisman, 2013; Nagarajan, Jones, Oram, Massanet-Nicolau, & Guwy, 2022). The inhibition of acidogenic fermentation by high hydrogen gas concentration has been observed and modeled by Das et al. finding that when concentrations are below 10% in batch reactor headspace little inhibition is observed; however when hydrogen partial pressures approach 30-50%, fermentation is strongly inhibited (Das, Calay, Chowdhury, Nath, & Eregno, 2020).

Clogging behavior has been observed in several LBR studies and is correlated with lower yields. The behavior has been observed at leachate circulation rates from less than 0.375 L/hr by Hussain et al, to at 13 L/hr by Saha (Hussain, Filiatrault, & Guiot, 2017; Saha S. , 2019). The wide range of flow rates for which clogging is observed suggests that the mechanisms of inhibition may be more complicated than low flow rates causing products to be retained in the container with the FW. The presence of several inhibitory factors as well as the unique biochemical environments of the container and the leachate may cause a clogged reactor to change the metabolic processes which dominate fermentation in a leach bed reactor. The first objective of this study was to test the hypothesis; reactor clogging changes the metabolic processes of fermentation by changing the microbial community and the dominant enzymatic pathways. This would represent a larger shift in reactor behavior than if clogging affected reactor performance only by reducing the rate at which fermentation proceeds and is relevant to the effective modeling of mixed-culture fermentation systems.

There is evidence that recirculation rate, which is the reactor condition most effected by clogging, affects the microbial community. In a 2014 study on the effect of different recirculation rates and solids loading rates on acidogenic fermentation, Xu et al. found that the circulation rate affected the composition of the microbial community (Xu, Karthikeyan, Selvam, & Wong, 2014). Specifically, higher recirculation was found to increase the prevenance of a γ-proteobacterium species while lower recirculation was found to improve the survivability of Lactobacillus panis (Xu, Karthikeyan, Selvam, & Wong, 2014). By testing

the microbial culture in the biofilm and the leachate Xu et al. also determined that the γ-proteobacterium species was only prevalent in leachate.

While many anaerobic and anoxic bacteria share acidogenic pathways, different microbial communities can follow different enzymatic pathways creating different products in different proportions. For example, one of the dominant bacterial genera in the acidogenic FW fermenting LBR used by Xiong et al. was Bifidobacterium, which only produces acids and no hydrogen by the Bifidum pathway (Xiong, Hussain, Lee, & Lee, 2019; Shah, 2011). While Bifidobacteria do produce hydrogen indirectly though cross-feeding with Clostridium species, this pathway favours acetic acid and lactic acid formation over the formation of gases (Xiong, Hussain, Lee, & Lee, 2019). In contrast, the γ-proteobacteria observed exclusively in leachate by Xu et al. were likely related to common anoxic enterobacteria which produce high biogas yields though the production of formate and formate dehydrogenase, the dominance of these bacteria in a leach bed reactor would favour hydrogen production over acid production (Willey, Sherwood, & Woolverton, Chapter 17: Microbial Taxonomy and Evolution of Diversity, 2011, p. 537).

To test if the metabolic processes of fermentation had been changed by clogging, the reactor design incorporated a mechanical disturbance to the FW container to create a reliably, unclogged reactor with identical inputs. If changes in the products or relative proportions of products are observed between the clogged and disturbed reactors and the removal of COD from each system is similar, then the metabolic processes of fermentation are likely different.

The second objective of this study was to test the hypothesis; a clogged leach bed reactor performance is improved by a mechanical disturbance. The mechanical disturbance is like the periodic turning of windrow compost piles rather the constant mixing in a continuously stirred tank reactor (CSTR) or fluidized bed reactor. There is evidence that a periodic disturbance can improve the production of hydrogen gas in LBR. In a 2009 study, a mechanical disturbance to a drum reactor was used in the efficient production of hydrogen gas in an uninoculated dry acidogenic digestor by Wang & Zhao (Wang & Zhao, 2009). The semi-continuous rotating drum reactor was fed with only pulverized 17% solids food waste rotating for 3 minutes, 5 times per hour. At peak efficiency the reactor generated 118 mL H2/ g VS. Outperforming most continuously stirred tank reactors and leach bed reactors without pre-treatment (Wang & Zhao, 2009). Periodic mechanical disturbances can encourage high-yield acidogenic fermentation by disrupting the biofilm without damaging the cell density or requiring a large investment of energy, as hydrogen and acid generation are biologically coupled in most LBR AF systems. The study by Wang and Zhou did not compare the performance of equivalent systems, this study made a direct comparison between identical reactors when one is disturbed and another is not.

The performance of the reactor was measured for both the disturbed and undisturbed conditions, yielding insights into factors which can be considered when designing and optimizing leach bed bioreactors for acidogenic fermentation, anaerobic digestion, or other biological solid waste treatment systems. These waste valorisation systems have demonstrated waste can be treated more effectively with less net energy and less net cost than traditional landfill and composting systems and waste valorization systems can be improved by better understanding bacterial kinetics in high solids environments.

3.2 Methods

3.2.1 Reactor Start-up

The reactor was inoculated with mesophilic anaerobic digestion sludge sampled from the Waterloo wastewater treatment plant (Ontario, Canada). The sludge was heated in a water bath at 90°C for two hours to inactivate methanogens, before being added to food waste consisting of mostly fruit peels and unsold salad from the residence cafeteria at the University of Waterloo (Waterloo, Canada). Food waste was chopped to pieces no longer than 20 mm mixed to homogenize and frozen at 20°C. The reactor was operated anaerobically at 22°C for three months for the anaerobic culture in the reactor to be firmly established. During this start up period food waste was replaced every two weeks. Leachate was removed every two weeks, centrifuged and the pellet was returned to the reactor suspended in 2.5 L of distilled water.

3.2.2 Reactor Design

Two leach bed reactors were used for this study. The first was adapted from the design Xiong et al. (Xiong, Hussain, Lee, & Lee, 2019), adding two modified food waste containers to reducing clogging, one with the FW divided between two vertically stacked shelfs (B) and one closed on both sides which was flipped over once per day (C). Then the design was improved, and a second iteration was built to obtain more insight into the differences in behavior of clogged and disturbed reactors. The first reactor, used in trials labelled A, B, and C in various modified forms, is shown in Figure 3. The reactor is a 7.5 L cylinder constructed from acrylic with a 1 inch (25.4 mm) internal diameter outlet in the bottom and the top, 5 x ½ inch (12.75 mm) inlets for mixing and instrumentation in the body as indicated in Figure 3, and 2 x ½ inch (12.75 mm) outlets for gas collection and emergency pressure release (EPR). The food waste and inoculant sludge were mixed and added to an approximately one litre container. Leachate was recirculated through the FW container using a Masterflex L/S® Standard Digital Drive (P1) (Masterflex, Radnor, PA, USA). Leachate was mixed with a centrifugal pump (P2) (MD-70RLZT Iwaki Co, Tokyo, Japan). pH was controlled with a pH meter (9157BN, Thermo Fisher Scientific, MA, USA) set in the

leachate, and a pH controller (MC122, Milwaukee Instruments, Inc., NC, USA) connected to a peristaltic pump (Masterflex L/S® Analog Variable-Speed Modular Drive, Masterflex, Radnor, PA, USA) for delivering 5 M NaOH (83076-580, Anachemia Canada Inc, ON, Canada). Biogas was collected first in an empty gas bag, then once the bag had been filled excess gas was counted with a silox-filled gas counter (MilliGascounters, Dr.-Ing. RITTER Apparatebau GmbH & Co. KG, Germany). This gas system accurately represented the net production from a batch but not daily gas production. Daily gas production was estimated from changes in concentration and net production values.

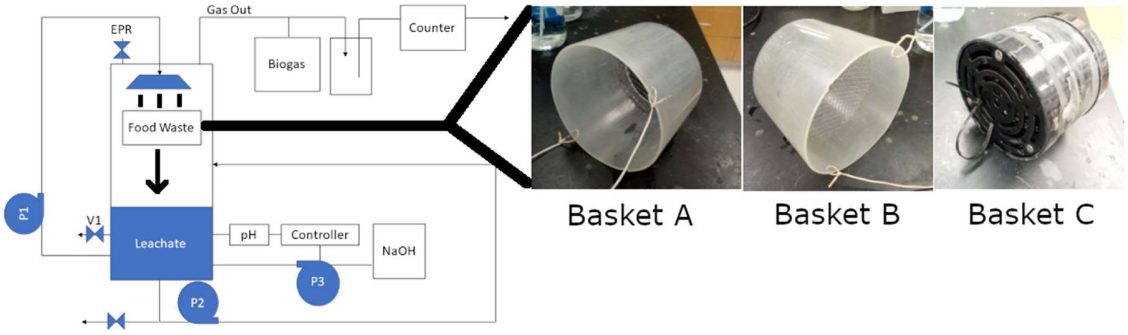

Figure 3: LBR diagram and photos of containers. Container A has mesh only on the bottom, container B has two shelves of mesh, and container C is capped at both ends with floor drain. Abbreviations: EPR (Emergency Pressure Release), V1 (Sampling Valve), P1 (Recirculation pump, peristaltic), P2 (mixing pump, centrifugal), P3 (pH correction pump, peristaltic).

The revised leach bed reactor, which was used in trials labeled D and E was designed to test the if a difference in metabolism or performance exists between a disturbed reactor and clogged reactor while keeping the gas composition in the reactor constant. The design also incorporated a method for sampling the reactor conditions inside the FW container and inside the leachate below the container. As shown in Figure 4, the reactor is a 4 L cylinder constructed from acrylic with seven one half inch (12.7 mm) internal diameter holes in the sides and the top. The three top holes are fitted with a gas tight bearing and mixer, an emergency pressure release valve, and the recirculation outlet. The two holes shown on the left side of the diagram are fitted with a gas outlet connected to a silox-filled gas counter (MilliGascounters, Dr.-Ing. RITTER Apparatebau GmbH & Co. KG, Germany) and a leachate outlet connected to a ½ in (12.7 mm) ball valve for sampling leachate. The two holes shown on the right side of the diagram were fit with a ¼ in (6.35 mm) hose from the top hole to the middle of the container and a ½ in (12.7 mm) hose from the bottom hole to bottom of the reactor. The top hole was attached to a 5 mL syringe on the outside of the reactor by which samples from the container could be collected. The bottom hole was attached to

the inlet of recirculation loop to prevent a settled zone from being formed in the bottom of the reactor. All reactors were custom build by the Civil and Environmental Engineering Department at the University of Waterloo.

In the improved reactor, the food waste and inoculant sludge were mixed and added to an approximately 1.5 L litre container. Leachate was recirculated through the FW container using a Masterflex L/S® Standard Digital Drive peristaltic pump (P1) (Masterflex, Radnor, PA, USA). pH was controlled with a pH meter (9157BN, Thermo Fisher Scientific, MA, USA) set in the leachate, and a pH controller (MC122, Milwaukee Instruments, Inc., NC, USA) connected to a peristaltic pump (Masterflex L/S® Analog Variable-Speed Modular Drive, Masterflex, Radnor, PA, USA) for delivering 3 M NaOH (83076-580, Anachemia Canada Inc, ON, Canada). NaOH was added to the recirculation line at the point of measurement to improve the responsiveness of pH control.

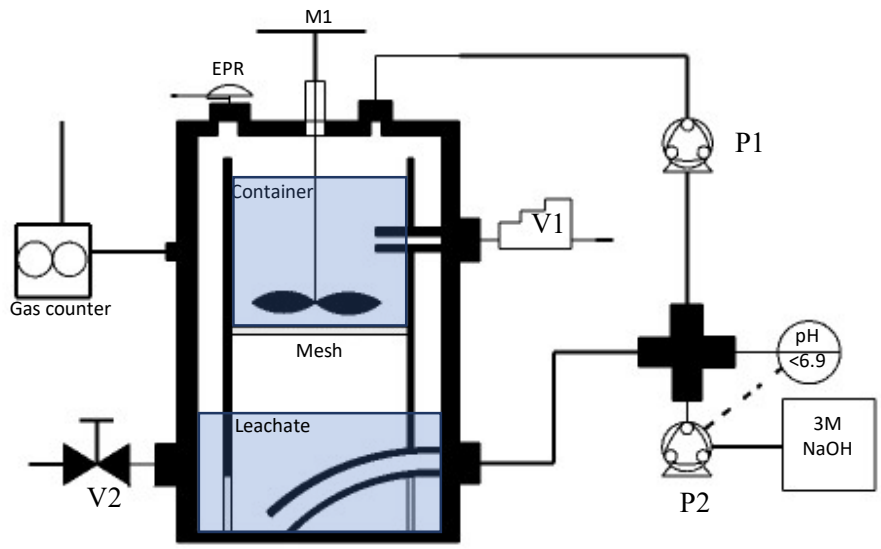

Figure 4: Diagram of the leach bed reactor used in conditions D and E, described in Table 3, with a hand mixer (M1) in the food waste container and sealed with a gas tight bearing used to compress FW into a puck at the bottom of the container to induce clogging in condition D and to mix food waste once per day to prevent clogging in condition E. Samples were taken of the conditions in the container from a ball valve at V1 and of the conditions in the leachate by a syringe at V2. The peristaltic pump P1 is used to pump leachate into the container. The peristaltic pump P2 pumps 3 M NaOH into the recirculation line and is attached to a pH controller set to turn on above pH 6.9.

Gas is released through the gas counter and a 20 PSI emergency pressure release valve (EPR) was installed in the top of the reactor.

3.2.3 Reactor Operation

Reactor operation was similar to the reactor operation of Xiong et al., with the goal of consistent batch AF with moderate recirculation (Xiong, Hussain, Lee, & Lee, 2019). Clogging was observed as this established procedure was followed with the same container as Xiong et al. (container A), clogging was also observed following the established procedure when food waste was divided between two vertically stacked shelfs in the food waste container (container B), clogging was not observed following the established procedure in the unmixed container D in the improved reactor but clogging was induced by compressing food waste against the mesh at the bottom of the container. Clogging was not observed in either of the disturbed (flipped or mixed) reactors (containers C and E respectively). A map of the experiments performed and the containers used is show in Figure 5.

In all trials, food waste and inoculum were mixed, and the container was filled with approximately 1 kg of wet substrate. 2.5 L of distilled water was added to the reactor in conditions A, B, and C, 1 L of distilled water was added to the reactor in conditions D and E. Then the FW container was placed in the top of the reactor and the reactor was sealed. Once the reactor was sealed it was purged with 15 L of nitrogen gas (99.8 %) though the sampling port V1. In the first reactor (conditions A, B, and C) the leachate was then circulated at 30 L/h for one minute out of every six for an average circulation rate of 5 L/h and the leachate was mixed at vigorously for 15 minutes every 2 hours. In the improved reactor (conditions D and E) the leachate was then circulated at 24 L/h for one minute out of every three for an average circulation rate of 8 L/h and leachate was not mixed.

In the first reactor (conditions A, B and C) 15 mL samples of leachate were collected daily from V1. 3 mL gas samples were collected daily from a septum in the gas line between the reactor and gas bag. The reactor was operated for 8 days, unless the daily change in sCOD was over 5% at the last measurement, in which case operation was continued until the daily change in sCOD fell below 5%.

In the improve reactor (conditions D and E) 5 mL leachate samples were collected six times in the first 24 hrs, three were taken over the following 24 hours and samples were taken daily for the following five days. Gas samples were collected 3 times in the first 24 hours then following the same sampling regime as leachate samples.

3.2.4 Experimental Conditions

To test for metabolic differences between clogged and unclogged fermentation the conditions which consistently yielded clogged or unclogged operation in this reactor needed to be established. Two modified containers (B and C) were initially tested against the control container (A) used by Xiong et al. (Xiong, Hussain, Lee, & Lee, 2019). It was established through these first trials that both the control container (A) and the container where food waste was split between two vertically stacked shelves (B) were consistently clogged and the food waste container that was flipped over once per day (C) was consistently not clogged. Having established that a disturbance to the food waste container would consistently prevent clogging the improved reactor was designed to monitor differences between fermentation in clogged and disturbed reactors more closely. A simple and undisturbed container was used for the clogged condition (D) and the same container mixed once daily was used as the disturbed condition (E).

Container “A” was constructed from a 1500 mL polypropylene measuring cup with the handle removed and the bottom replaced with a ¼ inch (6.35 mm) galvanized steel mesh, as shown in Figure 3. Container “B” was constructed identically to container A, with an additional layer of ¼ inch (6.35 mm) galvanized steel mesh placed 2 inches (50.8 mm) above the bottom mesh. Container "C” was constructed from two 4-inch (101.4 mm) floor-drain pipe-ends creating a pipe with ¼ inch (6.35 mm) slots cut in both ends. The total volume of container C was 983 mL (see Figure 3).

Container “A” and container “B” were left undisturbed and reactor operation proceeded as described in section 2.3.3. Container “C” was turned over 180° daily to disturb the biofilm on the food waste. When the container was turned over the reactor was opened to the air, the container was turned over, then the reactor was resealed and purged with 15 L of nitrogen.

In trial D3 and E3 the FW container was 111.12 mm in diameter, and 152 mm tall made of clear polystyrene resin. The bottom was replaced with a ¼ in (6.35 mm) galvanized steel mesh. In condition “D” the container was left undisturbed and reactor operation proceeded as described in section 3.2.3. In condition “E”, the mixer in the reactor was turned 360° daily to disturb the food waste.

To control for the variability in the food waste, trials were run in pairs with a single homogeneous batch of food waste, each pair consisting of one clogged control trial and a trial where the reaction conditions were modified from those used by Xiong et al.. For this reason, six trials were conducted: a clogged trial with container A and the first batch of food waste (trial A1); a trial with container B was conducted with the same food waste and was found to be clogged (trial B1); a clogged trial with container A and the second batch of food waste (trial A2); a trial with container C conducted with the second batch

of food waste which was disturbed and not clogged (trial C2). Finally, the clogged condition D and the disturbed and not clogged condition E were tested with a common batch of food waste, as indicated by trial names D3 and E3. The six trials are shown as arrows in Figure 5.

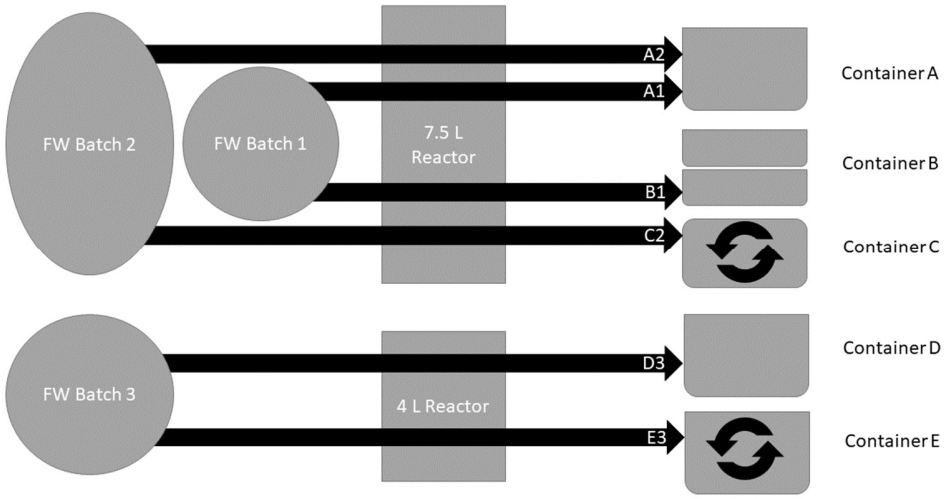

Figure 5: Outline of trials performed to test reactor clogging. Each black arrow represents one trial with each arrow identifying a common batch of food waste collected from the UW cafeteria with the reactor and food waste container used. Containers C and E were both disturbed daily. Container C was disturbed by being removed from the reactor and flipped 180° and container E was disturbed by turning a propeller-type mixer 360°.

For trials 1A and 1B, the inoculum was the pellet from centrifuged leachate, and the food waste from the cafeteria was rich in fats and sugars. The inoculum to substrate ratio (ISR) in trial 1A was 0.17 g VS/g VS and the ISR in trial 2A was 0.09 g VS/g VS. The drop in ISR is due to low inoculum recovery from leachate in trial 1A. To better conserve culture in the reactor and stabilize the ISR between subsequent batches, a portion of the used food waste was used as inoculum for trial 2A and 2C. In these trials 200 g of wet spent food waste was mixed with 800 g of new food waste before it was loaded into the reactor. The ISR in trial 2A was 0.24 g VS/g VS and the ISR of trial 2C was 0.12 g VS/g. The change in ISR is due to a change in moisture content of the spent food waste between trials. Digested food was also used as the inoculum in trial D3 and E3, for these trials the solids content was standardized and masses of food waste and inoculum were adjusted to achieve a total mass of 1kg substrate and an ISR 0.2.

Table 3: Experimental conditions used in the study of clogging in leach bed reactor

	Food Waste Batch	Starting COD (g)	ISR	Inoculum	Gas Purge	Container Design
A1 (Clogged)	Batch 1	390.1	0.17	Leachate Solids	At Start-up	A
B1 (Clogged)	Batch 1	395.4	0.09	Leachate Solids	At Start-up	B
A2 (Clogged)	Batch 2	284.7	0.24	Fermented FW	At Start-up	A
C2 (Disturbed)	Batch 2	165.5	0.12	Fermented FW	Daily	C
D3 (Clogged)	Batch 3	116.4	0.2	Fermented FW	At Start-up	D
E3 (Disturbed)	Batch 3	104.0	0.2	Fermented FW	At Start-up	E

3.2.5 Gas Composition

Gas production, specifically hydrogen and carbon dioxide production are strong indicators of metabolic actively and their relative proportion can indicate what bacteria are dominant in a reactor. Gas composition was measured to identify the concentrations of hydrogen, methane, carbon dioxide, and nitrogen and the change in concentration was compared with the total biogas production by the reactor to determine specific gas production rates. In the improved reactor (trials D and E), oxygen concentration in the reactor was measured as well. Two 0.5 mL samples were taken using a gas-tight syringe to determine the H_2, N_2 O_2 partial pressures in the reactor. Each 0.5 mL sample was injected into a gas chromatographer equipped with a thermal conductivity detector (GC-TCD) purged with argon at 50°C ramping 110°C over 8 minutes, sampling at 5 Hz, using an S.S. Molecular Sieve 5A Packed Column (8600-PK2B**,** SRI Instruments, CA, USA). The area was compared to 5% H_2 calibration gas and air to determine the partial pressure of H_2, O_2 and N_2. The same procedure was followed using two 1 mL syringe injections for CH_2, CO_2, and N_2, for this test the GC-TCD analyser (Model 310, SRI Instruments, CA, USA) was purged with helium holding at 40**°**C for 2 minutes, sampling at 5 Hz, using a Porapak Q 80/100 2 m, 2 mmID Packed Column (Restek, PA, USA). A 2-point calibration curve was used to calculate the partial pressure of N_2, CH_4, and CO_2. The calibration curve was prepared with 20% CO_2, 20% N_2, 60% CH_4 calibration gas and pure CH_2, CO_2, and N_2. The total of the partial pressure was then normalized to 1 atm.

Gas production was calculated by measuring the change in partial pressure (P) multiplied by the volume of the head space in the reactor ($V_{head\ space}$) and adding the average gas composition for a period multiplied by the amount of gas produced at the gas counter during that period ($\Delta V_{counter}$) as shown in Equation 9.

$$Gas\ Produced = (P_{t+1} - P_t)V_{head\ space} + \frac{(P_{t+1}-P_t)}{2}(\Delta V_{counter}) \quad (9)$$

3.2.6 Leachate

Leachate and fluid from the food waste container was tested for range of water quality metrics to monitor the chemical conditions in the reactors over the course of each batch. This monitored product formation, the disintegration and destruction of substrate, and product yield. These measures indicated when fermentation being inhibited, and when different pathways lead to different products. In the first reactor with a working volume of 3.5 L (containers A, B, and C), leachate samples were tested for total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VSS). This was not repeated in the improved reactor with a working volume of 2 L (containers D and E) as there was not sufficient reaction volume to remove these samples. All samples were tested for soluble chemical oxygen demand (sCOD), and VFA concentrations. Solids in the leachate were measured using duplicates of 2 mL samples, according to APHA Standard Methods (2540B, 2540D, and 2540E , APHA, 2002). sCOD measurements were made using 1/40 diluted leachate, vacuum filtered though 0.45 µm nylon filters (15945-27, Antylia Scientific, IL, USA). tCOD measurements of leachate used unfiltered 1/40 diluted leachate. All COD measurements were conducted in duplicate using HACH high range colorimetric COD determination method using HACH high range prepared vials, a HACH 3900 spectrometer and a HACH DRB200 digestor (HACH, ON, Canada) according to (ASTM D1252-95, 2006). VFA concentrations were determined from 2.5 mL samples diluted in 50ml of total volume and filtered through 0.2 µm nylon filters (UZ-32816-02, Antylia Scientific, IL, USA). The samples were acidified with 10 µL of concentrated phosphoric acid and frozen before analysis with an HP 5890 Series 2 gas chromatographer with a flame ionization detector (GC-FID) (Hewlett Packard, CA, USA) equipped with a capillary column (30 m × 0.53 mm × 0.5 µm PAG, Supelco, Bellefonte, PA). The injector temperature was maintained at 220°C and the detector temperature was maintained at 280°C. The oven temperature for VFA analysis was maintained at 150°C for 2 minutes, then increased to 190°C at a slope of 4°C/minute and maintained at 190°C for 3 minutes. Ethanol concentrations were determined from the same samples as VFA concentrations using the same equipment using the same detector and injector temperatures and gas flow

rates. For the ethanol analysis method, the oven temperature for VFA analysis was maintained at 40°C for 3 minutes, then increased to 60°C at a slope of 6°C/minute and maintained at 60°C for 6 minutes.

3.2.7 Food Waste Solids

Samples of the food waste solids and inoculum which were retained in the FW container were tested before and after fermentation. This was used to calculate the hydrolysis and acid yield as well as how effective clogged and disturbed reactors are at treating waste. 50 g samples blended into 500 mL of water were used for tCOD measurements and 5 g samples were used for determining total and volatile solids. All COD measurements were conducted in duplicate using HACH high range colorimetric COD determination method using HACH high range prepared vials, a HACH 3900 spectrometer and a HACH DRB200 digestor (HACH, ON, Canada) according to ASTM D1252-95 (ASTM D1252-95, 2006). Total and volatile solids were determined according to APHA Standard Methods analysis for thick media 2540 G (APHA).

3.2.8 Cell Growth Measurement in Heterogeneous Media

The partially hydrolysed and suspended food waste creates a challenge for common measures of cell density in a fermenter. Measures of turbidity such as volatile suspended solids and OD600 similarly cannot distinguish between turbidity caused by undigested food waste and turbidity caused by microbial growth. These measures provide a sum of these two turbidity sources and as microbial activity increases it converts FW to soluble metabolites, therefore they cannot be separated by fixed baseline correction. To overcome this challenge the cell density was estimated in each sample by measuring the cell growth over the course of four hours, cell growth was measured by recording the change in OD600 as described in Prescott's Microbiology 8th ed. (Willey, Sherwood, & Woolverton, Chapter 7: Microbial Growth, 2011, p. 171). Over the course of 4 hours the live cell culture consumed the sCOD in the vial and demonstrated cell growth according to the Monod model shown in Equation 10.

$$\mu = \mu_{max} \frac{S}{S+K_s} X \quad (10)$$

Assuming the cell growth observed in the sample vial is aerobic, all the measured sCOD will be oxidized. Therefore, substrate concentration (sCOD) is high at the start of the reaction the equation can be simplified to Equation 11.

$$\mu = \mu_{max} X \quad (11)$$

Since the measured rate is directly proportional to the cell density when fermentation starts, this measurable parameter was used to assess changes in cell density in the fermenter. One 2 mL sample of

leachate was taken from the leachate and the container six times in the first twenty-four hours then three times in the following twenty-four hours followed by sampling once per day for five more days. The baseline OD600 was measured using the HACH 3900 single wavelength method using deionized water as a blank. The absorbance was measured over the course of four hours and the rate in the first half and hour was interpolated.

3.3 Results and Discussion

3.3.1 Solubilization of FW and treatment efficiency

Clogging is most easily identified by the retention of solids in the FW container and the infiltration rate though the FW container approaching zero. Since infiltration rate could not be accurately measured in the systems studied and clogging has been reported at a wide range of infiltration rates this study uses the retention solids in the FW container and overflow of leachate from the top of the container as indicators that a reactor is clogged. Solids are removed from the FW container by two processes – the disintegration of FW where food waste is broken up into small insoluble chunks which can be washed though the mesh at the bottom of the container this will appear as an increase in turbidity in the leachate. The second process is the dissolving of FW where food waste is hydrolysed to soluble products which will appear as an increase in sCOD in the leachate. While disintegration of FW improves the reactor's ability to treat waste by reducing the mass of FW which will need to be composted after fermentation is complete, only dissolved food waste can be converted to fatty acids and biogas.

The clogged condition B1 which added a shelf into the middle of the food waste basket, showed less VS removal from the leachate over the corresponding clogged control condition A1, and a very low VFA/VS ratio suggests that fermentation in B1 was not complete (Table 5). The container with the shelf (B1) also showed the slowest increase in turbidity, as indicated by VS in the leachate in Figure 6, of any reactor suggesting that the shelf made clogging worse. When the FW basket was turned over 180° (C2), the reactor opened, and the gas purged a dramatic increase in VS in the leachate over its corresponding clogged condition (A2) was observed. The disturbed reactor (C2) also showed much higher VS removal from the system at the end of the batch, shown in Table 5. This is likely due to higher gas production discussed in section 3.3.4. When the FW container in the improved reactor was mixed daily by a mechanical mixer (E3) there was an increase in turbidity and a small increase in total VS removal over the clogged trial with the same reactor (D3). This increase is also explained by the higher gas production in E3 which is discussed further in section 3.3.4.

Table 4: Summary of the percent change in performance metrics for clogging interventions

	Difference VS removal from FW container (%)	Difference in VS removal FW container and leachate (%)
A1 – B1	-4	-21
A2 - C2	-8	+84
D3 – E3	+12	+5

VS in the leachate increased dramatically in the reactor with the flipped FW container C2 after the first day. The VS in the leachate fell as VS was consumed by bacteria for VFA and H_2 production (Figure 6).

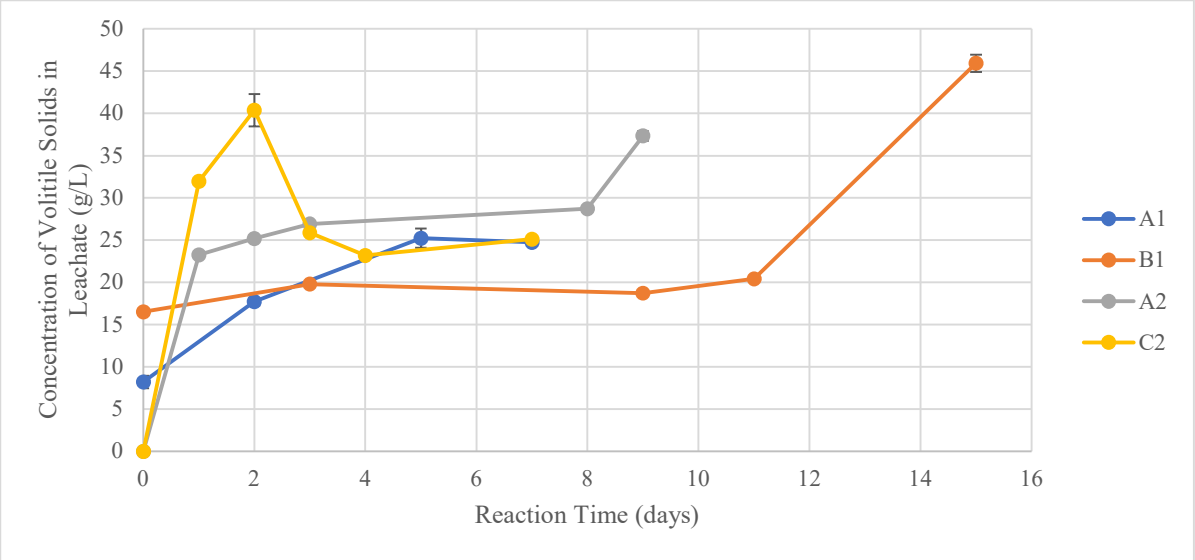

Figure 6: VS concentration in leachate over the reaction time in the reactor used in the first four survey tests, described in Figure 3. Batch A1 used a simple 1 L container with single layer of mesh and high COD food waste. B1 used the same food waste in a 1 L container with 2 layers of mesh separated by 2 inches filled with food waste. A2 used the same simple container used in A1 with lower COD food waste. C2 used a container which unlike container A and B was closed at both ends with mesh and was turned over 180° once per day.

Examining the VS concentration in the leachate in the first three clogged trials (A1, B1, and A2), VS only increased in the leachate after fermentation was nearly complete. When an additional shelf was added in condition B1 the time to reach 35 g/L VS in leachate was doubled.

The trials in the improved reactor (D and E) tested if the disintegration of FW in C2 was dramatically increased by the disturbance to the container or by the changing gas composition. Hydrolysis can be improved by maintaining a low hydrogen partial pressure, so the purging of gas from reactor C2 may have increased the hydrolysis rate (Cazier, Trably, Steyer, & Escudie, 2015). Since gas was not purged from the improved reactor the differences in disintegration and hydrolysis indicate the difference between clogged and unclogged fermentation.

While the large samples required for VS determination could not be taken from the smaller reactor, the turbidity and sCOD were measured regularly to identify when the solids in the container were disintegrated and dissolved. Very little difference in turbidity was observed in the leachate the first two days as shown in Figure 7. This indicates that the turbidity observed at this reaction time was easily suspended/dissolved FW. After two days, the turbidity in the leachate of the disturbed reactor was higher suggesting that the disturbance improved the transport of solids from the container into the leachate.

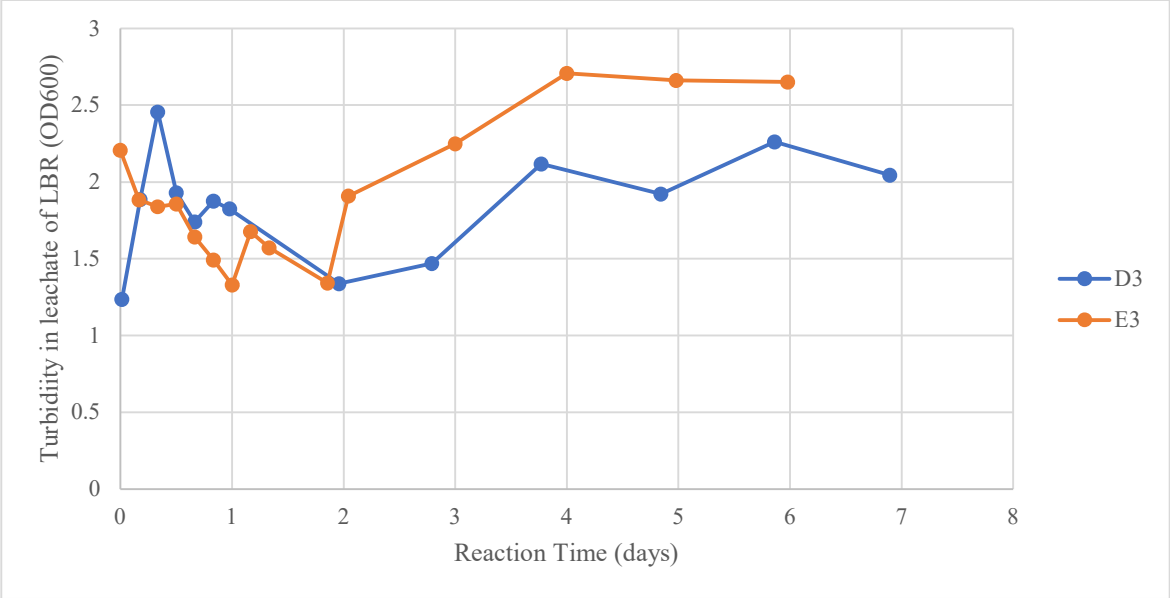

Figure 7: Turbidity (OD600) in the leachate of the improved reactor used for the last two batches. The reactor added a mixer and FW container sampling port as shown in Figure 4. Turbidity was comparable with volatile suspended solids which closely followed volatile solids in all tests. D3 represents the batch where food waste was pressed to induce clogging, the clog at the mesh was fully hydrolysed after 4 days. E3 represents the batch where food waste was mixed every 24 hours and no clogging was observed. Both batches used the same low COD food waste.

3.3.2 VFA Production

Fatty acid production was measured in every trial and no trial showed a significant difference in the production rate or final concentration. When the VFA concentration and production efficiency are considered in context with other reactor conditions some insights are apparent. The total VFA concentration in the leachate of all trials started below 5 gCODeq/L. The rate of total VFA production in the first reactor (trials A1, B1, A2, C2) was approximately 2.5 gCODeq/L/day as shown in Figure 8, while the starting concentration in the container with the shelf (B1) was notably higher and the lag phase in B1 was longer. From these tests there appears to be no impact of clogging on VFA production. The leachate concentrations in the improved reactor (D3 and E3) measured at V2 confirm this. Both showed a lag time of approximately two days followed by a slow increase in the VFA production rate to 5 gCOD/L/day until seven days when production slows as shown in Figure 9. This suggests that the VFA production is not improved by a disturbance to the FW container.

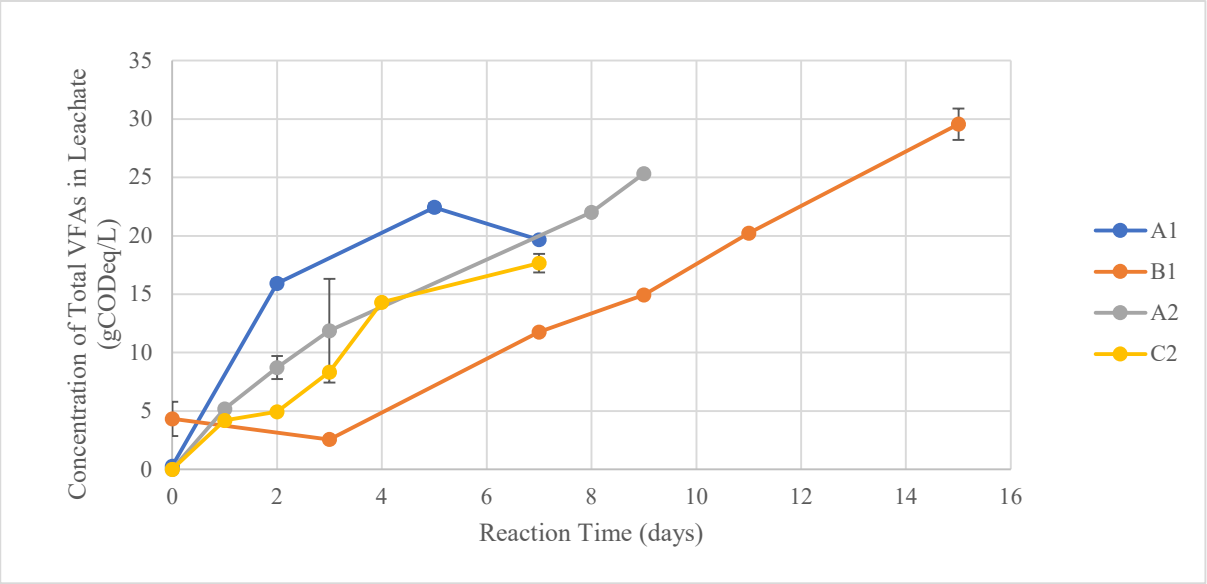

Figure 8: Total VFA concentration in leachate over the reaction time in the reactor used in the first four survey tests, described in Figure 3. Batch A1 used a simple 1 L container with single layer of mesh and high COD food waste. B1 used the same food waste in a 1 L container with 2 layers of mesh separated by 2 inches filled with food waste. A2 used the same simple container used in A1 with lower COD food waste. C2 used a container which unlike container A and B was closed at both ends with mesh and was flipped 180° once per day.

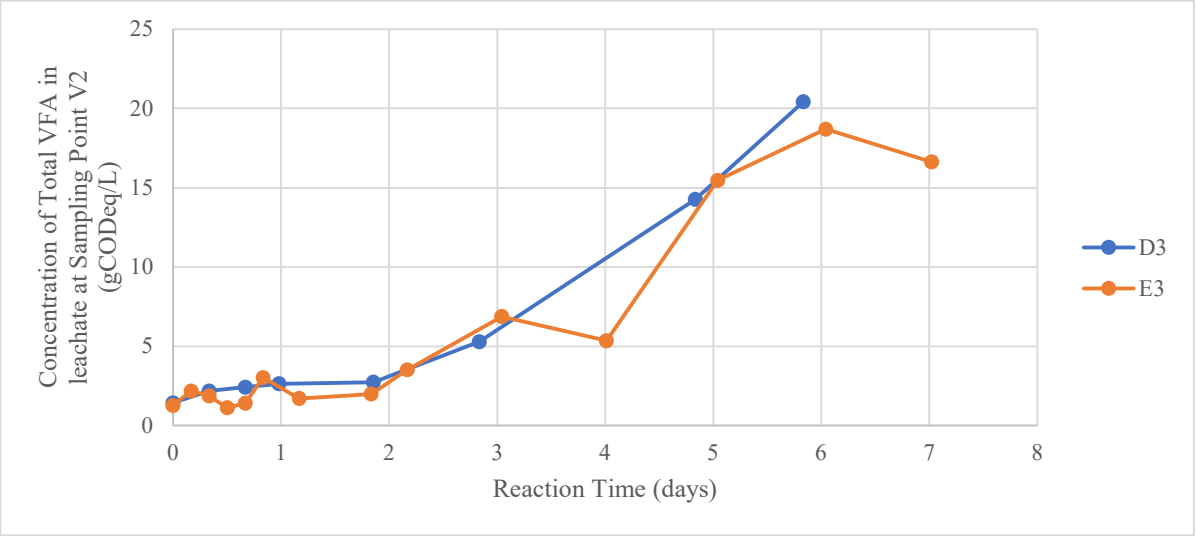

Figure 9: Concentration of total VFA in the leachate of the improved reactor used for the last two batches at sampling point V2, below the FW container. The reactor added a mixer and FW container sampling port as shown in Figure 4. D3 represents the batch where food waste was pressed to induce clogging, the clog at the mesh was fully hydrolysed after 4 days. E3 represents the batch where food waste was mixed every 24 hours and no clogging was observed. Both batches used the same low COD food waste.

VFA concentrations of approximately 20 gCODeq/L were generated in both clogged and disturbed tests in the improved reactor (D3 and E3 respectively), a maximum of 20.4 gCODeq/L for D3 and a maximum of 18.69 gCODeq/L for E3 as shown in Figure 9, Figure 10, and Figure 11. In both reactors significant acetic acid formation started after 1.8 days and the rate of acid production increased after the hydrogen concentration in the fermenter decreased, around four days. While the clogged reactor conserved and generated significantly more ethanol than the disturbed reactor, no significant difference in acid production was observed. We see that in both fermenters approximately 2 gCODeq/L of VFA was present in leachate until 1.9 days and then increased at a steady 5 gCODeq/L/day as shown in Figure 9. These findings are similar to those of Browne at al. in that although disintegration of FW can be improved by higher flow rates or a disturbance this does not improve VFA formation (Browne, Allen, & Murphy, 2013). Browne et al. tested a leach bed reactor fed with food waste with two flow rates, 0.7 L/h which is similar to clogged infiltration rates and 4.25 L/h similar to unclogged infiltration rates. At the slow infiltration rate food waste was dissolved at the same rate it was converted to products, in this case biogas while when the fast infiltration rate food waste was disintegrated faster than it could be converted to VFAs (Browne, Allen, & Murphy, 2013). The fact that VFA production remains slow when the barrier of

FW hydrolysis has been removed suggests that VFA production is subject to product inhibition by acids as well as hydrogen partial pressure.

A difference between clogged and disturbed reactors was observed in the production of the ethanol in the improved reactor. Both clogged and disturbed trials produced ethanol upon start up, likely due to a sugar-rich FW. In the disturbed reactor (E3), that ethanol was consumed immediately while a concentration of 5.3 gCODeq/L of ethanol was maintained in the leachate of the clogged reactor (D3), shown in Figure 12. This change suggests a difference in the microbial communities of the disturbed and clogged trials. The difference is even more striking comparing the ethanol concentration and VFA concentration in the clogged container D3 at V1 and in the clogged leachate D3 at V2 shown in Figure 10. In the clogged container where FW is retained VFA production is high reaching 12.7 gCODeq/L after only 3 days while only 5.2 gCODeq/L of VFA is present in the leachate below the container. At the same time the ethanol concentration in the leachate reached 6.9 gCODeq/L while no ethanol was present in clogged FW container. This suggests that different microbial communities are dominant in the clogged container than I the leachate. If this is true it is very likely that when there is more transport between the leachate and container that the microbial community will be changed.

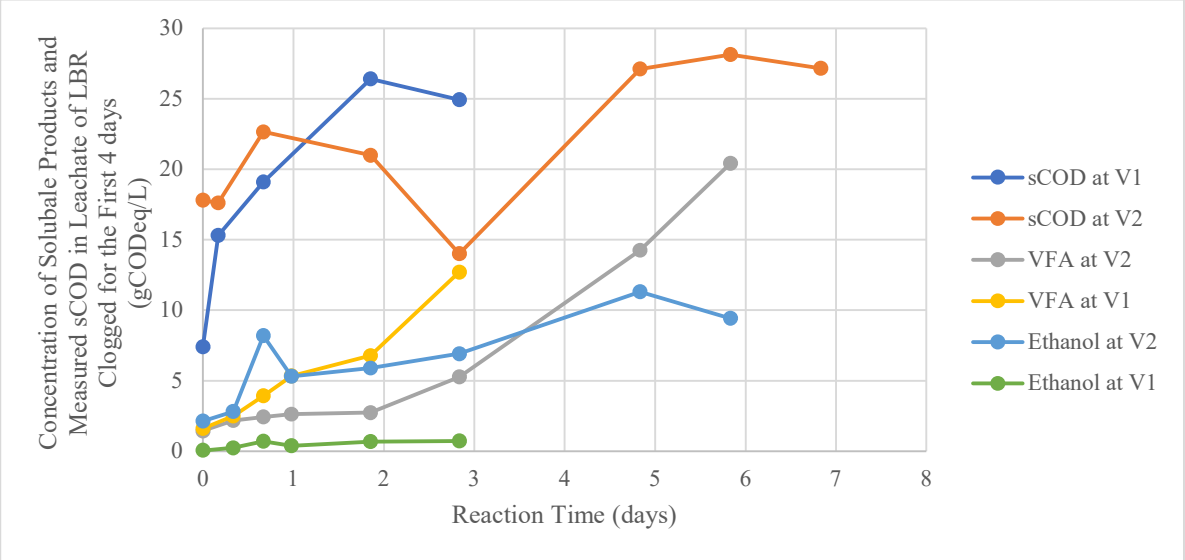

Figure 10: Concentration of total VFA, ethanol and sCOD in leachate of batch D3. VFA and ethanol concentrations were determined by GC-FID and sCOD was determined by colorimetric HACH test. This batch was deliberately clogged and remained clogged with a low infiltration rate for the first four days of reaction. During the first four days samples were taken from both the liquid trapped in the container (V1) and the liquid which had leached though the container (V2). A diagram of these sampling point is shown in Figure 4.

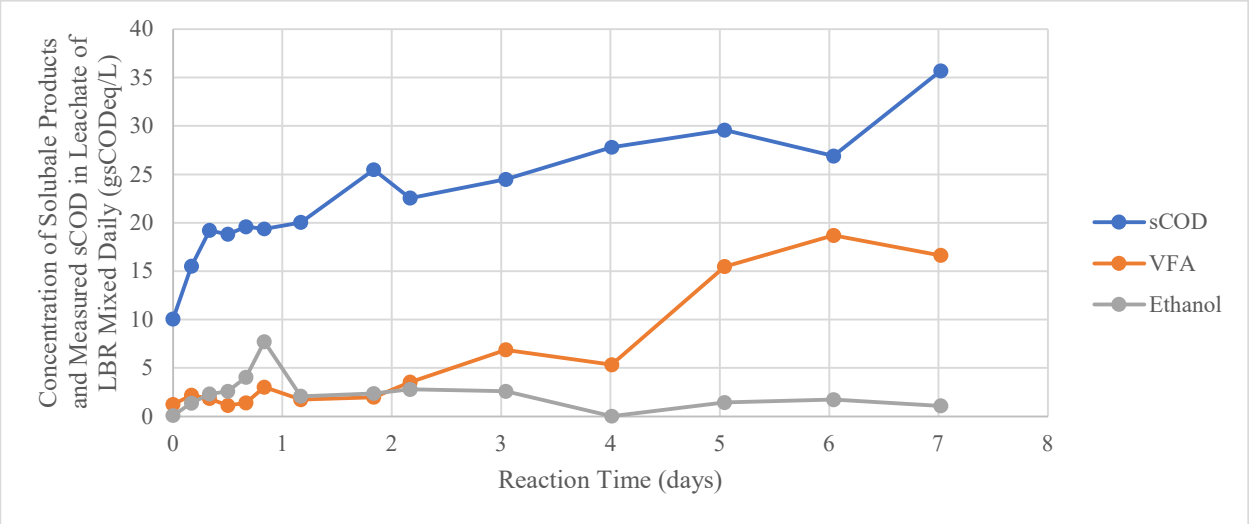

Figure 11: Concentration of total VFA, ethanol and sCOD in leachate of batch E3. VFA and ethanol concentrations were determined by GC-FID and sCOD was determined by colorimetric HACH test. This batch was mixed once per day in the container by a mixer and no clogging was observed.

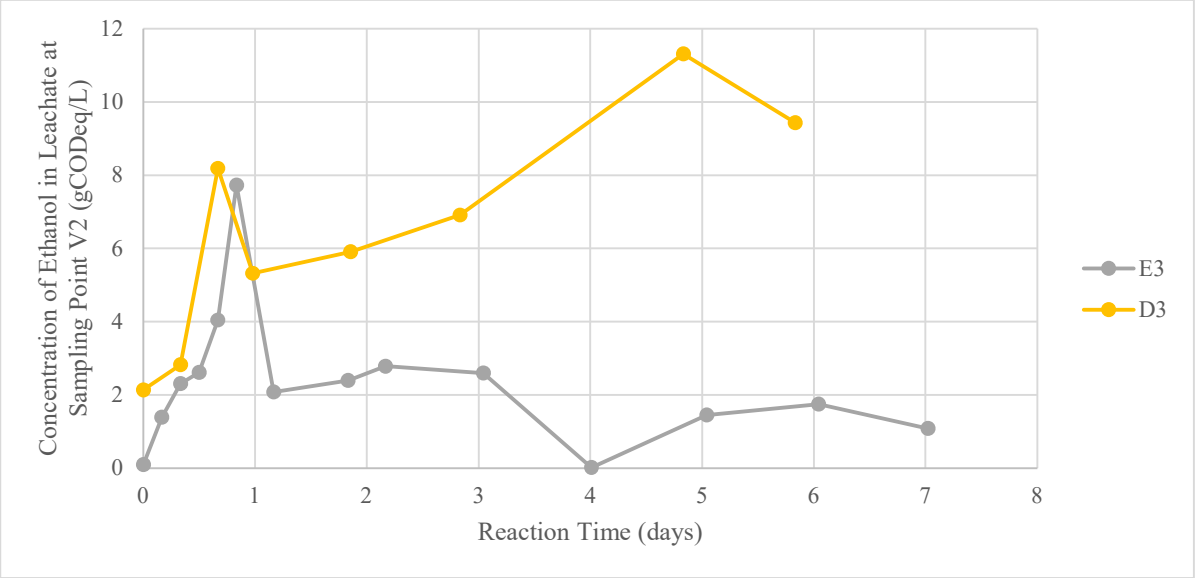

Figure 12: Concentration in the leachate of the improved reactor used for the last two batches at sampling point V2, below the FW container. The reactor added a mixer and FW container sampling port as shown in Figure 4. D3 represents the batch where food waste was pressed to induce clogging, the clog at the mesh was fully hydrolysed after 4 days. E3 represents the batch

where food waste was mixed every 24 hours and no clogging was observed. Both batches used the same low COD food waste.

3.3.3 Hydrolysis

The tCOD of the food waste that was fermented in the first reactor (A1, B1, A2 and C2) was higher than the tCOD of the food waste that was fermented in the improved reactor (D3 and E3). D3 and E3 started with a tCOD of 58.17 gCOD/L and 52.00 g/COD/L respectively compared to 81.33 gCOD/L for A2, 47.14 gCOD/L for C2, 111.46 gCOD/L for A1 and 122.97 gCOD/L for B1. To consider the hydrolysis yield in these diverse starting conditions, the sCOD in leachate was divided by the starting tCOD in each batch and plotted over the course of the batch as shown in Figure 13. Figure 13 shows the lowest rate of hydrolysis and lowest percent hydrolysis was observed in the clogged reactor A2. In the corresponding unclogged reactor with the flipped container (C2), the total COD of the FW waste was converted to soluble COD much faster. This trend of a disturbance to the reactor improving hydrolysis yield over a clogged reactor was replicated in the trials D3 and E3. Reactor D3 was clogged from the first day of reaction to the fourth day. During this time the percent of FW hydrolysed in the leachate appeared to fall. These results do not consider the sCOD loss due to gas formation, but since 2.6 g COD was lost to gas formation in the clogged reactor (D3) and 5.8 g COD was lost in the mixed reactor (E3) this does not explain an apparent drop in dissolved COD. This drop in sCOD was likely the capture of undissolved substrate in the clogged container allowing sCOD to be consumed faster than tCOD was hydrolysed in this area. The overall hydrolysis rate in D3 was less affected as shown by the hydrolysis yield of D3 converging with the hydrolysis yield in the mixed reactor E3 after the clog at the mesh is hydrolysed. The mixed reactor E3 achieved the highest hydrolysis yield in all tests, approaching 70% hydrolysis. The hydrolysis rate appeared to be improved by approximately 20% when the FW container was disturbed (mixed or flipped). While this increase in hydrolysis rate did not appear to increase the rate of VFA production substantially it does suggest that a small disturbance to the container can improve LBRs ability to treat organic waste.

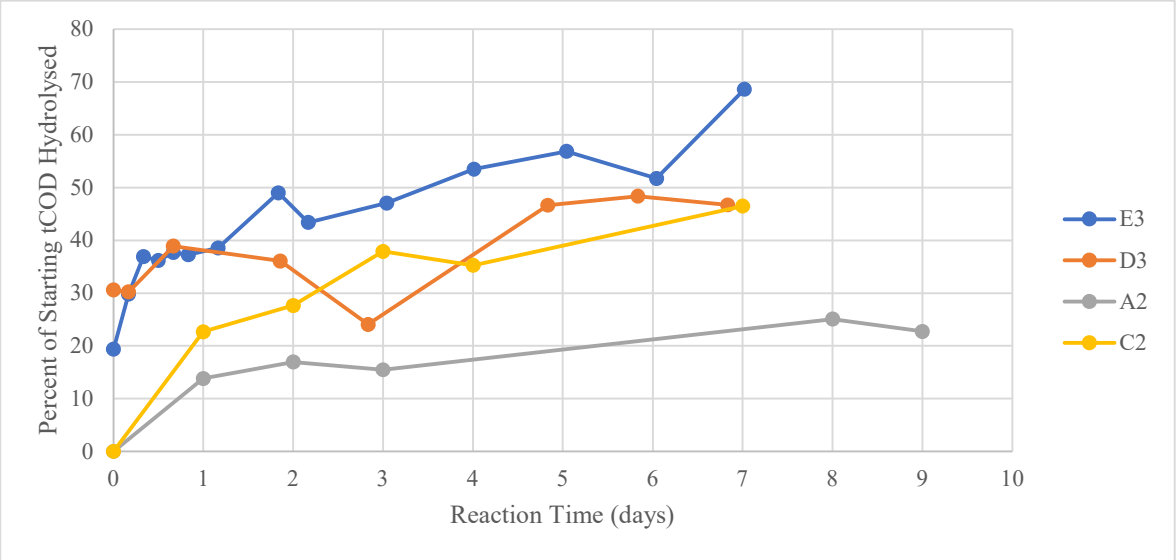

Figure 13: Hydrolysis yield as % of starting tCOD in the leachate of the improved reactor used for the disturbed batches and their control batches measured in the leachate below the FW container. A2 used a simple 1 L container with a ¼" mesh on the bottom with higher COD food waste. C2 used the same FW in a container which was closed at both ends with mesh and was flipped over 180° once per day. D3 represents the batch where food waste was pressed to induce clogging in a lower COD FW, the clog at the mesh was fully hydrolysed after 4 days. E3 represents the batch where food waste was mixed every 24 hours and no clogging was observed. Both batches used the same low COD food waste.

3.3.4 Gas Composition

In the clogged trials A1 and B1 where a static FW container had one or two layers of mesh respectively, gas production was measured. In both reactors most of the gas generated was generated in the first two days with gas production trailing off and hydrogen being replaced slowly by CO_2. In both cases hydrogen production stopped at a pressure of 0.1 atm. Since gas production and hydrogen consumption continued after this point, it is possible that inhibition of the hydrogen producing bacteria was being observed above this pressure. This is below the 0.3 atm that Das et al. observed hydrogen inhibition, however since these containers are clogged it is possible that bubbles of hydrogen trapped in the FW matrix are increasing the local hydrogen pressure in the container above the 0.3 atm threshold or it is also possible that fermentation has been dominated by non-gas producing bacteria such as Bifidobacterium (Das, Calay, Chowdhury, Nath, & Eregno, 2020). In either case changes in the metabolism of the clogged reactor are affecting gas production.

In the C2 trial where the reactor was opened and the FW container was flipped 180° each day, CO_2 and H_2 production was high for the first 2 days then fell. The C2 trial generated 490 mL of H_2 and 6,060 mL of CO_2 over four days, with no gas production after four days of fermentation. In comparison, the clogged A2 trial with the same food waste but no gas purging produced 590 mL of H_2 and 2,570 mL of CO_2 over 9 days with gas production still taking place after 8 days. When the reactor was opened and purged daily it is possible that a low concentration of oxygen remained present in the head space. This could have aerobically oxidized a small amount of food waste causing the higher CO_2 production observed in the C2 trial; however H_2 production was not adversely affected. Finally, the halt of gas production observed in the C2 trial after 4 days suggests that the C2 condition reduced the batch time to 4 days for hydrogen production. The difference in gas production might suggest a difference in the microbial community of the clogged A2 and disturbed C2 trials, however with the substantially different head space composition it is impossible to draw conclusions from this data.

Since the improved reactor used in trials D3 and E3 is substantially smaller, the concentrations of gasses in the reactor were much more responsive to changes in concentration than trials A1, B1, A2 and C2, making it more valuable for evaluating differences between clogged and disturbed reactor metabolism. When the improved reactor was mixed once per day it maintained lower concentrations of CO_2 and higher concentrations of H_2 than when it was deliberately clogged as shown in Figure 14 and Figure 15. This difference supports the hypothesis that different fermentations occurred in the clogged and disturbed reactor as well as the leachate and container of the clogged reactor. Specifically, that ethanol production was dominant in the leachate portion of clogged reactor as ethanol generating fermentation does not generate hydrogen. Oxygen was present in both reactors at approximately 0.1 atm at the start of the reaction time and was quickly consumed in both, higher concentration of oxygen was observed in the clogged D3 trial as hydrogen consumption and low gas production rates induced a larger negative pressure inside the headspace. The low hydrogen production in the clogged FW container when considered with the high VFA production rate in clogged FW container may suggest that the clogged container favours fermentation by Bifidobacteria or a consortium of Bifidobacterium and Clostrium species, similar to the cultures observed by Xiong et al (Xiong, Hussain, Lee, & Lee, 2019).

While the mixed reactor (E3) showed only a 4% increase in partial pressure over the clogged D3, the mixed reactor produced 5458 mL of gas measured by the counter while the clogged reactor produced only 1492 mL. Examining the production rates of these two reactors, shown in Figure 16 and Figure 17, it is clear that the mixed reactor produced similar amounts of CO_2 in the initial generation of ethanol and aerobic cell growth, but the disturbed reactor produced dramatically more hydrogen and more CO_2 during the acidogenic fermentation phase. This finding suggests that a small daily disturbance can generate far

more hydrogen in acidogenic fermenters. This is consistent with the findings of Wang et al. in their rotating drum reactor (Wang & Zhao, 2009). The increase in hydrogen production in the absence of higher VFA production suggest that an Enterobacterium species might be dominant in the leachate of the disturbed reactor similar to the γ-proteobacterium observed in the leachate of the LBRs tested by Xu et al.(Xu, Karthikeyen, Selvam, & Wong, 2014).

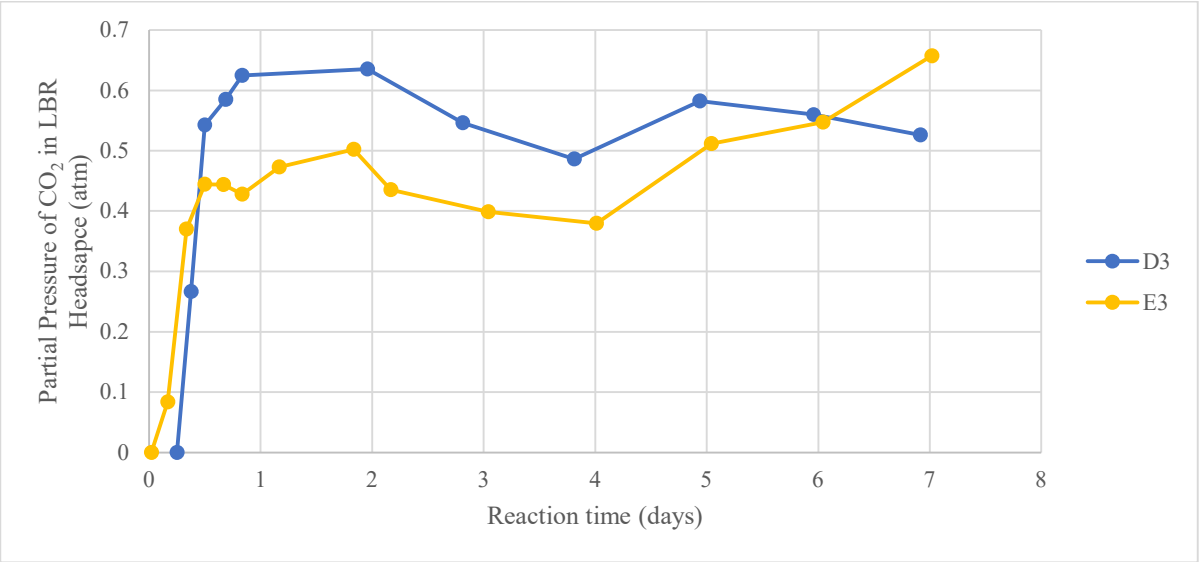

Figure 14: Partial pressure of CO_2 determined by GC-TCD over the course of the final two batches. Both batches were fermented in the reactor that included the mixer and FW container sampler (described in Figure 4). D3 represents the batch where food waste was pressed to induce clogging, the clog at the mesh was fully hydrolysed after 4 days. E3 represents the batch where food waste was mixed every 24 hours and no clogging was observed. Both batches used the same low COD food waste.

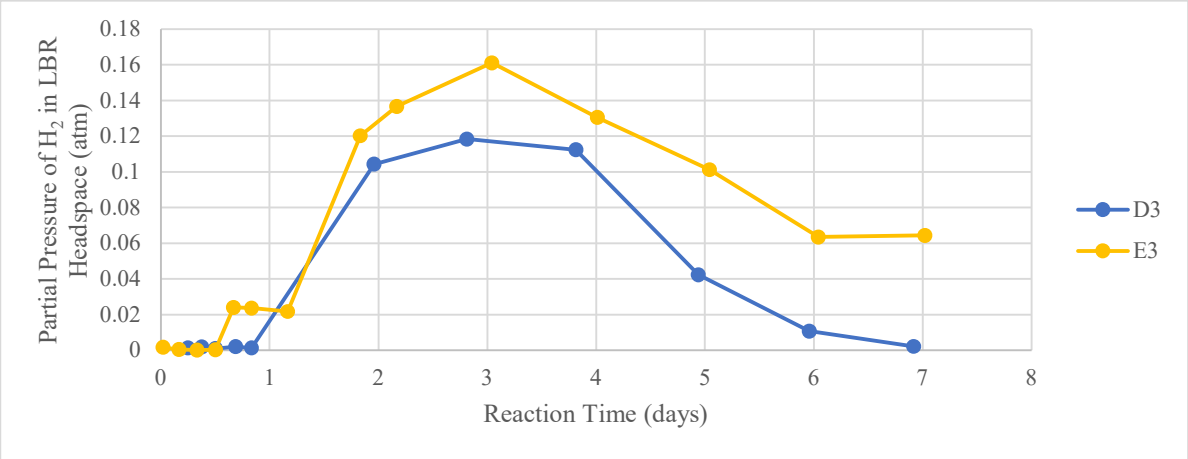

Figure 15: Partial pressure of H_2 determined by GC-TCD over the course of the final two batches. Both batches were fermented in the reactor with the mixer and FW container sampler added which is described in Figure 3. D3 is the batch where food waste was pressed to induce clogging, the clog at the mesh was fully hydrolysed after 4 days. E3 is the batch where food waste was mixed every 24 hours and no clogging was observed. Both batches used the same low COD food waste.

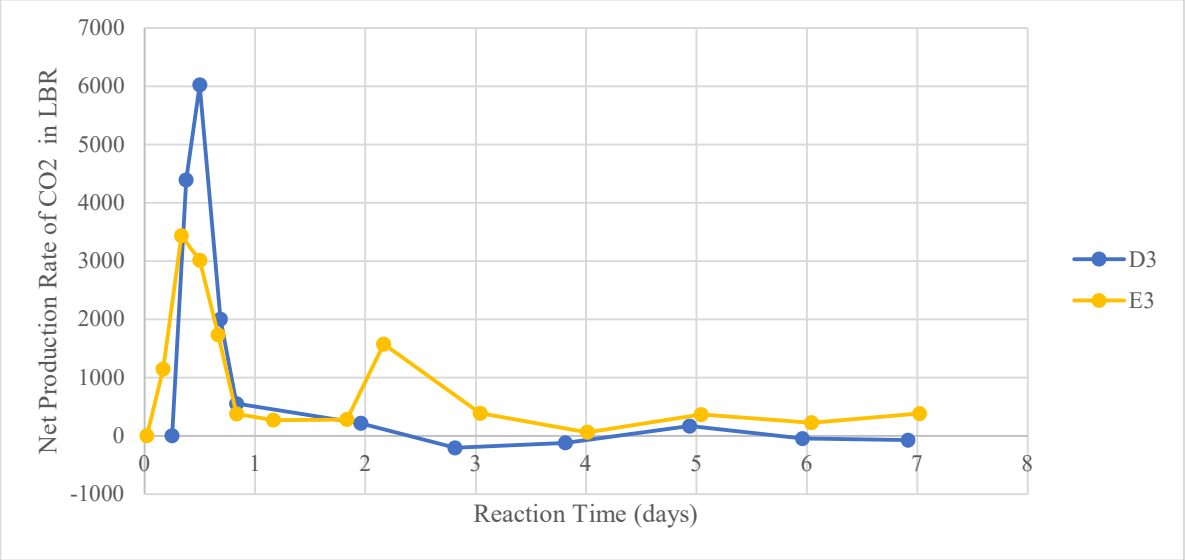

Figure 16: Net production rate of CO_2 determined by GC-TCD and gas counter over the course of the final two batches. Both batches were fermented in the reactor that included the mixer and FW container sampler (described in Figure 4). D3 represents the batch where food waste was pressed to induce clogging, the clog at the mesh was fully hydrolysed after 4 days. E3 represents the batch where food waste was mixed every 24 hours and no clogging was observed. Both batches used the same low COD food waste.

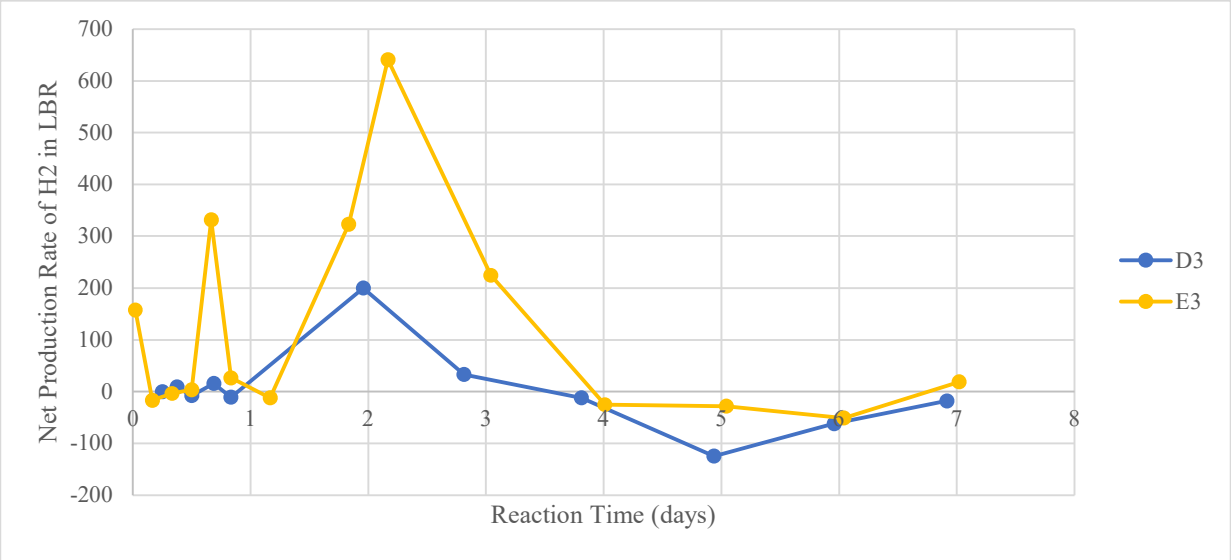

Figure 17: Net production rate of H_2 determined by GC-TCD and gas counter over the course of the final two batches. Both batches were fermented in the reactor that included mixer and FW container sampler (described in Figure 4). D3 represents the batch where food waste was pressed to induce clogging, the clog at the mesh was fully hydrolysed after 4 days. E3 is the batch where food waste was mixed every 24 hours and no clogging was observed. Both batches used the same low COD food waste.

Hydrogen production in stirred systems such as CSTRs and upflow anaerobic sludge blanket (UASB) reactors is well studied, and common behaviors can be identified to explain the difference between clogged and non-clogged operation metabolically. In a study of hydrogen production in a continuous UASB reactor fed with sugar cane molasses, Vilela et al. found that increasing the organic loading rate only increased the rate of hydrogen production up to a rate of 120 g COD/L/day (Vilela, et al., 2019). In our system the highest organic loading from the food waste container to the leachate was 32 g VS/L/day or approximately 53 g COD/L/day. Since the loading is not limiting in the leachate, it is not surprising that hydrogen production followed the addition of VS to the leachate.

Figure 6 shows the transport of VS from the container to the leachate at a rate of less than 5 g VS/L/day for the first three "clogged" trials (A1, B1, A2) after start-up. In the C2 trial, VS was added to the leachate at a much higher rate high hydrogen production rate is expected. The hydrogen production rate from the disturbed C2 followed the VS concentration in just this way suggesting that the VS in the leachate was partially hydrolysed food waste which accelerated fermentation. In the clogged control trials A1 and A2, CO_2 and H_2 production slowed over the course of the trial. At the same time the VS change in

the leachate of these trials was slow, this may suggest that hydrogen production and therefore acidogenic fermentation was limited by the mass transfer rate from the FW to the leachate. In the disturbed C2 trial, CO_2 and H_2 production was high when VS was high and stopped after VS stabilized. Hydrogen production increased at the same time that turbidity in the leachate increased in the disturbed test E3 as well shown in Figure 7 and Figure 17. This supports the connection between flow from the container to the leachate and hydrogen production and further that a small disturbance increases hydrogen production. However, in the clogged test D3 hydrogen increased before turbidity increased in the leachate showing that transport out of the FW container is not required for hydrogen production. Hydrogen is produced by acidogenesis and differences in the VFA production rate in the container and leachate of the clogged trial D3 explain this apparent discrepancy. This is discussed in section 3.3.3.

3.3.5 Cell Density

The estimated cell density as defined in section 3.2.8, provides insights in the cycles of cell growth and death observed in each reactor and helps explain the trends identified in the product concentration data. The dramatic difference in growth with a similar production of fatty acids suggests that the microbial community in the disturbed reactor is less efficient at producing fatty acids than the microbial community in the clogged reactor. The results of this test are shown in Figure 18, each point on the chart shows the change in OD600nm of the sample over 30 from when the sample was taken. In some early samples no growth was observed as the cell concentration was too low, this created negative values as some VS did not remain in suspension, however all negative values represent zero cell growth.

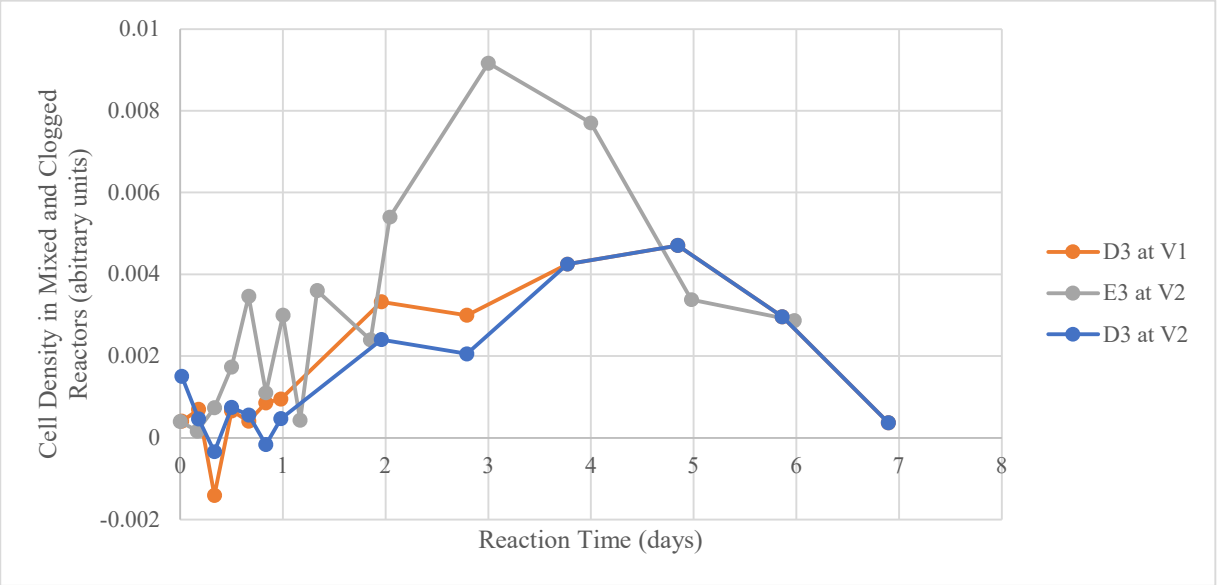

Figure 18: Cell density in samples taken from the clogged batch D3 and the mixed batch (E3). Batch D3 was deliberately clogged and remained clogged with a low infiltration rate for the first four days of reaction. During those first four days samples were taken from both the liquid trapped in the container (V1) and the liquid which had leached though the container (V2). A diagram of these sampling point is shown in Figure 3.

Both reactors showed very little cell growth in the first day. The cell density then climbed in both reactors, climbing faster in the container of the clogged reactor D3 sampled at V1 than the leachate of the clogged reactor D3 sampled at V2, and fastest in the leachate of the mixed reactor E3 sampled at V2. In the mixed reactor the peak cell density was twice that observed in the clogged reactor and reached its peak after three days, two days before the peak cell density was reached in the clogged reactor. After the peak density was reached in both reactors cell death was fast - approaching start-up levels by the end of the run.

The lag phase, or the period of very low cell growth at the start in both batches corresponds to the period of ethanol generation before acidogenesis starts. The peak cell density in the mixed reactor occurred when the hydrogen concentration in the reactor reached its peak and hydrolysis measured by sCOD continued to increase after cell growth stopped. In contrast, the cell growth in in the clogged reactor stopped when sCOD stopped increasing and the more importantly when all sCOD had been converted to alcohol or VFA. The behavior observed in the clogged reactor is consistent with cell growth limited by substrate concentration, according to Monod kinetics. While the cell growth observed in the disturbed reactor appears to be limited by some inhibitory factor, such as VFA, hydrogen or cell

concentration. The concentration of VFAs in both reactors is similar and it is assumed that biofilm thickness in the disturbed reactor is thinner than in the clogged reactor, therefore it is likely that a high hydrogen concentration is limiting cell growth in the disturbed reactor. This suggests that hydrogen concentration would have been higher if hydrogen was continuously removed providing further evidence that the disturbed reactor is better for producing hydrogen than the clogged reactor.

3.4 Modeling

3.4.1 Model Development

A model based on the work of Yunardi et al. and Simeonov and Stoyanov was implemented to estimate the parameters associated with reactor kinetics from cell density, sCOD and VFA concentrations (Simeonov & Stoyanov, 2003; Yunardi, Rinaldi, & Fathanah, 2018). Specifically, differences in the parameters that explain the unique behaviour between the disturbed and clogged reactors. Two cell growth models were compared, the Contois and Monod models and six independent parameters were estimated as if they were constant across both systems and as if they were unique to each reactor state. The Monod cell growth model is generally used to model most waste treatment processes in mixed culture, it was refined to match the hydrolysis-acidogenesis-methanogenesis pathway by Simeonov and Stoyanov in the form shown in Equation 12. Where μ is the growth rate, S is the available hydrolysed substrate made up of simple compounds such as sugars and amino acids reported in sCOD/L, X is the cell density determined in this study by OD600 measurement and reported in arbitrary units, and K_s is the half saturation constant, half the substrate concentration above which no gain in growth rate is obtained.

$$\mu = \mu_{max} \frac{S}{S+K_s} X, (12)$$

The Contois cell growth model assumes that cell density is not proportional with growth rate and has an inhibitory effect. This replaces Ks with Kc a factor which corresponds to the degree that biofilm thickness inhibits cell growth as is shown in Equation 13. Both models treat the cell death rate as proportional to cell density as described in Equation 14 where b is the death rate, b_c is the specific death rate.

$$\mu = \mu_{max} \frac{S}{S+K_c \cdot X} X, (13)$$

$$b = b_c \cdot X, (14)$$

Combining Equation 12 or Equation 13 with Equation 14 yields Equation 15. The substrate concentration can also be calculated by Equation 16, where Y_{XS} is the cell yield in gCOD/L consumed per unit of cell produced.

$$\frac{dX}{dt} = \mu - b, (15)$$

$$\frac{dS}{dt} = -\mu \cdot Y_{XS}, (16)$$

From the cell growth model defined by Equation 15 and Equation 16 the product generation rate of volatile fatty acids is defined by Equation 17. A is volatile fatty acid concentration in gCOD/L, Y_{XA} is the is the acid yield in gVFA per unit of cell.

$$\frac{dA}{dt} = \mu Y_{XA} (17)$$

Finally, the model includes the step of hydrolysis of food waste to simple substrates usable by the cell, measured by the sCOD minus the VFA concentration in CODeq. Hydrolysis rate was assumed to be proportional with cell density based on the work of Simeonov and Stoyanov (Simeonov & Stoyanov, 2003). Therefore Equation 16 becomes Equation 18 and Equation 19 is added to describe the concentration of unhydrolyzed FW in the system. The parameters of interest and the initial conditions are described in Table 5.

$$\frac{dS}{dt} = X \cdot FW \cdot Y_{hydrolysis} - \mu \cdot Y_{XS}, (18)$$

$$\frac{dFW}{dt} = -X \cdot FW \cdot Y_{hydrolysis}, (19)$$

Table 5: Summary of fitting parameters and initial conditions for modeling of clogged and disturbed acidogenic fermentation. μ_{max} is the maximum cell growth rate, K_s is the half-saturation constant and K_c is a constant associated with the degree to which the biofilm inhibits substrate availability., B_c is the specific cell death rate, Y_{xs} is the amount of sCOD that is consumed to generate one arbitrary unit of cell, Y_{xa} is the amount of CODeq of acid generated by each arbitrary unit of cell, $Y_{hydrolysis}$ or Y_{Hy} is the amount of insoluble COD converted to soluble COD by each arbitrary unit of cell. X is the cell density, S is the soluble substrate density and A is total fatty acid concentration.

Parameter	Units	Initial Conditions	Units
μ_{max}	Day^{-1}	X_0	cell (arbitrary)
K_s or K_c	gCOD/L or gCOD/L $cell^{-1}$	FW_0	gCOD/L
b_c	Day^{-1}	S_0	gCOD/L
Y_{xs}	gCOD/L $cell^{-1}$	A_0	gCODeq/L
Y_{xa}	gCOD/L $cell^{-1}$		
$Y_{hydrolysis}$	$cell^{-1}Day^{-1}$		

The simulated values of X, S, and A were fit to the collected data using ODE45 and lsqcurvefit in MATLAB. The function tolerance was set to 10^-4 and the step tolerance was set to 10^-5. The sum of normalized residuals was used as measure of the goodness of fit. The regression was first performed using Monod cell growth equation and common parameters across all conditions. Then regression was performed again for each parameter, with a unique parameter for each reaction condition and the sum of the normalized residuals was compared with the sum of normalized residuals that was calculated with common parameters. The regression was then performed using common parameters using the Contois model and a common K_c and the Contois model and unique K_cs for each trial.

3.4.2 Results of Model Test

The lowest residual was obtained by applying a unique cell yield (Y_{xs}) to the disturbed reactor, the clogged container, and the clogged leachate shown in Table 6. With low residuals also found for unique hydrolysis rates and death rates. The findings found that the disturbed reactor showed a higher cell yield per unit sCOD consumed, a higher hydrolysis rate, or a lower death rate than the cells in clogged reactor. When seen together this shows that the microbial culture in the disturbed reactor is more metabolically efficient, hydrolysis food waste faster and has better access to the substrate than the culture in the clogged

reactor and that microbial communities that form in a clogged reactor are less metabolically efficient. The best fit results and the common parameter results are shown in Figure 19 and Figure 20.

Table 6: Results of parameter finding regression to identify potential metabolic differences between clogged and mixed reactors D3 and E3. The measured yield of fatty acids per gCOD removed was 0.435 g/g in clogged reactor and 0.775 g/g in disturbed reactor, the calculated yield appears in the table as a secondary test of fit. Unique parameters were set one parameter per each condition, in the table condition 1 ($X_0$1 ect.) refers to the mixed reactor E3 sampled below the container at V2, condition 2 refers to the clogged reactor D3 sampled in the container at V1, and condition 3 refers to the clogged reactor D3 sampled below the container at V2.

	Normalized Sum of Residuals	Parameters	Starting Cell Density	Yield
Common Parameters	9.1592*10^3	µmax = 9.7359 Ks = 214.4021 Yxs = 0.8084 Yxa = 0.2692 Bc = 0.1235 Yhy = 0.0375	$X_0$1 = 17.4682 $X_0$2 = 7.8515 $X_0$3 = 3.8035	Ysa = 0.3330
Unique µmax	9.0814*10^3	µmax1 = 6.3820 µmax2 = 4.7508 µmax3 = 20.7542 Ks = 98.5777 Yxs = 0.7129 Yxa = 0.1684 Bc = 0.1635 Yhy = 0.0453	$X_0$1 = 11.4261 $X_0$2 = 6.3958 $X_0$3 = 0.2491	Ysa = 0.2362
Unique Ks	1.2020*10^4	µmax = 14.3403 Ks1 = 102.3116 Ks2 = 168.3540 Ks3 = 54.9872 Yxs = 0.6574 Yxa = 0.1468 Bc = 0.1917 Yhy = 0.0490	$X_0$1 = 5.0567 $X_0$2 = 3.2727 $X_0$3 = 0.1793	Ysa = 0.2233

Unique Yxs	7.6147*10^3	µmax = 9.7218 Ks = 197.7778 Yxs1 = 0.4382 Yxs2 = 0.6555 Yxs3 = 0.7783 Yxa = 0.1752 Bc = 0.2279 Yhy = 0.0368	$X_0$1 = 12.2491 $X_0$2 = 5.9369 $X_0$3 = 3.6703	Ysa1 = 0.3998 Ysa2 = 0.2673 Ysa3 = 0.2251
Unique Yxa	1.0674*10^4	µmax = 17.0803 Ks = 298.9347 Yxs = 0.9819 Yxa1 = 0.2518 Yxa2 = 0.3817 Yxa3 = 0.2151 Bc = 0.0709 Yhy = 0.0449	$X_0$1 = 15.9474 $X_0$2 = 7.0294 $X_0$3 = 4.0638	Ysa1 = 0.2564 Ysa2 = 0.3887 Ysa3 = 0.2191
Unique Bc	8.9475*10^3	µmax = 15.8835 Ks = 325.6177 Yxs = 0.6032 Yxa = 0.1967 Bc1 = 0.1410 Bc2 = 0.2491 Bc3 = 0.2914 Yhy = 0.04163	$X_0$1 = 13.0500 $X_0$2 = 6.0677 $X_0$3 = 2.8905	Ysa = 0.3261
Unique Thy	8.557*10^3	µmax = 17.2242 Ks = 247.2904 Yxs = 0.4655 Yxa = 0.1468 Bc = 0.2460 Thy1 = 0.0371 Thy2 = 0.026 Thy3 = 0.0089	$X_0$1 = 12.2787 $X_0$2 = 6.4192 $X_0$3 = 5.2655	Ysa = 0.3154

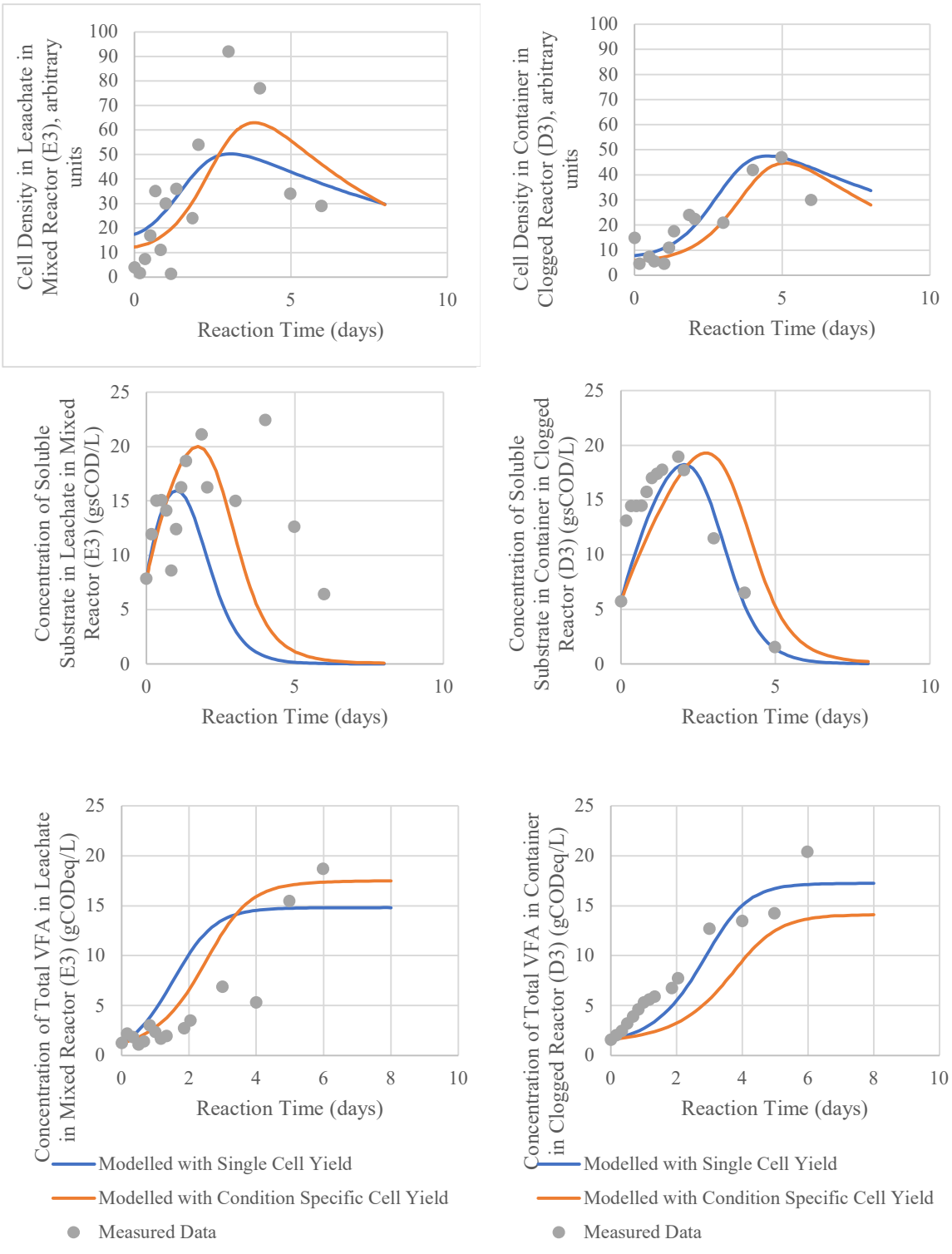

Figure 19: Modelling results with single cell yield (Yxs) and with condition specific cell yield (Yxs1 for E3V2, Yxs2 for D3V1, and Yxs3 for D3V1) compared with measured data in leachate of the

mixed reactor E3 at V2 and the container of the clogged reactor D3 at V1. Cell yield is the amount of sCOD which is consumed in the growth of one unit of cell and the conditions specific case assumes that the amount of sCOD consumed to produced one unit of cell is different in the mixed leachate (Yxs1), the clogged container (Yxs2) and the clogged leachate (Yxs3).

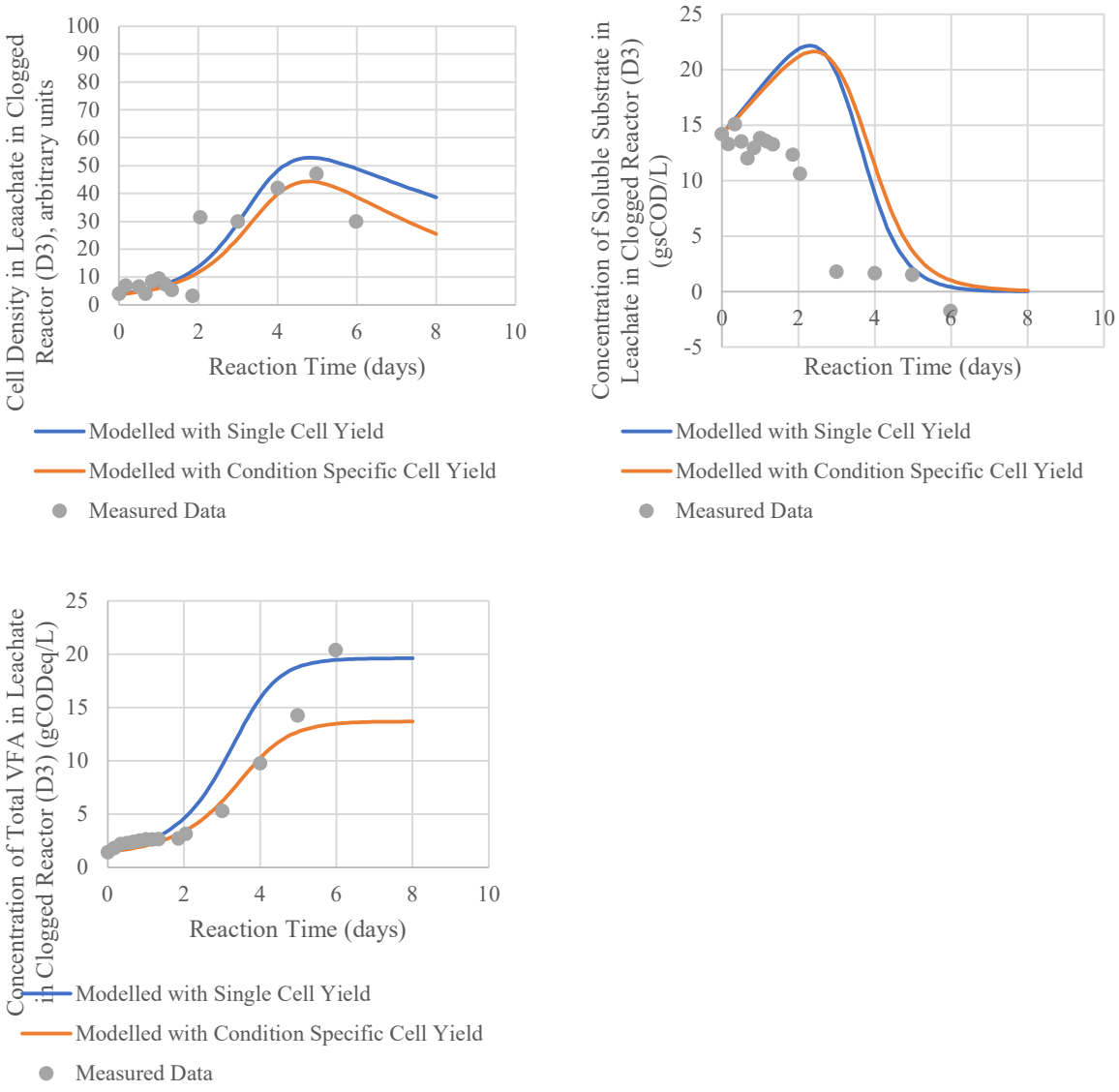

Figure 20: Modelling results with single cell yield (Yxs) and with condition specific cell yield (Yxs1, Yxs2, and Yxs3) compared with measured data in leachate of the clogged reactor D3 at V2. Cell yield is the amount of sCOD which is consumed in the growth of one unit of cell and the conditions specific case assumes that the amount of sCOD consumed to produced one unit of cell is different in the mixed leachate (Yxs1), the clogged container (Yxs2) and the clogged leachate (Yxs3).

The Contois model did not fit the data as well as the Monod model when compared by total absolute residual, shown in Table 7. This suggests that cell growth was not limited by the cell density and biofilm thickness in any condition. When the Kc was calculated independently the coefficient of inhibition (K_c) was nearly identical in the disturbed reactor (K_{c1}) and in the clogged reactor (K_{c2}). This suggests that the differences in clogged fermentation are not related to inhibition of fermentation by the biofilm.

Table 7: Results of parameter fitting to test the validity of the Contois model in clogged and unclogged leach bed reactors.

	Normalized Residual	Parameters	Starting Cell Density	Yield
Contois Common Parameters	9.7797*10^3	Umax = 97.5542 Kc = 104.3338 Yxs = 0.7354 Yxa = 0.2061 Bc = 0.1304 Thy = 0.0372	$X_0$1 = 18.9364 $X_0$2 = 6.6436 $X_0$3 = 0.0044	0.2803
Unique μmax	9.2800*10^3	Umax = 12.6131 Kc1 = 18.2168 Kc2 = 17.7165 Kc3 = 10.2920 Yxs = 1.0992 Yxa = 0.2987 Bc = 0.0487 Thy = 0.0485	$X_0$1 = 19.0198 $X_0$2 = 7.395 $X_0$3 = 0.2685	0.2717

3.5 Conclusion

Reactor clogging is only one of many inhibitory processes at play in leach bed reactors as organic loading rate is increased. As the rate of soluble substrate loading is increased so does the rate of cell growth, hydrogen concentration, and VFA concentration. These processes put selective pressure on bacterial populations favouring certain bacteria in unclogged reactors where FW is well disintegrated in leachate and other bacteria in clogged reactors where acids are concentrated in container with food waste. Regular disturbances to the FW container prevent clogging, increase cell growth and the rate of gas production, and improve hydrolysis. In this respect there is evidence supporting the hypothesis that a mechanical

disturbance to the FW container improves performance, so long as high hydrogen production is desired. However, fatty acid formation is not significantly improved. This is likely due to a combination of the difference in metabolic pathways favoured in the disturbed reactor and the product inhibition from hydrogen and fatty acids. In this study similar FW feedstock always produced similar VFA production rates and peak VFA production. There was no evidence in the experimental or modelling studies that showed that either inhibition of cell growth, hydrolysis, or product transport explained the inhibition of fatty acid formation across all tests. While it has been repeatedly shown that VFA production is slow in clogged systems, preventing clogging in these systems will not necessarily increase VFA production. This is likely due the changes in the metabolism caused by the increased transport of FW to the leachate and the subsequent production of hydrogen. This supports the hypothesis that clogging changes the microbial community in the fermenter and as consequence changes the dominant enzymatic pathways.

Acidogenic fermenters are of industrial interest to produce fatty acids and to produce hydrogen. This study finds that a small daily disturbance of the FW container improves the disintegration and solution of FW in high solids reactors and this disturbance favors hydrogen formation. If optimization for rapid VFA production is desired other limiting factors must be addressed.

Chapter 4
Chain-Elongation of Fatty Acids

4.1 Introduction

Acidogenic fermentation has been identified as a pathway to reclaim value from organic waste streams with higher potential than composting and methanogenic aerobic digestion. In their 2015 techno-economic analysis, Bonk et al. found that the highest cost associated with a VFA production was due to the concentration and purification stage (Bonk, Bastidas-Oyanedel, & Schmidt, 2015). This high concentration and purification cost is due to the high affinity that short-chain fatty acids have for water and their small molecular weight. Medium-chain fatty acids are easier to purify due to their hydrophobicity and high molecular weight and are similarly valuable per unit carbon. Short-chain fatty acids can be converted by acidogenic fermenters to medium-chain fatty acids when ethanol, lactic acid or other 2-carbon energy sources are available.

While chain elongation has been demonstrated in leach bed and stirred reactors at mesophilic and thermophilic conditions little work has been done to identify how the process cost can be reduced. An understanding of the efficiency gains attained by heating and stirring fermenters as well as the efficiency gains by operating slowly at trickle flow rates is needed to justify the costs associated with these energy and space intensive reactor improvements.

Using a well studied psychrophilic leach bed reactor (LBR) with a high circulation rate and short solids retention time (SRT) this study tested the hypothesis that chain elongation using ethanol in an acidogenic fermenter was improved at temperatures below 25°C. Previous work has shown that without ethanol addition this reactor produces a ratio of short-chain to medium-chain VFAs of approximately 5 to 1. The current process yields approximately 2 g/L (C5-C7) from 1kg of food waste. This was compared to the yields obtained with ethanol addition and to mesophilic and thermophilic chain elongation studies as well as studies with longer SRTs and lower solids concentrations. The cost of these changes was compared with the yield to assess if psychrophilic fermentation warrants further investigation for the scale-up of these processes.

4.2 Methods

4.2.1 Bottle Tests

A 14-day serum bottle trial was performed to determine the ideal ethanol concentration. The substrate used was 10 g TS/L food waste blended to pass though a 4 mm sieve, the inoculum was dewatered LBR

leachate, the ISR was 0.12, starting pH was 7 and the bottles were buffered. Four treatment levels were tested: no ethanol, 5 g/L ethanol, 10 g/L ethanol, 25 g/L ethanol. Each bottle was run in duplicate. Bottles were tested at the start, after 4 days, and after 7 days, and after 14 days.

Bottles were tested for total solids (TS) and volatile solids (VS) following AWWA Standard Methods, sCOD following HACH high range method, and VFA content determined by GC-FID. The gas content was measured at the end of the 16-day trial using GC-TCD.

4.2.2 Bioreactor Design

The leach bed reactor (LBR) was adapted from the design published by Xiong et al. (Xiong, Hussain, Lee, & Lee, 2019). As shown in Figure 18, the reactor is a 7.5 L cylinder constructed from acrylic with a 1 inch (25.4 mm) internal diameter outlet in the bottom and the top, 5 x ½ inch (12.75 mm) inlets for mixing and instrumentation in the body as indicated in Figure 3, and 2 x ½ inch (12.75 mm) outlets for gas collection and emergency pressure release (EPR). The food waste and inoculant sludge were mixed and added to an approximately one litre container. Leachate was recirculated through the FW container using a Masterflex L/S® Standard Digital Drive (P1) (Masterflex, Radnor, PA, USA). Leachate was mixed with a centrifugal pump (P2) (MD-70RLZT Iwaki Co, Tokyo, Japan). pH was controlled with a pH meter (9157BN, Thermo Fisher Scientific, MA, USA) set in the leachate, and a pH controller (MC122, Milwaukee Instruments, Inc., NC, USA) connected to a peristaltic pump (Masterflex L/S® Analog Variable-Speed Modular Drive, Masterflex, Radnor, PA, USA) for delivering 5 M NaOH (83076-580, Anachemia Canada Inc, ON, Canada). Biogas was counted with a silox-filled gas counter (MilliGascounters, Dr.-Ing. RITTER Apparatebau GmbH & Co. KG, Germany), gas consumption was buffered by 2 L gas bag filled with N_2 gas.

4.2.3 Reactor Operation

Food waste and inoculum were mixed, and the container was filled with approximately 1 kg of wet substrate. 2.5 L of distilled water was added to the reactor and the container was placed in the top of the reactor and the reactor was sealed. The sealed reactor was purged with 15 L of nitrogen gas (99.8 %) though the sampling port V1. The leachate was then circulated at 24 L/h for one minute out of every six for an average circulation rate of 4 L/h and mixed at approximately 40 L/min for 15 minutes every 2 hours. 15 mL samples of leachate were collected daily from V1 for the first 7 days then every other day for the following 7 days. 3 mL gas samples were collected from a septum in the gas line between the reactor and gas bag daily for the first 7 days then every other day for the 7 following days. The reactor was operated for 15 days per batch. The entire system was maintained at room temperature of 22°C.

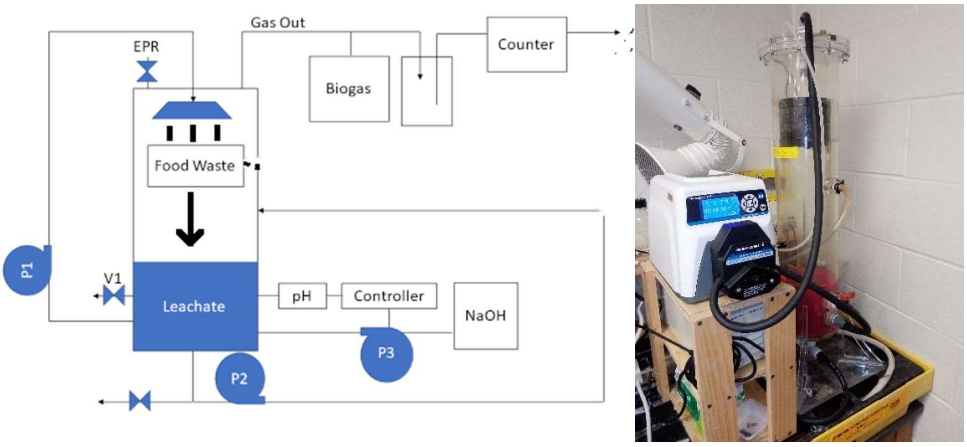

Figure 21: Diagram and photo of LBR used for this chain elongation study and previous work by Saha et al (Saha S. , 2019). Abbreviations: EPR (Emergency Pressure Release), V1 (Sampling Valve), P1 (Recirculation pump, peristaltic), P2 (mixing pump, centrifugal), P3 (pH correction pump, peristaltic).

4.2.4 Experimental Conditions

Three experimental conditions were tested to evaluate the effect of ethanol addition on chain length of fatty acids produced from AF. These conditions were without ethanol addition in batch reaction, with 5 g/L ethanol addition in a batch reaction and with 5 g/L ethanol addition in a semi-continuous reaction. In the semi-continuous reaction ethanol was added stoichiometrically to maintain an approximate concentration of 5 g/L and achieve a hydraulic residence time of 7 days (half the solids residence time). Two batches were tested without ethanol, two batches were tested with ethanol and the semi-continuous operation with ethanol was tested once. Across all studies the pH, temperature, recirculation rate, leachate mixing rate, solids loading rate and retention time remained the same.

The two ethanol-free batches followed the reactor operation described in section 4.3.3 exactly. The two batches with ethanol added 5 g/L ethanol to the 2500 mL of distilled water added at the start of the batch. The semi-continuous trial with ethanol included 2500 mL of 5 g/L ethanol in place of water on start-up and used a 4 L reservoir of 0.3 M NaOH and 0.6 M ethanol to adjust pH instead of 5 M NaOH. These concentrations were selected as they represent 1 mol of NaOH for every mol of acid generated and 2 mols of ethanol for every mol of acid generated – this concentration targeted completing 1 working volume dilution over the course of 14 days. The volume of the reactor was adjusted to 2.5 L of leachate daily and the removed volumes were retained for analysis.

4.2.5 Food Waste Analysis

Samples of food waste solids were tested before and after fermentation. 50 g samples blended into 500 mL of water were used for tCOD measurements and 5 g samples were used for determining total and volatile solids. All COD measurements were conducted in duplicate using HACH high range colorimetric COD determination method using HACH high range prepared vials, a HACH 3900 spectrometer and a HACH DRB200 digestor (HACH, ON, Canada) according to ASTM D1252-95 (ASTM D1252-95, 2006) Total and volatile solids were determined according to APHA standard water analysis method for thick media 2540 G (APHA).

4.2.6 Gas Composition

Partial pressures of H_2, O_2, N_2, CH_2, and CO_2 were measured. Two of 0.5 mL samples were taken using a gas-tight syringe to determine the H_2, O_2 and N_2 partial pressures in the reactor. Each 0.5 mL sample was injected into a gas chromatographer equipped with a thermal conductivity detector (GC-TCD) purged with argon at 50°C ramping 110°C over 8 minutes, sampling at 5 Hz, using an S.S. Molecular Sieve 5A Packed Column (8600-PK2B**,** SRI Instruments, CA, USA). 5% H_2 calibration gas and air were used for calibration of the GC. The same procedure was followed using two 1 mL syringe injections for CH_2, CO_2, and N_2, for this test the GC-TCD analyser (Model 310, SRI Instruments, CA, USA) was purged with helium holding at 40°C for 2 minutes, sampling at 5 Hz, using a Porapak Q 80/100 2 m, 2 mmID Packed Column (Restek, PA, USA). A 2-point calibration curve was used to calculate the partial pressure of N_2, CH_4, and CO_2. The calibration curve was prepared with 20% CO_2, 20% N_2, 60% CH_4 calibration gas and pure CH_2, CO_2, and N_2. The total of the partial pressures was then normalized to 1 atm.

4.2.7 Leachate Analysis

Leachate samples were collected every day for the first 5 days of operation, when gas production was taking place, then every second day after that. Leachate samples were tested for total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), soluble chemical oxygen demand (sCOD), volatile fatty acid (VFA) concentrations. TS, VS, and total chemical oxygen demand (tCOD) of the food waste was measured before and after each run. Solids in the leachate were measured using duplicates of 2 mL samples, according to the standard (2540B, 2540D, and 2540E , APHA, 2002). sCOD measurements were made using 1/40 diluted leachate, vacuum filtered though 0.45 μm nylon filters (15945-27, Antylia Scientific, IL, USA). tCOD measurements of leachate used unfiltered 1/40 diluted leachate. All COD measurements were conducted in duplicate using HACH high range colorimetric COD determination method using HACH high range prepared vials, a HACH 3900 spectrometer and a HACH DRB200 digestor (HACH, ON, Canada) according to (ASTM D1252-95,

2006). VFA concentrations were determined from 2.5 mL samples diluted 20 times and filtered through 0.2 µm nylon filters (UZ-32816-02, Antylia Scientific, IL, USA). The samples were preserved with 10 µL of concentrated phosphoric acid and frozen before analysis with an HP 5890 series 2 gas chromatographer with a flame ionization detector (GC-FID) (Hewlitt Packard, CA, USA) equipped with a capillary column (30 m × 0.53 mm × 0.5µm PAG, Supelco, Bellefonte, PA). The oven temperature for VFA analysis was maintained at 150°C for 2 minutes, then increased to 190°C at a slope of 4°C/minute and maintained at 190°C for 3 minutes. Ethanol concentrations were determined from the same samples as VFA concentrations using the same equipment using the same detector and injector temperatures and gas flow rates. For the ethanol analysis method, the oven temperature for VFA analysis was maintained at 40°C for 3 minutes, then increased to 60°C at a slope of 6°C/minute and maintained at 60°C for 6 minutes.

4.2.8 Medium-Chain Fatty Acid Analysis Method

Medium-chain fatty acid (5-10 carbon acids) concentration was analysed separately as caprylic acid and capric acid (C8 and C10 respectively) were not completely eluted from the column in the VFA analysis method described in 4.2.6. To overcome this, this method was used based on the work of Zeils et al. using 0.2 micron filtered 20x diluted samples (Ziels, et al., 2015).

1 mL samples were acidified and salted with 100 µL phosphoric acid and 300 µL 167g/L NaCl. The 1 mL sample was then mixed with 2 mL 1:1 hexane:MTBE solvent. The mixture was vortexed for 5 mins and centrifuged for 15 min @ 3000*g then the organic fractions and acid fractions were decanted into separate autosampler vials. The 1 µm acid and organic samples were each analysed using an HP 5890 series 2 gas chromatographer with a flame ionization detector (GC-FID) (Hewlitt Packard, CA, USA) equipped with a capillary column (PAG (USP G18) column 30 m x 0.53 mm). The injection temperature was 220°C, the oven temperature starts at 100°C and ramped to 220°C which was maintained for 10 minutes. The detector temperature was 300°C.

The method was repeated for three mixed calibrant solutions containing ethanol and 11 different fatty acids with molar masses from 60 g/mol (acetic acid) to 172 g/mol (capric acid). The retention times for each compound were determined and the areas of each were integrated for both the acid soluble and organic soluble fractions. The area in the acid soluble fractions were added to the areas in the organic soluble fractions and the total areas were plotted against the concentration to develop a composite calibration curve for each compound. Once each sample was analysed the areas of each peak from each compound in acid and organic fractions were summed and compared to the calibration curve to calculate the concentration. Since some impurity peaks were consistently observed in organic fraction blanks around the same retention time as butyric acid, caproic acid and capric acid a correction was made at

these concentrations, subtracting the largest area observed in the blank for a given compound from the total of each measured value.

4.3 Results

4.3.1 Bottle Tests

The bottle test found that 5 g/L ethanol produced the highest VFA yield and highest medium-chain VFA yield. This is consistent with the work of Andersen et al. 2017, who achieved 3.2 g/ L yield of caprylic acid while maintaining an ethanol concentration of 6 g/L (Andersen et al., 2017). Concentrations for non-caprylic fatty acids were relatively low due to very low starting VS and tCOD concentrations. This justified the use of 5 g/L ethanol in the LBR to improve medium-chain VFA.

Total solids and volatile solids in all bottles show 100% of the removal in the first 4 days as ethanol and soluble sugars are converted to CO_2, after which solids increased steadily for 8 days as cell growth continued. Then the concentration of solids in leachate stabilized as fermentation slowed in the last 4 days.

Very little COD removal was observed in any test. Most of the COD was removed in the first 4 days as ethanol and soluble sugars were absorbed by cells. Then some COD was returned to the soluble phase as cells produced products of anaerobic fermentation from hydrolysed food waste. The ethanol added was the largest source of sCOD in the 10 and 25 g/L bottles, the sCOD from ethanol and food waste were balanced in the 5 g/L test (Figure 22). The sCOD percent removal has also been plotted to show the trend more clearly (Figure 23). The 5 g/L ethanol bottles showed the highest sCOD removal, and there appeared to be inhibition of cell activity at higher ethanol concentrations.

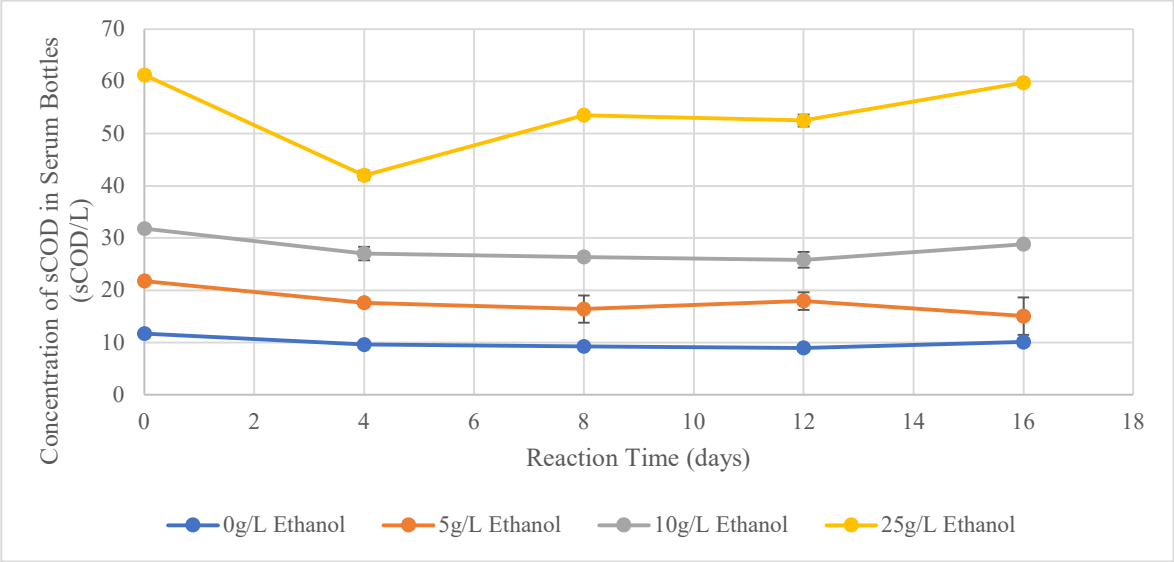

Figure 22: Concentration of sCOD in serum bottles with various starting concentrations of ethanol ranging from no ethanol added to 25 g/L added. Error bars show the maximum and minimum values observed between the two bottles with the same starting fatty acid concentration.

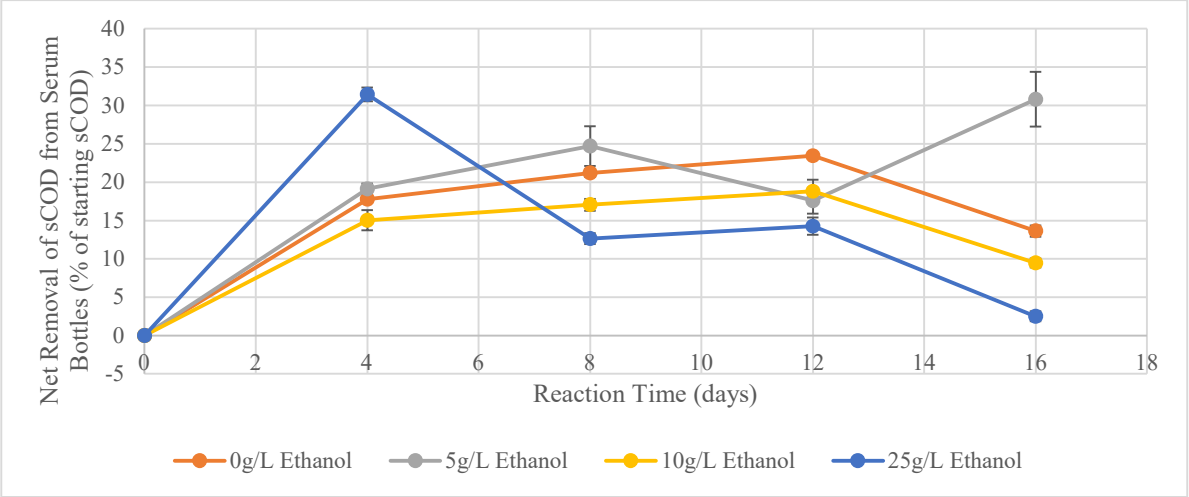

Figure 23: Net removal of sCOD from serum bottles with various starting concentrations of ethanol. Net removal is expressed as a percentage of starting sCOD after four, eight, twelve and sixteen days. Error bars show the maximum and minimum values observed between the two bottles with the same starting fatty acid concentration.

The concentration of acetate over time is presented in Figure 24. While it shows that acetate yields of 0.1 g/L to 0.7 g/L are achieved in all bottles (low compared to all LBR trials), no trend is observed due to low sample concentration.

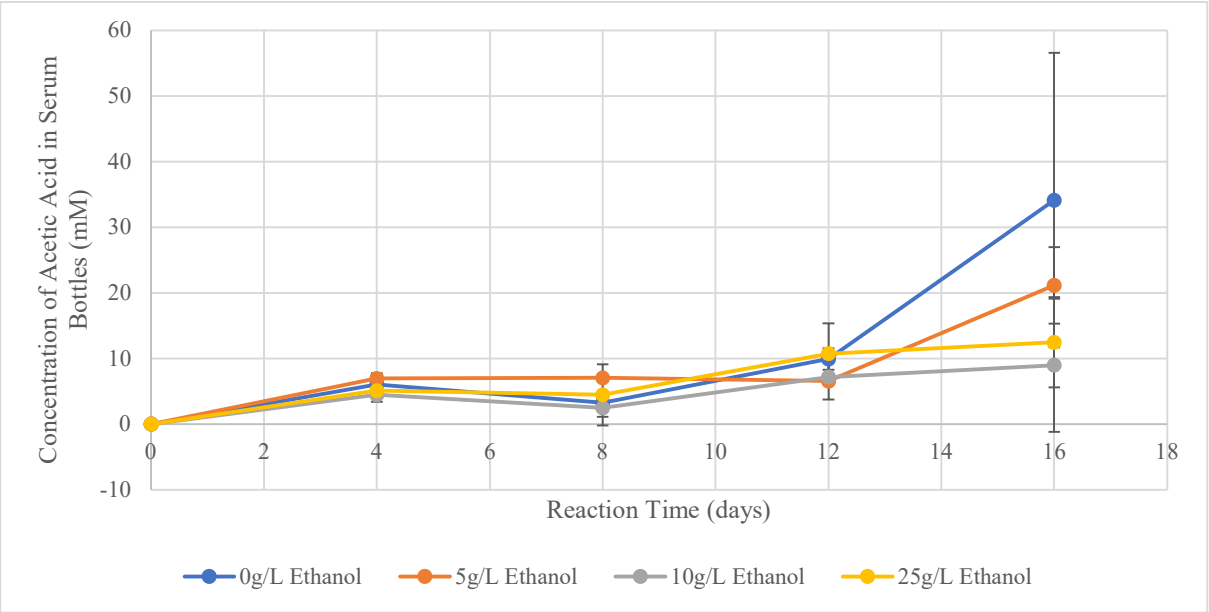

Figure 24: Concentration of acetic acid in serum bottles with various starting concentrations of ethanol. Error bars show the maximum and minimum values observed between the two bottles with the same starting fatty acid concentration.

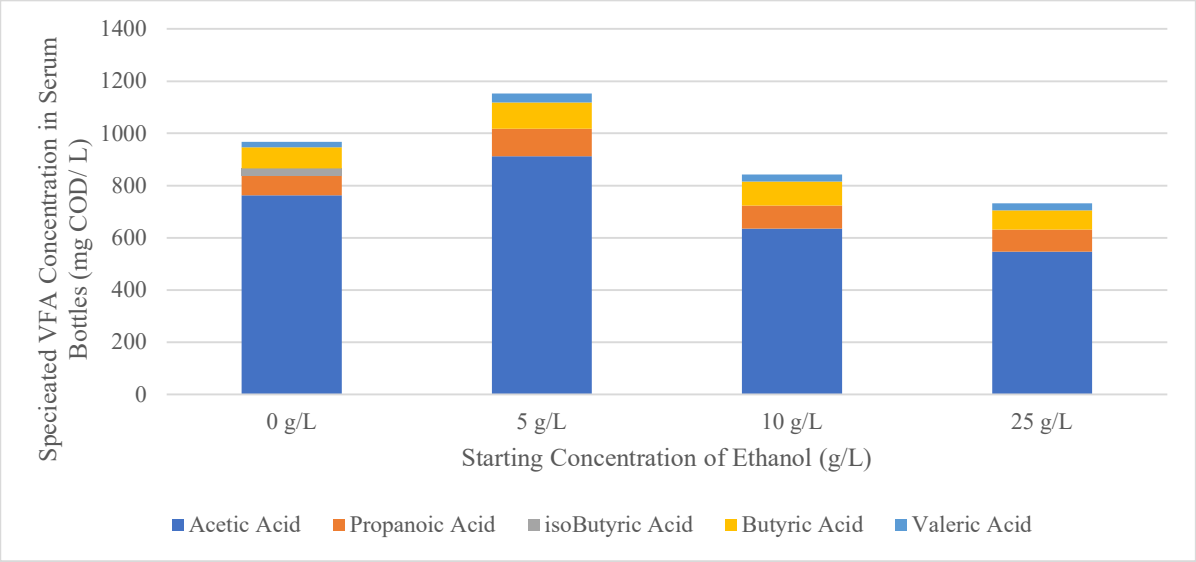

Figure 25: Speciated VFA concentration in serum bottles in with various starting concentrations of ethanol after 16 days.

GC-FID analysis was repeated for each bottle using a 4x diluted sample, in each case acetate dominated VFAs (shown in Figure 25). The 5 g/L ethanol bottles produced the highest concentration of VFAs generally and of valerate specifically. 5 g/L was selected as the concentration of ethanol using in future work.

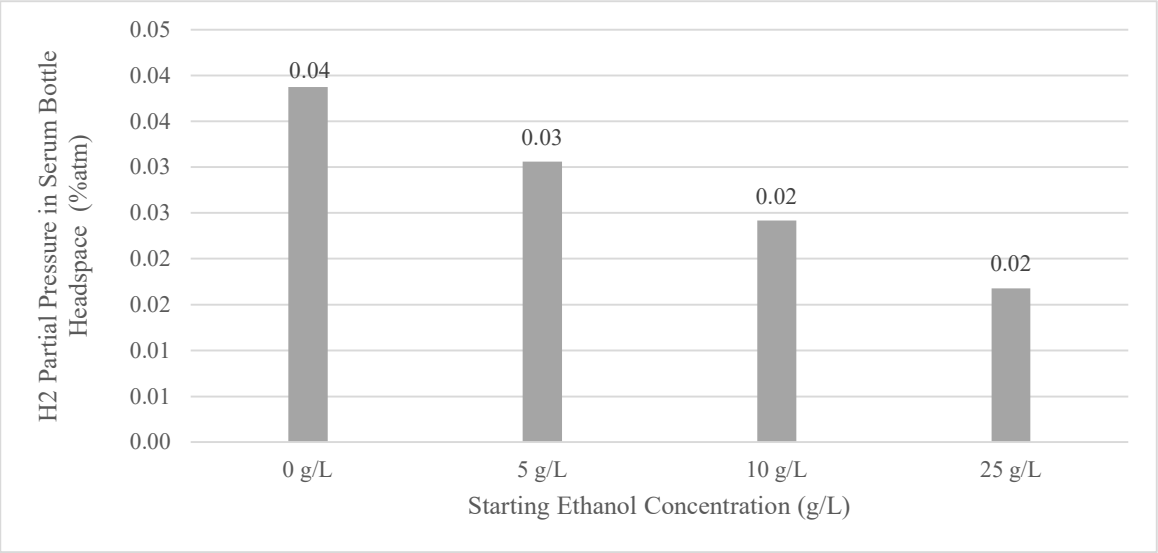

Figure 26: Hydrogen partial pressure in serum bottle headspace after 16 days of fermentation with varying amounts of starting ethanol.

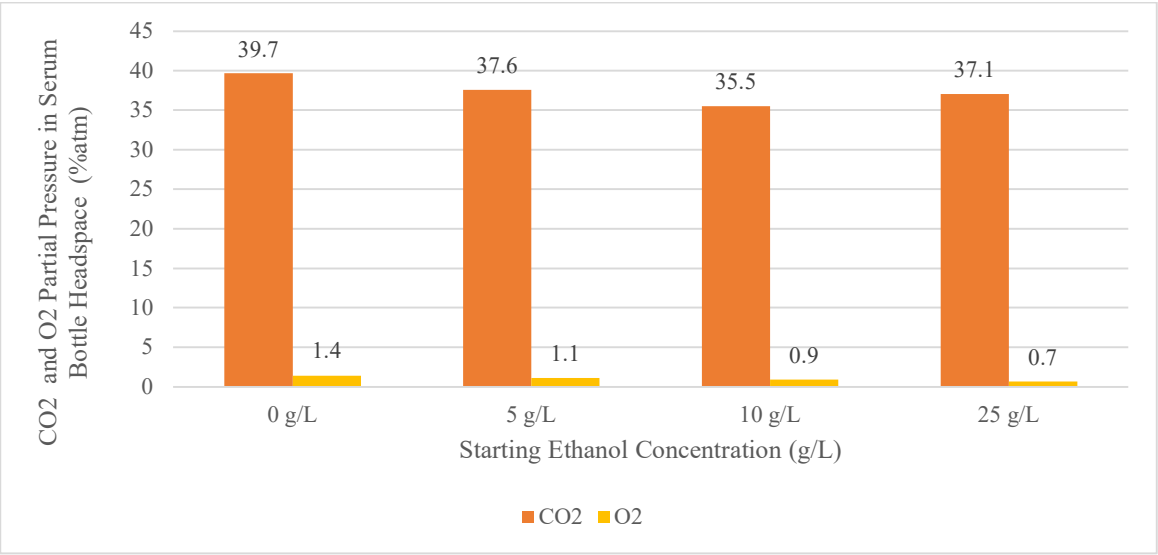

Figure 27: Gas composition in serum bottle headspace after 16 days of fermentation with varying amounts of starting ethanol.

The ethanol concentration affected hydrogen production and oxygen consumption in bottle tests. As ethanol concentration increased the hydrogen concentration and the oxygen concentration fell, as shown in Figure 26 and Figure 27. The increase in oxygen consumption is a consequence of higher COD in serum bottles. While hydrogen was observed in all but one bottle, the hydrogen production was negligible in all bottles and variations are not significant.

4.3.2 LBR Test Summary

The medium-chain fatty acid concentrations in batches without ethanol addition were comparability low. Less than 2 g of MCFAs were produced in approximately 2.5 L of leachate and were almost exclusively composed of valerate isomers, detailed results are shown in Table 9. The same operating conditions and food waste with 5 g/L of ethanol yielded more fatty acids overall and higher concentrations of valeric (C5), caproic acid (C6), and enanthic acid (C7). The addition of ethanol also increased the ratio of medium-chain fatty acids to total fatty acid from a maximum of 14% of in the control to a maximum of 45% in with ethanol addition (Figure 28). In the semi-continuous trial with 5 g/L ethanol the hydrolysis yield expressed as g sCOD/kg VS added increased significantly. This is an expected result of dilution as the presence of fatty acids has been shown by other authors to inhibit hydrolysis. The dilution increased the production of VFAs by a factor of 1.6, however the concentration of VFA fell by one third. It is possible that that this suggests that ethanol is preferred as a substrate to the hydrolysis products produced from food waste.

4.3.3 Performance

Comparing the performance of the chain elongation tests, the addition of 5g/L ethanol generated 428 g CODeq of VFA per kg VS added, 38% more than was produced without the addition of ethanol. Less reduction in VS was observed when ethanol was added to the trial and VFAs made up slightly less of the final sCOD suggesting that higher conversion rates are possible. Washout of acidogenic bacteria was observed in the continuous system, along with high hydrolysis rates and low yields and low VS removal. The performance indicates that 5 g/L of ethanol improves the production of MCFAs and continuous dilution provides no benefit at the product concentration of approximately 20 g/L.

Table 8: Comparison of the performance of VFA formation and MCFA formation in this study and previous work

	Final tCOD in Leachate (gtCOD/L)	Final sCOD in Leachate (gsCOD/L)	Final Total Fatty Acid Concentration in Leachate (VFA gCODeq/L)	Hydrolysis Yield (gsCOD/kg vs added)	Total Fatty Acid Yield (gCODeq VFA/ kg VS added)	Fatty Acid Ratio (gCODeq VFA/gsCOD)	Vs removal from FW container (%)
Saha ISR5	33	26	20	666	495	76	93
Saha ISR10	34	25	22	624	571	88	88
This Study ISR20	29 (±7)	25 (±1)	20 (±5)	879 (±429)	505 (±195)	84 (±17)	76 (±6)
This Study ISR20 5g/L ethanol	28 (±5)	26 (±5)	15 (±7)	1055 (±381)	330 (±99)	51 (±13)	46 (±5)
This Study ISR20 5g/L ethanol and 2x dilution	44	40	14	1173	363	41	33

4.3.4 Medium-Chain Fatty Acid Production

The addition of 5 g/L ethanol increased the proportion of valerate, caproate and enanthate relative to butyrate as shown in Figure 30 . This suggests that chain elongation occurred. The rate of chain elongation appears to increase around the time gas production slows down, between 2 and 3 days from start of the experiment. Once started, the production of medium-chain fatty acids continued at a steady rate until day 12 when acetogenesis starts to dominate, destroying medium-chain-fatty acids to generate acetic acid (Figure 28).

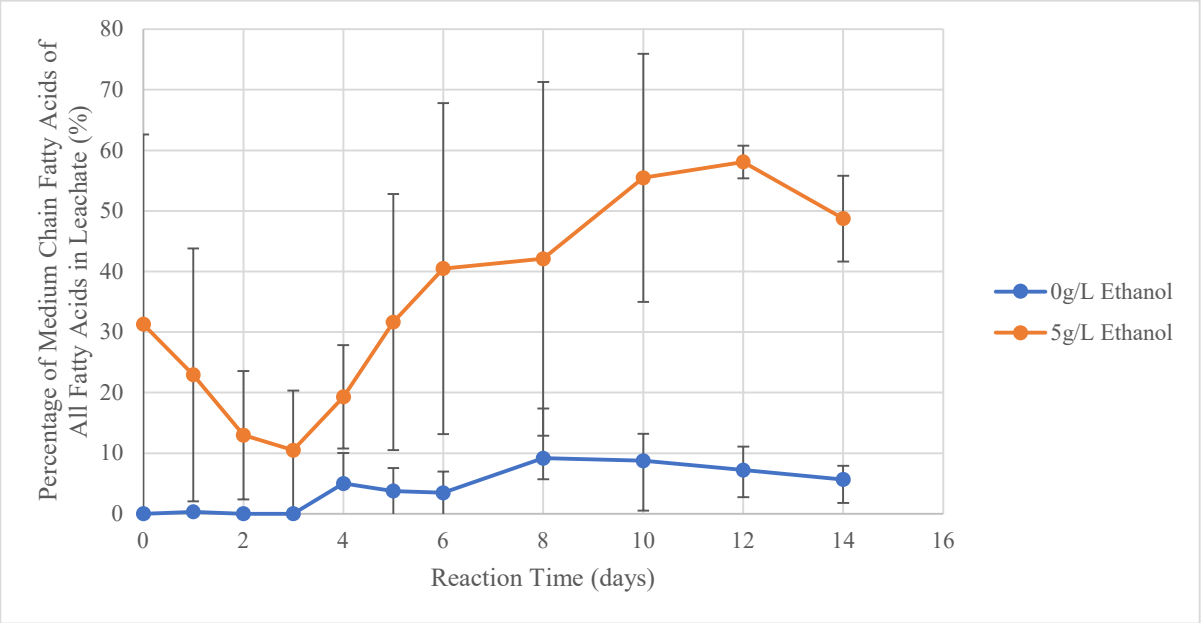

Figure 28: Average percent of fatty acids that are medium-chain fatty acids (C5-C10) on a CODeq basis in batches without ethanol addition and batches with ethanol addition over the course of the experiment. Error bars show the maximum and minimum values observed between the two trials.

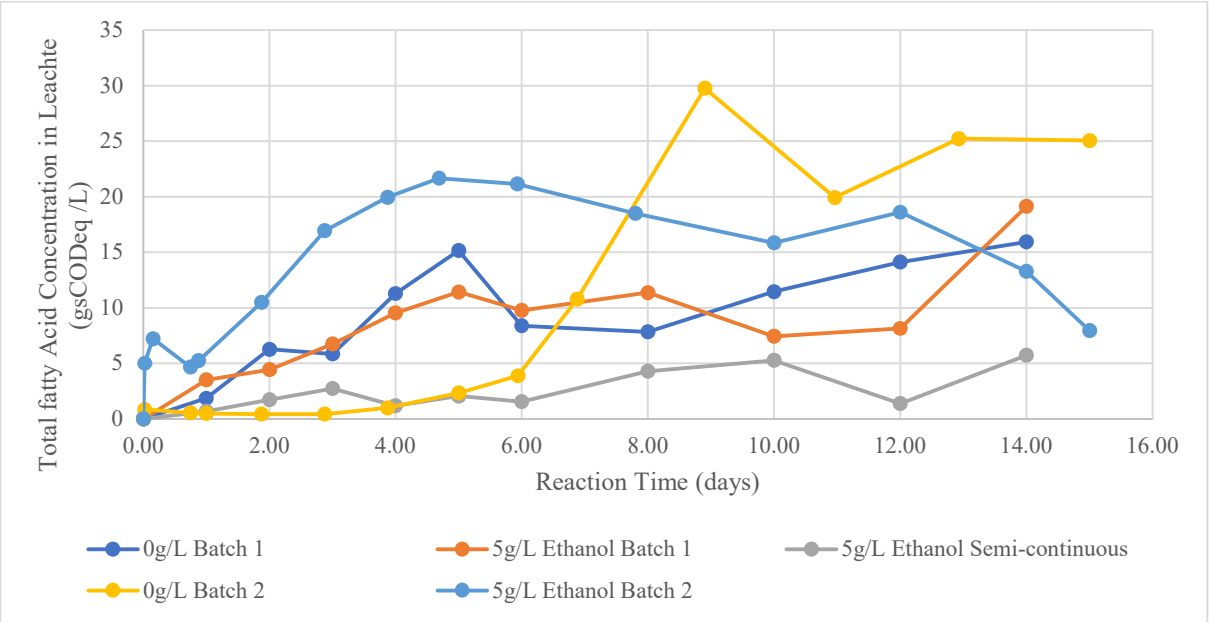

Figure 29: Total fatty acid concentration in leachate (expressed in gCODeq/L) over the course of all 5 batches. Batch 1 and the semi-continuous test used the same food waste and Batch 2 used a different food waste.

The rate of fatty acid production over the course of the 15-day experiment varied from trial to trial as with the lag time of the acidogenic bacteria being the most common difference between trials. The lag time is the time before the bacterial population of interest has reached a high enough concentration to grow exponentially (Willey, Sherwood, & Woolverton, Chapter 7: Microbial Growth, 2011, p. 168). The lag time is influenced by fermentation conditions – such as oxygen and hydrogen partial pressure, starting cell density, and available substrate. The lag times for the 5 trials are: two days and one day for the first and second ethanol addition batch tests, 2 days and 6 days for the first and second ethanol free batch tests, and 6 days for the semi-continuous ethanol addition test. There is no evidence that ethanol affected lag time, the starting conditions were similar in all trials, therefore differences in the density of viable acidogenic bacteria in the inoculum are the likely cause of difference.

When the difference in substrate availability and starting cell density is accounted for, some similarities in the fatty acid production curves become apparent. All cultures produce FAs at a similar rate for 4 to 5 days reaching a peak. In both cases the peak is higher for the alcohol-free batch and FA production slows gradually for the cultures with alcohol present as they approach peak concentration. Comparing the individual fatty acid concentrations near this peak, the consumption of ethanol and generation of medium-chain fatty acids can be seen at this time. This can be seen from day 8 to days 12 in the first batch and from day 4 to day 6 in the second batch. In later trials where fatty acids were generated more quickly, degradation of the fatty acids was observed, to compare the trials evenly on their ability to produced fatty acids an average concentration over the two days of highest total concentration was calculated. These peak fatty acid concentrations have been compared in Table 9.

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Table 9: Peak total fatty-acid concentrations for each batch by species. Peak concentrations are a two-day average over the period when total fatty acid concentration was the highest in each batch. The results of the semicontinuous trial is the concentration in the total leachate output.

	Acetic gCODeq /L	Propionic gCODeq /L	Butyric gCODeq /L	Valeric gCODeq /L	Caproic gCODeq /L	Enanthic gCODeq /L	Caprylic gCODeq /L	Capric gCODeq /L
Ethanol-Free Batch 1 Day 6-8	4.55	6.47	4.40	2.34	0.105	0	0	0
Ethanol-Free Batch 2 Day 9-11	11.02	4.74	8.11	0.976	0	0	0	0
5g/L Ethanol-Batch 1 Day 10-12	1.71	3.63	4.09	3.58	2.28	2.35	0	0
5g/L Ethanol Batch 2 Day 4-6	6.00	5.93	2.58	6.90	3.78	2.62	0.055	0
5g/L Ethanol with continuous dilution Averaged sum	1.15	1.95	7.44	0.588	1.21	0.321	0.055	1.54

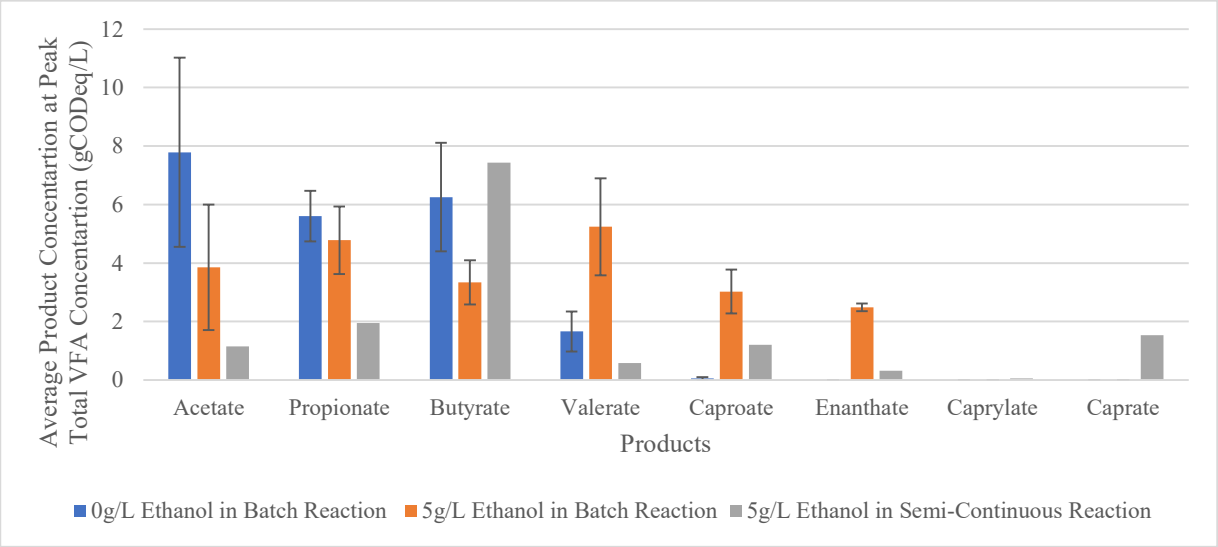

Figure 30: Soluble product concentration achieved gCODeq/L in leachate generated from 1 kg of food waste in leach bed reactor with a working volume of 3.5L (ISR 0.2 g vs/g vs). Product concentrations recorded at the peak total VFA concentration in leachate. Error bars show the maximum and minimum values observed between the two trials.

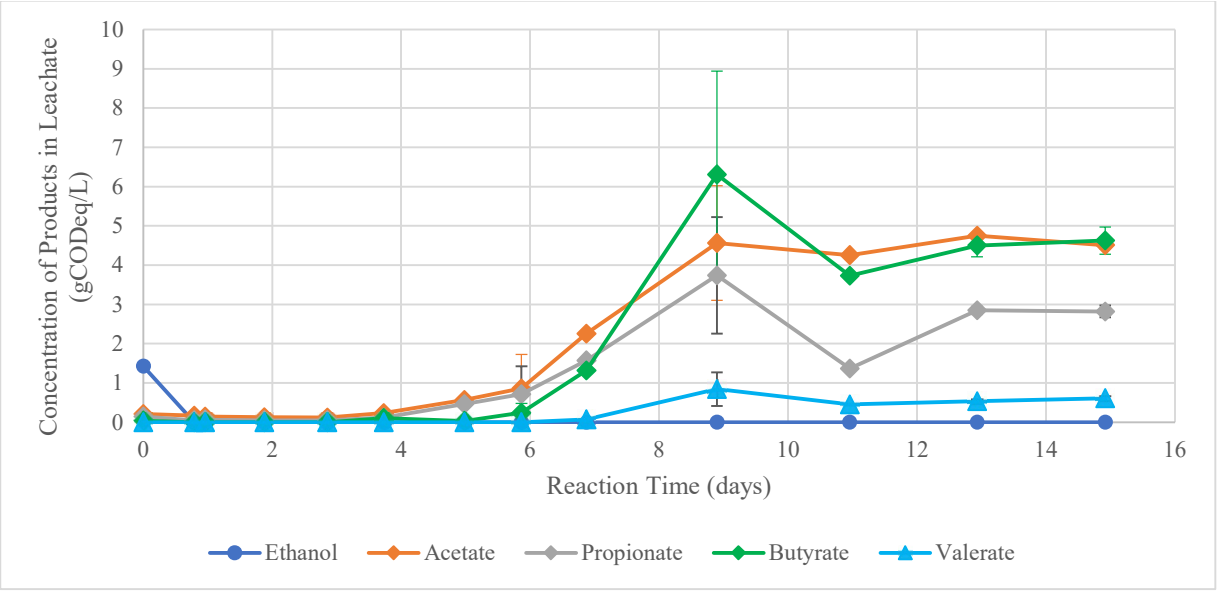

Figure 31: Soluble product concentration of most plentiful species in leachate generated from 1 kg FW in a leach bed reactor with working volume of 3.5 L with a starting ethanol concentration of 0 g/L over the course of the second batch. Error bars show the maximum and minimum of values measured by GC-FID.

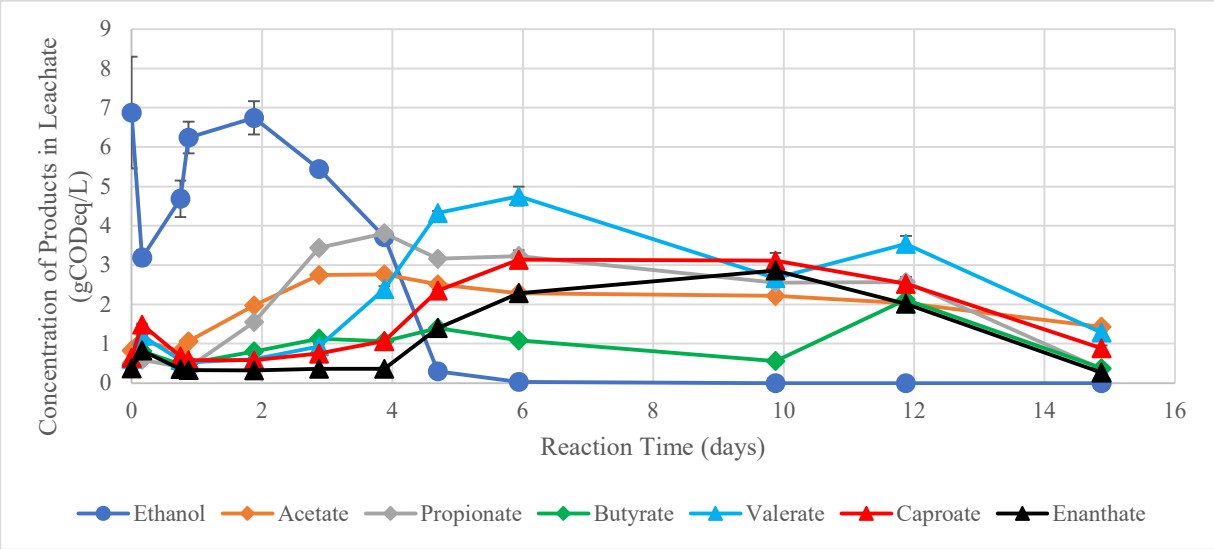

Figure 32: Soluble product concentration of most plentiful species in leachate generated from 1kg FW in a leach bed reactor with working volume of 3.5L with a starting ethanol concentration of 5g/L over the course of the second batch. Error bars show the maximum and minimum of values measured by GC-FID.

4.3.5 Gas Production

The two ethanol-free batches generated 3.2 and 0.79 L of biogas for the first and second replicates, the batches with 5 g/L of ethanol added generated 5.1 L and 1.7 L of biogas, and the semi-continuous trial with ethanol addition generated 4.0 L. The hydrogen partial pressure in each trial increased for the first two to three days then steadily fell over time. This is likely due to hydrogen consumption by bacteria in the presence of small partial pressures of oxygen. The partial pressure of carbon dioxide was the highest in the batches without ethanol addition with less CO_2 measured in semi-continuous operation than in batch operation. Lower concentrations of gases in semi-continuous operation were due to the constant removal of CO_3^{-2} saturated leachate and replacing it with basic ethanol solution with minimal dissolved CO_3^{-2}. For the same reason higher concentrations of oxygen were observed in the semi-continuous trial. The oxygen partial pressures were maintained below 0.05 atm in the reactor for most trials with oxygen concentration in batches with ethanol addition and semi-continuous operation with ethanol addition being consistently higher than ethanol free batches. The gas production overall from reactors with ethanol added was lower and hydrogen gas consumption was observed, therefore oxygen may also be over-represented due a sampling bias which draws samples from a gas line which has no mixing with reactor when the vessel is under zero or negative pressure. While continued acidogenesis and the generation of hydrogen

gas suggest that the concentration of oxygen was not high enough to allow aerobic digestion to proceed, the concentrations are more consistent with anoxic fermentation rather than true anaerobic fermentation. This mode may favour acidogenesis as methanogenesis was only observed when oxygen concentrations became very low.

In the final days of batches without ethanol addition some methane generation was observed, and this may account for the drop in fatty acid concentration as the reactor shifts to a methanogenic mode and consumes the medium-chain acids it has generated and converts them back to shorter chains. To avoid this behavior an SRT less than 14 days is likely desirable for the optimization of this process.

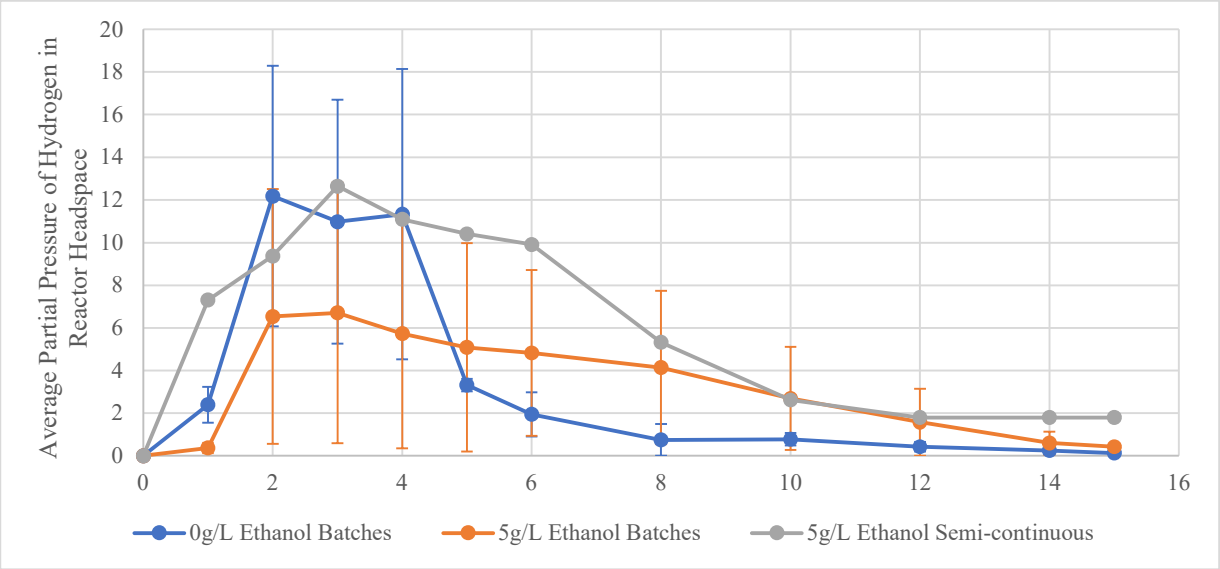

Figure 33: Average partial pressure of Hydrogen in reactor headspace for batches with starting concentrations of 0 g/L and 5 g/L and in semi-continuous operation with an ethanol concentration maintained at 5 g/L. Error bars show the maximum and minimum values observed between the two trials.

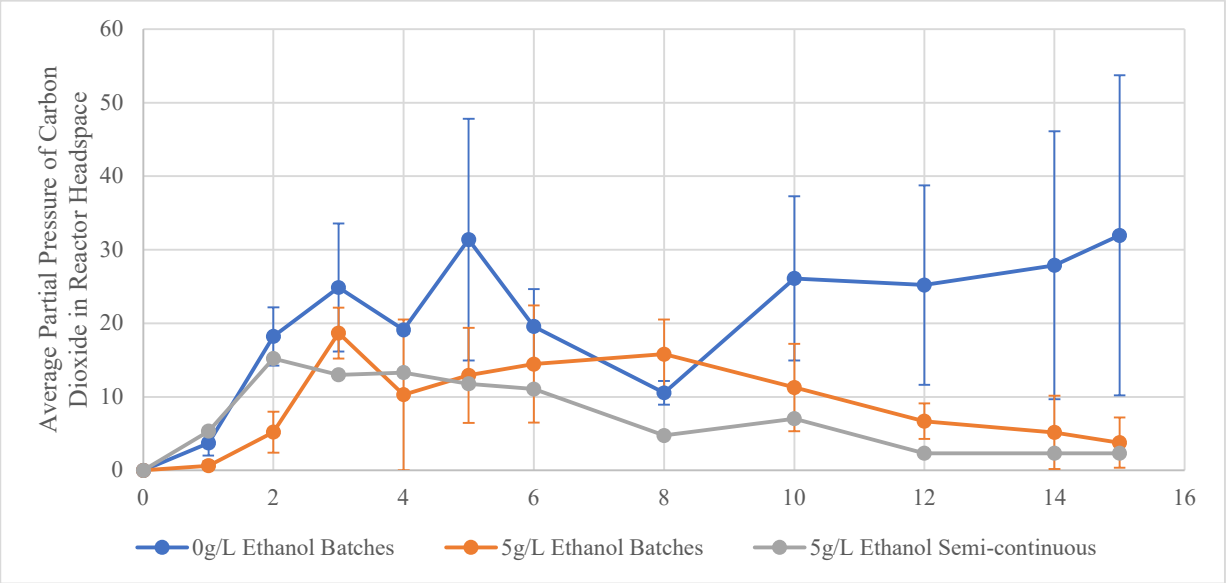

Figure 34: Average partial pressure of Carbon Dioxide in reactor headspace for batches with starting concentrations of 0 g/L and 5 g/L and in semi-continuous operation with an ethanol concentration maintained at 5 g/L. Error bars show the maximum and minimum values observed between the two trials.

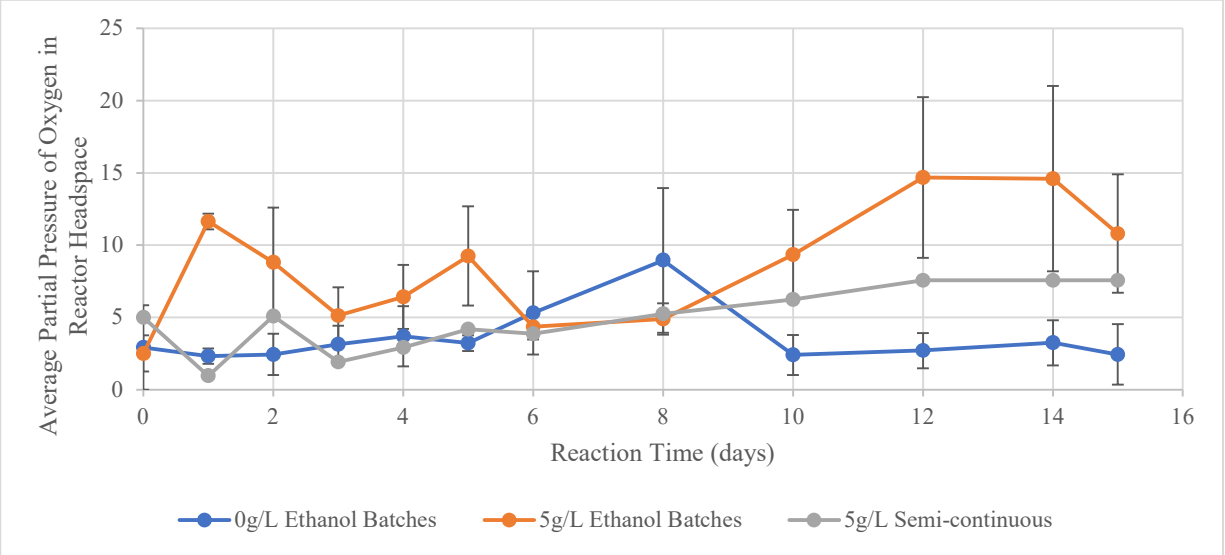

Figure 35: Average partial pressure of Oxygen in reactor headspace for batches with starting concentrations of 0 g/L and 5 g/L and in semi-continuous operation with an ethanol concentration maintained at 5 g/L. Error bars show the maximum and minimum values observed between the two trials.

4.4 Discussion

Low concentrations of ethanol in batch or semi-continuous processes work to effectively extend the chain lengths of fatty acids. This low-cost addition creates an effective method for improving the ratio of medium-chain to short-chain fatty acids, increasing the value of the leachate for extracting MCFA products and for bioplastic production. While the addition of ethanol to the fermenter achieved conversion of approximately half of the short-chain fatty acids to medium-chain fatty acids, the overall yield and efficiency of the reactor was lower than the comparable mesophilic tests performed by Nzeteu et al. (Nzeteu, Trego, Abram, & O'Flaherty, 2018). Nzeteu et al. used similar FW, a similar solids residence time, and similar inoculum to food waste ratio as well as a significantly lower recirculation rate. The high concentration of fatty acids in the Nzeteu et al. study can be attributed to partially to a higher concentration of solids, however the high yield of caproate relative to VS added suggests that VFA production was limited in this study by the low temperature. Psychrophilic conditions can be used for chain elongation, however due to the significant loss in efficiency it is unlikely to be an economically relevant pathway for industrial-scale processes.

Table 10: Comparison of caproic acid production and short-chain to medium-chain fatty acid ration of chain elongation reactors. *Grootscholten et al expressed SC:LC in g:g, all other results are reported in gCODeq/gCODeq

	Caproic Acid Concentration g/L	Caproic Acid Yield gCODeq/g VS	SC:LC
Nzeteu et al. 2018 7 days, recirculation rate of 0.05L/hr, 37C	10	0.2875	2.19:1
Grootscholten et al. 2013 15 days, 30C,	2.8	0.05545	1.22:1 *
Andersen et al. 2017 7.5 days	8.1	0.1845	1.22:1
This Study 4-6 days, 22C	1.4	0.1195	1.08:1

4.5 Conclusion

Ethanol addition to psychrophilic LBRs fed with food waste can convert up to half the VFA produced to valerate, caproate, enanthate and caprylate. These products are easier to isolate from leachate and are

valuable as commodity chemicals and as a mixed broth feedstock for generating high quality polyhydroxy-alkenoates. The reactor conditions favoured valerate production producing a maximum of 6.9 gCODeq/L of valerate in approximately five days. Ethanol did not significantly affect hydrogen production or fatty acid production rate; these parameters were dominated by quality of inoculum and FW. The hypothesis that chain-elongation was improved at low temperatures must be rejected as mesophilic chain-elongation outperforms psychrophilic chain-elongation. Since effective chain elongation was still observed the processes demonstrated in this study may present an opportunity for co-product biorefinery in existing low-temperature acidogenic fermenters.

Chapter 5
Conclusions for Acidogenic Leach Bed Reactor Scale-Up and Optimization

Acidogenic fermenters are potentially effective tools for biorefinery using mixed cultures and diverse feedstocks. Large scale fermenters are currently used as pre-treatment stage in methanogenic anaerobic digestion. These large-scale fermenters typically operate with low solids concentrations which require stirring and mesophilic conditions which require heating. Leach bed reactors are efficient acidogenic fermenters generating high concentrations of acids in leachates, using little energy for recirculation, and maintaining water use and small footprint. A better understanding of the potential and limitations of leach bed reactors will allow large scale reactors to be built and tested with minimal risk. The conclusions of this thesis can be applied to the scale up of these reactors for a range of applications, specifically for generating VFA rich broth for the production of PHBV and other PHAs using Pseudomonas putida, for generating VFA rich broth for efficient methanogenesis, for the biorefinery of fatty acids.

5.1 Conclusions for the Generation of Bioplastic

Fernández-Dacosta in a techno-economic analysis based on the generation of PHBV from wastewater sludge digestate estimated that to generate PHBV effectively the leachate must contain a least 22 g/L VFA (Fernández-Dacosta, Posada, Kleerebezem, Cuellar, & Ramirez, 2015). According to this guideline the reactors in this experiment can be economically used to generate PHAs so long as the cost of FW is negligible. The study described in chapter 3 found that a mechanical disturbance to the FW container in a leach bed reactor favoured hydrogen formation over MCFA formation, slower recirculation also allowed ethanol generating and acid generating cultures to coexist in a single reactor creating the conditions necessary for chain-elongation. Other authors have found that VFA formation is slowed in clogged leach bed reactors, the findings of this thesis suggest that disturbance of the food waste container will not be a reliable way to reduce the fermentation time if the goal is to generate a MCFA rich fermentation broth. Other methods of increasing the fermentation rate include pre-treatment of the food waste, higher temperature fermentation and culture enrichment.

The study described in chapter 4 found that small additions of ethanol to leachate (5 g/L) generated leachates which favoured producing valerate and caproate over acetate and butyrate. Valerate and caproate rich fermentation broth is necessary for generating PHBV and other medium-chain polyhydroxy alkenoates which are significantly more valuable than the acetate and butyrate derived PHB.

The study finds evidence that regardless of temperature and fermentation conditions small additions of ethanol dramatically improve the quality of PHAs in this supply chain.

If the leachate generated in this study was fed to a system like the PHA production system used by Amulya et al. and was able to maintain the same yield of 0.17 gCODeq PHA/gCOD in leachate, then approximately 6.63 gCODeq of PHA could be produced per kg of wet FW added with an SRT of 5 days. (Amulya, Srinivas Jukuri, & Venkata Monhan, 2015; Rodriguez-Pereza, Serrano, Pantión, & Alonso-Fariñasc, 2018). This is equivalent to 3.97g of polymer per kg of FW, even at the high price of $10 per kg this yields $0.04 per kg FW. Approximately 0.04 m^3 of renewable natural gas can be produced with the same kg of FW can be produced, at the current Ontario price of renewable natural gas (RNG) ($1.10/$m^3$), this bioplastic pathway is not currently an economically competitive treatment method. In markets where a premium cannot be charged for RNG there may be economic potential for bioplastic generation as an income source for FW treatment plants.

5.2 Conclusions for the Generation of Biogas

Conventional biogas generation uses the mesophilic or thermophilic anaerobic digestion process. The process uses a mixed culture of microorganisms to hydrolyse waste, generate fatty acids from the hydrolysate, convert those fatty acids to acetic acid which is then consumed by methanogens to generate methane and CO_2. Specific acidogenic reactors are used in biogas generation in two ways, improving conditions for complete acidogenic fermentation of waste and capturing hydrogen generated as biogas. Hydrogen is a potential substitute for methane as a fuel in most applications and can be blended with methane. This substitution is being used by gas systems as it reduces the carbon dioxide emitted by burning the fuel, reducing the environmental impact of burning biogas. Hydrogen is also a valuable industrial input which is typically generated from fossil fuels, if pure fossil-free hydrogen can be produced from fermentation it can replace so-called grey hydrogen and its associated environmental costs. The study described in chapter 3 found that hydrogen production was improved when the FW container was disturbed by inverting or stirring the container once per day. This disturbance could be incorporated into an industrial scale reactor through a live bed or screw conveyor system using substantially less energy than the constant mixing required in stirred or upflow tank. The study suggests that this reactor design could maintain a high solids content and convert nearly all the solids to a highly concentrated leachate which would then be efficiently digested in an anaerobic digestor or hydrogen specific fermenter. Long and medium-chain fatty acids are detrimental to the efficiency of the methanogenic and hydrogen generating fermenters as they increase the fermentation time required for “aceotogenisis” the conversion of fatty acids to acetic acid before conversion to gas. Even small concentrations of ethanol were found to

significantly increase the amount of valerate and caproate, therefore it is recommended that conditions which encourage ethanol formation be avoided. The disturbance of the FW container was also found to effectively reduce the ethanol concentration, an additional reason that container disturbance should be considered in the design of acidogenic fermenters optimized for biogas formation. While the findings of this work can be applied to the biohydrogen generating reactors, the highest hydrogen yield observed was 11 cm^3/g of VS. This yield is much lower than the 50-70 cm^3/g of VS found in previous studies. The relatively low hydrogen production is likely caused by hydrogen consuming bacteria in the reactor and the relatively low temperature of the fermentation.

5.3 Conclusions for the Biorefinery of Fatty Acids

The biorefinery of fatty acids has been identified as a potential pathway for value recovery from food waste. Challenges to this biorefinery pathway include the need to incorporate heterogeneous feedstocks, the need to achieve high fatty acid concentrations, and the need to achieve low purification costs. The addition of ethanol can lower the purification cost by increasing the fraction of less soluble medium-chain length fatty acids. In these studies the highest concentration of MCFA observed was 6.23 g/L, this was composed of mostly valerate with caprate and enanthate making up the remainder. This is lower than the yields demonstrated in the 37°C reactor tested by Nzeteu et al. which produced 10g/L of MCFAs producing composed of only caproate (Nzeteu, Trego, Abram, & O'Flaherty, 2018). The disturbance of the FW container will not improve the reactors potential for biorefinery as it does not increase the concentration of fatty acid produced unless other inhibitory factors are addressed. The highest fatty acid concentration achieved in the study was 30 gCODeq/L with most tests reaching approximately 20 gCODeq/L never reaching the 40-60 gCODeq/L seen in mesophilic and thermophilic reactors. VFA yields all fell in the lower end of the typical range, varying from 0.45-0.7 gCODeq/g VS added. No interventions increased the yield from the control. Variability was observed between trials associated with the inoculum and food waste used. Previous authors have identified bioaugmentation, the addition of specific concentrated microbial culture to mixed culture fermentation, as a potential path for improving VFA yield. While this study shows that several psychrophilic organisms can effectively produce fatty acids from food waste, work is required to optimize inoculum in large scale reactors as the technology develops.

5.4 Concluding Remarks

The strength of AF in mixed culture lies in its simplicity and resilience to change. AF is performed by diverse range of organisms many of which are spore forming and can survive extreme pH and temperature shocks. In addition, many of these mixed culture bacteria perform well in LBRs which

require minimal instrumentation for control and mixing - further lowering potential operating costs. This thesis has addressed how acidogenic reactors work at the limits of operation.

In this thesis three hypotheses were tested: that reactor clogging changes the metabolic processes of fermentation by changing the microbial community and the dominant enzymatic pathways; this a clogged leach bed reactor performance is improved by a mechanical disturbance; and that chain elongation using ethanol in an acidogenic fermenter was improved at temperatures below 25°C. The studies of reactor clogging were performed by iterating the reactor design, testing interventions to prevent clogging and monitoring how product formation and reactor kinetics were affected. Changes in the gas production, in substrate solubilization, and in cell growth kinetics suggest that clogging does change the microbial community. This change in the microbial community in the disturbed reactor improved the gas production rate and therefore reactor performance is improved by a disturbance for this application. The production of fatty acids in clogged and unclogged reactors was the same, so a disturbance is not useful for bioplastic production or fatty acid isolation. To test the performance of chain elongation at low temperatures an LBR was operated at 22°C starting with and without 5g/L ethanol in the leachate. Almost 50% of the fatty acids in the leachate were elongated into valerate, caproate, and enanthate. While chain elongation appears to work well at this temperature the initial production of fatty acids was dramatically lower than higher temperature reactors. Due to this low fatty acid production rate low temperatures are not preferable to produce MCFAs.

This technology is currently at TRL6 and to extend to TRL7 a complete FW to bioproduct system must be developed at pilot-scale. Clogging will affect fermentation with a FW container deeper than 4 cm with certain food wastes. Pilot reactor designs might incorporate a live bed that can disturb the food waste at the bottom of the container. To better understand the behavior of pilot-scale reactors, monitoring of conditions withing the bulk of the food waste will likely show useful insights – as this work demonstrates, conditions can vary between the FW container and the leachate. An investigation into the composition of the microbial cultures and enzymatic pathways favored by different infiltration rates and reactor modes would be valuable to understand how physical reactor design can be used to achieve consistent products from mixed cultures. If MCFAs are desired from a pilot-scale fermenter, chain elongation will not be stopped by wide variations in temperature. However, if low solids residence times are desired then temperatures should be maintained above 30°C and high ratios of desired products should be sought in the purification stage which must follow the fermentation to optimize the bioplastic generation process. Further work into systems which can remove and concentrate fatty acids from solution during fermentation must be done to explore the potential of bioplastic generation from waste as well as the potential of fatty acids as a revenue stream for organic waste treatment plants.

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