ABSTRACT

Title of Thesis: GENERATING BIOENERGY AND HIGH-VALUE PRODUCTS FROM HIGH SALINITY FOOD WASTE

Emily Lim McCoy, Master of Science, 2024

Thesis Directed By: Professor Stephanie Lansing, Department of Environmental Science and Technology

Bioenergy generation and volatile fatty acids (VFAs) production from household food waste and high salinity food processing waste were explored using anaerobic digestion and dark fermentation processes, respectively. This study tested adding value to three organic waste streams: household food waste, high salinity food processing waste (composed of glycerin sludge from biodiesel production), and residual solids from VFAs separation after dark fermentation of food waste. The investigations were conducted using batch and semi-continuous systems in mesophilic conditions (35°C). Methane (CH_4) potential tests were conducted to determine the bioenergy production of food waste and residual solids, including the addition of dark fermentation gas at four ratios of hydrogen (H_2) to carbon dioxide (CO_2) (1:1, 1:2, 1:3, 1:5) into the liquid portion of the reactor to enhance CH_4 production and three inoculum to substrate ratios (1.5:1, 2:1, 4:1). Additionally, a semi-continuous dark fermentation study was used to determine the VFA production from household food waste and high salinity food processing waste combinations over 62 days.

The anaerobic digestion of residual solids from VFAs separation had similar bioenergy potential as household food waste when normalized by volatile solids (VS) added (492 ± 11 mL

CH_4/g VS and 470 ± 11 mL CH_4/g VS, respectively). Dark fermentation gas added into the liquid portion of the reactor during anaerobic digestion decreased CH_4 yields, especially at low H_2:CO_2 ratios, suggesting that only dark fermentation reactors that produce high H_2:CO_2 ratios should have the gas sparged into anaerobic digestion systems. When the residual solids from dark fermentation were fermented at three inoculum to substrate ratios (1.5:1, 2:1, 4:1), the lowest inoculum to substrate ratio (1.5:1) had the highest VFAs concentration (28.05 ± 0.89 g/L) after nine days of fermentation, which showed that residual solids can be fermented with low inoculum levels, allowing more room for substrate fermentation.

Additionally, the mono- and co-fermentation of household food waste and high salinity food processing waste showed that the high salinity waste improved VFA production due to the high pH (9 – 10) and high organic loading (6.3 – 17.8 g VS/L-day), even with high salinity levels (21.4 – 85.6 g/L Na) in this waste. There was significantly higher VFA production in high salinity food processing waste (36.04 ± 0.54 g/L) compared to household food waste (9.29 ± 1.01 g/L). The maximum VFA concentration (36.04 ± 0.54 g/L) was achieved after 51 days of high salinity food processing waste semi-continuous fermentation.

The findings in this study can be used to improve operations of anaerobic digestion and dark fermentation systems by using residual solids for bioenergy generation or VFA production. The testing of mono- and co-fermentation of household food waste and high salinity food processing waste showed high VFA production in fermenting high salinity food processing waste. This work showed the valorization of three organic waste streams through bioconversion to both bioenergy and high-value products (VFAs), which redirected these waste products from municipal solids landfills and into resources, thereby reducing CH_4 released into the atmosphere from landfills and reducing global warming potential.

GENERATING BIOENERGY AND HIGH-VALUE PRODUCTS FROM HIGH SALINITY FOOD WASTE

by

Emily Lim McCoy

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Advisory Committee:
Professor Dr. Stephanie Lansing, Chair
Associate Research Scientist Dr. Amro Hassanein
Assistant Professor Dr. Guangbin Li

Dedication

This thesis is dedicated to my family, friends, and most importantly, my forever-puppy Belle. My family assisted my undergraduate education at the University of Maryland which led me to the 4+1 program in the ENST department and graduate school. My friends, Sarah Na, Juliana Guerra, and Dylan Celli, who were always there for me throughout this entire journey. Thank you for being my support system as I figured out how to navigate through graduate school. Thank you, Belle, for being the angel that you are and bringing me lots of joy and happiness every time I visit home.

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List of Abbreviations

Anaerobic Digestion (AD)

Anaerobic Dark Fermentation (ADF)

Biochemical Methane Potential (BMP)

Carbon Dioxide (CO_2)

Chemical Oxygen Demand (COD)

Dark Fermentation (DF)

Food Waste (FW)

High Salinity Food Processing Waste (HSFW)

Hydraulic Retention Time (HRT)

Hydrogen (H_2)

Inoculum to Substrate Ratio (ISR)

Methane (CH_4)

Microaerobic Dark Fermentation (MADF)

Organic Loading Rate (OLR)

Soluble Chemical Oxygen Demand (sCOD)

Standard Error (SE)

Total Solids (TS)

Volatile Fatty Acids (VFA)

Volatiles Solids (VS)

Chapter 1: Introduction

1.1 Impact of Food Waste in the United States

The increasing temperatures caused by greenhouse gas (GHG) emissions are becoming a major concern worldwide. The rate of warming temperatures has tripled from 0.06 to 0.20°C per year since 1982, with this past decade having the top ten warmest years since 1850 with 2023 being the highest (Lindsey et al., 2024). Anthropogenically produced GHG emissions, including carbon dioxide (CO_2), methane (CH_4), nitrous oxide, and fluorinated gases, are the main cause of rising temperatures (US EPA, 2024d; Lindsey et al., 2024). In 2021, the United States (US) contributed 14% of global CO_2 emissions, and transportation (28%), electric power (25%), industry (23%), and agriculture (10%) contributed to the total GHG emissions (Ritchie et al., 2023; US EPA, 2024e). The GHG emissions from the US are comprised of 79% CO_2, 12% CH_4, 6% nitrous oxide, and 3% fluorinated gases (US EPA, 2024d). Although CH_4 emissions are 67% less than CO_2, it has 25 times more global warming potential than CO_2 (US EPA, 2023c). The three main contributors of CH_4 in the US are natural gas and petroleum systems (29%), enteric fermentation (25%), and landfills (15%) (US EPA, 2024d).

The US disposes 40% of produced food, resulting in $218 billion (USD) of economic losses annually (US EPA, 2024c). The EPA Wasted Food Report (2023) found that most wasted food comes from households (40%), manufacturing and processing (38%), and restaurants (28%) (US EPA, 2023a). There are several disposal routes, such as food donations, animal feed, and composting. However, the main methods for food waste disposal in the US are landfill (60%) and controlled combustion (15%) (US EPA, 2023a). Food waste constitutes 24% of municipal solid waste landfills and decays quickly due to conducive temperature, moisture, and pH conditions (J. Buzby, 2022; US EPA, 2023a). Food waste results in 58% of CH_4 emissions from

municipal solid waste landfills (US EPA, 2023a). The most common food waste disposal methods, landfills and controlled combustion, contribute to GHG emissions but proper treatment of food waste can be expensive. Potential solutions include anaerobic digestion and dark fermentation, which are biological processes that can treat food waste while producing bioenergy and high-value products.

1.2 Anaerobic Digestion of Food Waste

Anaerobic digestion (AD) is a biological process that utilizes bacteria to break down organic matter, i.e. volatile solids (VS), such as animal manure and food waste, into products such as CH_4-rich biogas and fertilizer. The AD process involves four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolytic bacteria use extracellular enzymes to convert complex organic material into soluble substrates: carbohydrates, fats, and proteins. During acidogenesis and acetogenesis, bacteria use soluble compounds from hydrolysis and turn them into CO_2, hydrogen gas (H_2), and volatile fatty acids (VFA), i.e., short-chain organic acids. Methanogenesis is the last step, where methanogenic bacteria convert the H_2 and VFAs into biogas, mainly CH_4 and CO_2, with trace amounts of hydrogen sulfide and ammonia (Hassanein et al., 2022).

Anaerobic digesters are the systems that contain the organic waste, process it through AD, and collect any biogas or fertilizer that exits the system. There are three main types of anaerobic digesters: stand-alone, on-farm, and wastewater treatment plants (US EPA, 2023b). Stand-alone digesters can accept various feedstocks for tipping fees, mainly waste from the food and beverage industry; however, co-digestion with other organic materials such as yard waste and wastewater solids is common (US EPA, 2023b). On-farm digesters handle manure, primarily dairy and hog farms, or crop residues. Digesters at wastewater treatment plants process

wastewater solids (US EPA, 2023b). As of 2023, there are 473 operational on-farm AD systems, 1,269 wastewater digesters, and 102 stand-alone food waste digesters in the US (US EPA, 2024a). Anaerobic digesters produce biogas that can be converted into electricity and used either on-site or sold to the electrical grid. The electricity output from digesters can be impactful. In 2022, AD systems generated 2.42 million MWh of electricity, which can power 220,469 homes annually (US EPA, 2024a, 2024b). Manure-based AD systems reduced GHG emissions by 10.4 million metric tons of CO_2 equivalent ($MMTCO_2e$) and 9.5 $MMTCO_2e$ of direct CH_4 emissions, which is equivalent to not using 71 coal-fired power plants for a year (US EPA, 2024a, 2024b).

Several parameters are involved in designing anaerobic digesters that influence bioenergy production in the form of CH_4, such as temperature, pH, organic loading rate (OLR), and feedstock. There are two main temperature ranges in which CH_4-producing bacteria thrive: mesophilic (30 – 40°C) and thermophilic (50 – 60°C). Several studies report that thermophilic AD has a CH_4 yield 17 – 20% higher than mesophilic AD (Balasundaram et al., 2024; H. He et al., 2023; L. Zhang et al., 2019). An additional benefit of thermophilic AD is foodborne pathogen reduction (Pandey et al., 2016). While CH_4 yields can be improved through thermophilic temperatures, additional energy consumption can increase overall costs with increasing digestion temperatures (Ghanimeh et al., 2019). The pH can also determine the success of an anaerobic digester. Several studies suggest that a pH range of 6.0 to 8.0 is best for CH_4-producing bacteria; however, it varies among substrates such as tannery waste sludge, dairy manure, and food waste (Mpofu et al., 2020; Witarsa & Lansing, 2015; Zou et al., 2020). For example, the tannery waste sludge achieved 108 – 118 mL CH_4/g VS with a pH at 6.5 and the food waste achieved 402 mL CH_4/g VS with a pH at 5.0 (Mpofu et al., 2020; Zou et al., 2020). The pH at 5.0 is below the methanogenic bacteria pH range, but food waste produced more CH_4

than tannery waste sludge, most likely due to the feedstock type.

The OLR can also fluctuate depending on digester size and feedstock. Blasius et al. (2020) and Liu et al. (2017) tested food waste digestion with 250 and 400 mL of reactor volume and OLRs of 0.90 and 1.5 g VS/L-day, respectively. Liu et al. (2017) had 38% more reactor volume and 40% greater OLR but only achieved 5% more CH_4 (391 mL CH_4/g VS) than Blasius et al. (2020) (371 mL CH_4/g VS). Blasius et al. (2020) had a food waste mixture comprised of only vegetables while Liu et al. (2017) digested a combination of rice, meat, noodles, eggs, and vegetables. Food waste composition and digester design (lab-scale and pilot-scale) will impact the OLRs and overall CH_4 production (Jiang et al., 2022; Malinowsky et al., 2021; Parajuli et al., 2022).

There are challenges to standardize AD parameters, such as pH and OLR, due to fluctuations based on feedstock types. More research is needed into how various feedstocks, pH, OLR, and temperature affect food waste digestion to increase the number of AD systems that accept food waste, as only 5.5% of operational digesters in the US process food waste (US EPA, 2024a).

Technologies that improve CH_4 production, including H_2 gas sparging, have been tested to increase the percent CH_4 in biogas produced from AD systems. Deschamps et al. (2021) studied cattle manure AD in two anaerobic digesters. The first digester was fed with fresh cattle manure while the second was fed with the digestate of the first digester and pumped with pure H_2 gas. After two years of continuous biomethanation, the CH_4 production increased by 32.8% and the percent CH_4 increased from 67% to 98%. This is attributed to the Sabatier process, where four H_2 molecules react with one CO_2 molecule to form one CH_4 and two water molecules (Wang et al., 2022). The increase in percent CH_4 is promising, but there is an issue of high H_2

partial pressure when injecting H_2 gas. Typical homoacetogenesis, the process where acetogenic bacteria release H_2 and CO_2, functions below a H_2 partial pressure of 10 Pa (Fukuzaki et al., 1990). When excess H_2 is introduced, it increases the rate of homoacetogenesis and consumes 35% more H_2 than typical homoacetogenesis in AD (R. Liu et al., 2016). This can result in lower CH_4 outputs with high H_2 partial pressure.

Other studies have tested combinations of H_2 and CO_2 gas injections into anaerobic digesters to avoid high H_2 partial pressure from pure H_2 gas sparging. Kozak et al. (2022) tested the difference in CH_4 production in municipal wastewater digestion with H_2 and CO_2 injections at mesophilic and thermophilic temperatures. At a 4:1 H_2:CO_2 injection ratio, a percent CH_4 of 79% at mesophilic conditions and 81% at thermophilic conditions was accomplished. Bassani et al. (2015) achieved a percent CH_4 of 89% in mesophilic and 85% in thermophilic at the same 4:1 H_2:CO_2 ratio. Kozak et al. (2022) and Bassani et al. (2015) increased CH_4 content from typical AD without gas injections which is 50 – 75% CH_4 (US EPA, 2024a). Previous studies have not investigated the H_2 and CO_2 ratios seen from dark fermentation systems (1:5 H_2:CO_2 ratio).

The literature showed successful bioenergy generation from various feedstocks by adjusting the temperature, pH, OLR, and H_2 sparging additions. Common feedstocks include wastewater, manure, and yard waste. The feedstock is a major parameter in how an anaerobic digester functions due to the inherent pH and organic content. A challenging feedstock is food waste due to the fluctuation of composition in vegetables, fruits, grains, proteins, and dairy products. Food waste anaerobic digesters can be difficult to operate as each one must be tested based on the composition of the food waste feedstock. This may be a reason why operational stand-alone food digesters in the United States are 78 and 92% fewer than on-farm manure and wastewater digesters, respectively (US EPA, 2024a). Further insights into food waste AD,

including H_2 and CO_2 sparging for bioenergy enhancement, can improve digester performance and redirect more food waste from landfills to AD for bioenergy.

1.3 Dark Fermentation of Food Waste

Dark fermentation comprises the first three steps of AD (hydrolysis, acidogenesis, acetogenesis) but inhibits methanogenesis, resulting in CO_2, H_2, and VFAs (Srisowmeya et al., 2020). There are strategies to inhibit methanogens from converting VFAs to CH_4 so the intermediate products, VFAs and H_2, can be utilized as high-value products such as pharmaceuticals, bioenergy, and bioplastics (Agnihotri et al., 2022). This includes operating at a low hydraulic retention time (HRT) of 3 – 10 days and a low pH of 5.0 – 6.0. However, these parameters can vary depending on the substrate including food waste, wheat powder, cheese whey powder, molasses wastewater, and rice winery wastewater (Elbeshbishy et al., 2017; Lim et al., 2008; Swiatkiewicz et al., 2021; Weide et al., 2019). For example, Lim et al (2008) reported that the dark fermentation of food waste had a maximum VFA production of 25 g/L VFAs at 35°C with an 8-day HRT and a pH at 6. However, sugary wastewater produced 42% less VFAs (14.5 g/L VFAs) with a 7-day HRT and a pH of 7 (Weide et al., 2019).

The same parameters that influence anaerobic digesters also impact dark fermentation reactors. The temperature, pH, HRT, and OLR can determine the amount of VFA production. A lower pH of 5.0 – 6.0 can prevent methanogenic bacteria growth in a dark fermentation reactor, but there has been success in VFA production with pH at 6.0 – 8.0 (Grzelak et al., 2018; Hemalatha et al., 2019; Sarkar & Venkata Mohan, 2020; Slezak et al., 2021). For example, Hemalatha et al. (2019) observed a total of 1.3 g/L VFAs with a heat shock-pretreated culture of Azolla biomass at a pH of 6 and 8, a 48 h HRT, and mesophilic (37°C) conditions. Sarkar & Mohan (2020) had a maximum VFA production of 5.4 g/L VFAs from synthetic wastewater

processed by facultative anaerobes in an anaerobic microenvironment with a pH at 6.5, a 48 h HRT, and ambient conditions (28 ± 2°C). Sarkar et al. (2020) exceeded both these values with 6.5 g/L VFAs in food waste fermentation in a saline microenvironment with a pH at 6, a 48 h HRT, and ambient (28 ± 2°C) conditions. Dark fermentation of kitchen-based food waste in mesophilic conditions yielded higher VFAs compared to other substrates, such as the Azolla biomass and wastewater. Grzelak et al. (2018) found that dark fermented food waste at a pH 7 and 8 produced 19.5 g/L VFAs within 48 h. Slezak et al. (2021) also analyzed dark fermented kitchen-based food waste with a pH at 8 and 4-day HRT, resulting in 13.9 g/L VFAs. These studies demonstrated that pH and HRT influence the VFA production of fermented organic matter. Food waste with an acidic pH (5), fermented at longer HRTs (15 days) has not been explored to determine if longer fermentation periods with acidic pHs can achieve high VFAs values.

The OLR in dark fermentation can be much higher than AD, as the biological process can manage higher loads in a short period (3 – 10 days). A wide range of OLRs have been tested, including 5.0, 25.5, and 48.2 g VS/L-day (Slezak et al., 2017, 2021; Swiatkiewicz et al., 2021). Slezak et al. (2017) found a maximum of 9.81 g/L VFAs from an OLR of 48.2 g VS/L-day after 4 days of kitchen-based food waste dark fermentation. More recently, Slezak et al. (2021) observed a maximum of 13.9 g/L VFAs from an OLR of 25.5 g VS/L-day at a 4-day HRT from a similar substrate. At a much lower OLR (5 g VS/L), Swiatkiewicz et al. (2021) achieved a maximum of 22.3 g/L VFAs at a 10-day HRT with food waste dark fermentation. In addition to the previously mentioned substrates, duckweed, brewery wastewater sludge, and defatted algal biomass have been tested for VFA production through dark fermentation (Kumar et al., 2018; Mu et al., 2020; Sim et al., 2020; L. Zhang et al., 2020). As shown in these studies, OLR varied

among substrates and impacted the VFA production. The capacity to handle higher OLRs in dark fermentation could be a potential solution for managing large loads of food waste, as AD is limited in the amount of food waste that can enter the system and is a 20 – 30 days slower process.

Dark fermentation can divert food waste from landfills while producing high-value products like VFAs that can be used for biodegradable bioplastics production. The VFAs can be used by specific strains of bacteria cultures that can produce and accumulate polyhydroxyalkanoate (PHAs), which are the monomers for synthesizing biodegradable bioplastics (Gottardo et al., 2022; Lagoa-Costa et al., 2022; Nielsen et al., 2017). The process of food waste dark fermentation for VFA production results in a liquid and solid slurry. The slurry is centrifuged, and the two fractions are physically separated. The liquid portion has accessible VFAs that can be processed for PHA production. The remaining solid fraction is a by-product of this system and is not used further. These remaining solids, i.e. residual solids, are carbon-rich and can be fermented for VFAs or digested for bioenergy. Another by-product of food waste DF for VFA production is the DF gas composed of H_2 and CO_2. This gas can be incorporated into AD to enhance bioenergy outputs. The literature has not extracted residual solids from the VFA separation process for additional products or used DF gas as a source of H_2 and CO_2 for CH_4 production. Optimizing this system can improve bioplastics and bioenergy outputs and create cost-competitive products against fossil fuel-based plastics and energy while simultaneously diverting food waste from landfills.

1.4 Dark Fermentation of High Salinity Food Processing Waste

Biodegradable plastics made from PHAs can be formed by halophilic bacteria that requires high salinity to survive. Creating PHAs from a halophilic bacteria requires additional materials

and costs from the addition of salt. One solution is to replace fresh salt with high salinity food processing waste for fermentation to decrease salt additions in the PHA production process with high salinity food processing waste providing the salinity required for halophilic bacteria. However, fermentation of high salinity food processing waste can be challenging, as the salinity disrupts cellular osmolarity (Gunde-Cimerman et al., 2018).

High salinity food processing waste can be found in kitchen seasonings with high levels of sodium chloride (NaCl), seafood processing facilities (Y. Wang et al., 2023; L. Zhang et al., 2016), or from processing glycerin from food oil-derived biodiesel. Previous studies have used sodium chloride (NaCl) to test salinity ranges from 0 to 50 g/L NaCl to determine the effect of salinity on VFA production in dark fermentation. Some studies found an indirect relationship between increasing salinity and decreasing VFA production, as the salt inhibited acidogenesis (Huang et al., 2022; N. Liu et al., 2017). Huang et al. (2022) tested the anaerobic fermentation of synthetic kitchen waste comprised of rice, noodles, vegetables, and pork, with six different salinity levels ranging from 0 to 20 g/L NaCl. The batch experiment concluded that low-salinity treatments (0 to 6 g/L NaCl) reached at least 80% of the VFA of regular food waste in three days, while high-salinity treatments (10 to 20 g/L NaCl) only produced 43% of the VFAs of regular food waste. Liu et al. (2017) conducted a similar study using a synthetic food waste mixture comprised of rice, noodles, cabbage, and pork, fermented at four salinity levels of 3, 6, 9, and 12 g/L NaCl. The highest salinity of 12 g/L NaCl produced below 5 g/L VFAs while lower salt concentrations of 3, 6, and 9 g/L NaCl produced 36.2, 33.4, and 22.7 g/L VFAs, respectively.

Conversely, another study found that the salinity increased VFA production by 8% due to increased dehydrogenase enzyme activity, a microbial activity indicator (Sarkar et al., 2020).

Sarkar et al. (2020) used another type of food waste with vegetables, meat, and boiled spices, in combination with seven NaCl concentrations ranging from 1 to 50 g/L. The second highest salinity concentration (40 g/L NaCl) produced the highest amount of VFAs at 6.6 g/L after 48 h of fermentation. Lower concentrations at 0 to 30 g/L NaCl ranged from 6.1 to 6.5 g/L VFAs while the 50 g/L NaCl concentration had the least amount of VFAs at 5.9 g/L VFAs. The results from Sarkar et al. (2020) showed a positive relationship with high salinity, however, the total VFAs produced were 82% lower than presented in Liu et al. (2017) which had a maximum of 36.2 g/L VFAs at a 3 g/L NaCl. The mono-fermentation of salty food waste produced high amounts of VFAs ranging from 22.7 to 36.2 g/L VFAs as salinity decreased from 20 to 0 g/L NaCl as a result of cellular osmolarity disruption from high salinity. A potential solution to this inhibition is the co-fermentation with other organic substrates.

Co-fermentation of salty food waste and waste activated sludge was shown to improve hydrolysis—the rate limiting step of dark fermentation—but there was a limit as to how much salt was beneficial until it inhibited the microorganisms (10 g/L NaCl) (Li et al., 2021). The highest VFAs achieved in Li et al. (2021) was 29.5 g/L VFAs after two days of fermentation at 10 g/L NaCl. When the salinity increased to 20, 30, 40, and 50 g/L NaCl, the VFA production decreased by 22, 14, 26, and 73%, respectively. The results demonstrated the benefit of salt in solubilizing organic material for food waste fermentation and the disruption of cell envelopes of the waste activated sludge, but only under low salinity concentrations. The fermentation of both substrates under low salinity conditions improved solubilization therefore, increased VFA production.

Other sources of high salinity waste can be obtained from glycerol, a by-product of biodiesel production. Over the past decade, glycerol production has notably increased due to the growing

demand for biodiesel (Attarbachi et al., 2023). The biodiesel production process entails high-speed centrifugation of vegetable oils and animal fats, separating methyl ester fatty acids and crude glycerol into distinct layers (Bagnato et al., 2017). Crude glycerol contains both salt and organic matter, which present opportunities for fermentation into valuable VFAs (Anitha et al., 2016). Previous studies encountered challenges in mono-fermentation of glycerol for VFA production from impurities that impact cell growth (Kaur et al., 2020; Montiel-Jarillo et al., 2021; F. C. Silva et al., 2013); however, recent efforts have explored strategies such as controlled oxidation-reduction potential, direct interspecies electron transfer (DIET), and two-stage AD to enhance glycerol processing (Im et al., 2019; Silva et al., 2018; Vesga-Baron et al., 2021). Co-fermentation with another waste stream, like regular food waste, can be another potential solution, but has not been explored in the literature. The co-fermentation of food waste and high salinity food processing waste can divert two abundant organic wastes from landfills to VFAs, that can be converted into bioplastics and create a competitive product against fossil fuel-based plastics.

1.5 Study Objectives

Understanding how to manage food waste and high salinity food processing waste through biological processes such as AD and dark fermentation can remove these abundant organic wastes from landfills, reducing the greenhouse gas impact of landfills. In addition, bioenergy and bioplastics can be produced as a result of processing these organic wastes, creating competitive products against fossil fuel-based plastics and energy. There are challenges with digesting and fermenting food waste and high salinity food processing waste such as inhibition from high organic loads, acidity, and high salinity. This research determined how to combat these challenges through co-fermentation. Additionally, dark fermentation of food waste for bioplastic

production only used the VFA-rich liquid fraction, resulting in two main by-products including dark fermentation gas comprised of H_2 and CO_2 and remaining solids after the VFA separation process. This research valorized the residual solids by-product through dark fermentation for further VFA production, and AD for bioenergy production. This study also tested the dark fermentation gas by-product by incorporating it in AD to enhance bioenergy production. Managing the challenges in AD and dark fermentation of food waste and high salinity food processing waste and repurposing the by-products from food waste dark fermentation for VFA production could reduce the amount of organic waste entering landfills and decrease the dependence on fossil-fuels.

The first goal of this research was to determine how the by-products of food waste dark fermentation for VFA production could be incorporated into digestion for bioenergy and fermentation for VFA production. This research focused on the efficacy of residual solids AD compared to unfermented food waste AD for bioenergy production in the form of CH_4 with dark fermentation gas incorporation. Additionally, the VFA production from residual solids dark fermentation was compared to unfermented food waste. Understanding how residual solids and dark fermentation gas could be incorporated into anaerobic digesters and dark fermentation reactors could improve food waste management.

The second goal of this research was to provide knowledge on food waste and high salinity food processing waste (derived from glycerin processing waste) mono-fermentation and co-fermentation for VFA production. This research determined how co-fermentation of these organic wastes could combat the challenges of high organic loads, acidity, and high salinity. The mono-fermentation of food waste was set to expand previous literature and the co-fermentation with high salinity food processing waste would fill a literature gap. Determining how to handle

these challenging substrates could expand the application of managing food waste and high salinity food processing waste through dark fermentation for VFA production to be converted into bioplastics, creating a competitive product against fossil fuel-based plastics.

The results from this study could be used to further the understanding of food waste and residual solids AD and dark fermentation, dark fermentation gas integration in AD systems, and high salinity food processing waste mono- and co-fermentation with regular food waste.

Chapter 2: Anaerobic Digestion and Dark Fermentation of Food Waste and Residual Solids

2.1 Introduction

Food waste is a growing concern in the United States (US) as 40% of produced food is wasted, resulting in $218 billion (USD) in economic losses annually (US EPA, 2024c). Landfills receiving organic waste produce methane (CH_4), a greenhouse gas (GHG) that has 25 times more global warming potential than carbon dioxide (CO_2) (US EPA, 2019). Anaerobic digestion (AD) produces bioenergy from organic feedstock, such as food waste, while dark fermentation (DF) produces volatile fatty acids (VFAs), which could be used in bioplastics, chemicals, or other value-added product formation. The AD process involves four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During acetogenesis, bacteria use soluble compounds from two prior steps to form hydrogen gas (H_2), CO_2, and VFAs i.e. short-chain organic acids. The combination of these three steps, hydrolysis, acidogenesis, acetogenesis, is consideration dark fermentation (DF) (Srisowmeya et al., 2020). If the products of DF are used by methanogenic bacteria to produce biogas, composed of 50 – 75% CH_4, then the process is referred to as AD (Hassanein et al., 2022).

There are strategies to inhibit methanogens from converting VFAs to CH_4 so the intermediate products can be utilized for high-value products, such as polyhydroxyalkanoate (PHA) production, which are the monomers for synthesizing biodegradable plastics (Gottardo et al., 2022). This includes operating at a low hydraulic retention time (HRT) of 3 – 10 days and a low pH at 5.0 – 6.0 (Elbeshbishy et al., 2017; Lim et al., 2008; Swiatkiewicz et al., 2021; K. Wang et al., 2014). The VFA-rich liquid product from DF is separated from any remaining solids, i.e. residual solids, with the liquid portion used for PHA production. However, these residual solids from the separation process contain carbon that can be further fermented for VFA

production or digested for bioenergy.

Another product from this system is the DF gas, which is comprised of H_2, CO_2, and CH_4 (Slupek et al., 2019). A potential option to valorize DF gas is injecting the gas into an AD reactor for further CH_4 production. D'Silva et al. (2023) stated that DF is a useful technology for products like VFAs and H_2, but it should be coupled with another established technology, like AD, to reach industrial scale. There are several advantages to coupling DF and AD, including the production of both biohydrogen and biomethane, flexible reactor design, and higher feeding rates for AD. Studies have tested different ratios of H_2 and CO_2 in AD to simulate how DF gas with high amounts of H_2 effects CH_4 production. Agneessens et al. (2017) tested five ratios of H_2 to CO_2 ranging from 2:1 to 10:1 on sewage sludge digested at mesophilic temperatures. At the 4:1 ratio, a CH_4 content of 89% was achieved, however, ratios between 6:1 and 10:1 of H_2 to CO_2 were necessary to see 100% CH_4. A similar study injected 4:1 H_2 to CO_2 in labelled maize leaf AD, which increased the percent CH_4 from 67 to 89% (Mulat et al., 2017). Prior studies tested high H_2 to CO_2 ratios (4:1, 6:1, 8:1, 10:1), however, no studies observed how low H_2 to CO_2 ratios (1:1, 1:2, 1:3, 1:5), more similar to the concentrations of gases in the DF reactor headspace, perform.

The products of DF include VFA-rich liquid, residual solids, and DF gas. The residual solids are the remaining solids after VFAs separation as only the liquid fraction has accessible VFAs. The DF gas comprised of H_2 and CO_2 can be coupled with AD to enhance CH_4 production, however, the DF gas from a reactor with low H_2 and high CO_2 production has not been tested. The goals of this research were: 1) to compare the bioenergy potential of food waste to residual solids from the VFA separation process; 2) to compare the VFA production of food waste and residual solids from the VFA separation process; 3) to integrate DF gas in the AD

process to increase bioenergy production from food waste or residual solids from the VFA separation process; and 4) to determine the efficacy of utilizing residual solids for additional CH_4 and VFA production in food waste DF. The results from this study can provide insight into effective strategies for incorporating the by-products of food waste DF for VFA production, into additional VFAs and bioenergy production. Additionally, the data from this research can provide better knowledge on how useful the H_2 in DF gas is for increased bioenergy production.

2.2 Materials and Methods

Four experiments were conducted with food waste and/or residual solids from DF after VFA separation. The first two experiments only used residual solids. The first experiment (#1-AD) assessed the AD of residual solids for bioenergy potential. The second experiment (#2-DF) assessed the DF of residual solids to produce VFAs in anaerobic and microaerobic inoculum. The third experiment (#3-DF) assessed AD of food waste and residual solids for bioenergy potential with DF gas injections included during the AD process. The fourth experiment (#4-DF) assessed DF of food waste and residual solids to produce VFAs using multiple inoculum to substrate (ISR) ratios (Table 1).

Table 1. The names of the four experiments that were completed to determine the bioenergy and volatile fatty acids (VFA) production of residual solids (RS) and food waste in anaerobic digestion (AD) and dark fermentation (DF), respectively. The fourth experiment tested various inoculum to substrate ratios (ISR) for VFA production.

	Residual Solids	**Residual Solids & Food Waste**
Anaerobic Digestion	Exp. #1-AD: RS digestion for bioenergy	Exp. #3-AD: Digestion with various DF gas ratios added for bioenergy
Dark Fermentation	Exp. #2-DF: RS dark fermentation for VFAs	Exp. #4-DF: Dark fermentation with multiple ISRs for VFAs

2.2.1 Substrates

The residual solids after VFA separation were obtained from two semi-continuous, lab-scale (37.8 L) DF reactors located at College Park, Maryland, US. Both DF reactors were loaded with lab-prepared food waste adapted from the food waste characterization by the US Department of Agriculture (Table 2; Buzby et al., 2014). The food waste mixture had an average pH of 4.5 and average total solids (TS) and volatile solids (VS) concentrations of 21.6 ± 0.3% and 20.4 ± 0.3%, respectively. The two semi-continuous DF reactors had a 2.5 g VS/L-day organic loading rate (OLR) at mesophilic conditions (35°C) with the pH adjusted to 7 using 10% KOH once a week, and a 12-day HRT. One reactor operated under anaerobic conditions (810 days) while the other was microaerobic (739 days), which involved sparging with 0.5 L O_2/min for one hour, twice a week. This microaerobic condition was tested to improve VFA production by increasing hydrolysis efficiency and microbial diversity (Cao et al., 2022; Nguyen et al., 2019; C. Wang et al., 2020). The DF gas from the anaerobic reactor comprised of 15% H_2, 65% CO_2, and 5% CH_4. The DF gas from the microaerobic reactor was 10% H_2, 25% CO_2, and 1% CH_4.

Residual solids from DF were collected by centrifuging the content of the two 37.8 L DF reactors (5,000 rpm; 15 min; 0°C) and physically separating the liquid and solid fractions by hand, using a scoopula. The liquid fraction from the VFA separation process was filtered to 0.45 μm and used for PHA production. The residual solids from the VFA separation process were used in four experiments. The residual solids used in the four experiments were from the same 37.8 L DF reactors but were collected at different time points and thus had different starting conditions. For Exp. #1-AD (RS digestion for bioenergy) and Exp. #2-DF (RS dark fermentation for VFAs), the residual solids collected from the anaerobic DF reactor had a TS and VS of

10.9% and 9.90%, respectively. The residual solids collected from the microaerobic DF reactor had a 17.3 ± 0.2% TS and a 15.9 ± 0.7% VS. For Exp. #3-AD (digestion with DF gas injection ratios for bioenergy) and Exp. #4-DF (dark fermentation with varying ISRs for VFAs), only anaerobic DF residual solids were used based on the results of the first two experiments and had an average TS of 13.7 ± 1.6% and VS of 13.5 ± 1.8%. The lab-prepared household food waste mixture was used as substrate in Exp. #3-AD (digestion with DF gas injection ratios) for bioenergy and Exp. #4-DF (dark fermentation with varying ISRs for VFAs) in addition to the residual solids collected from the anaerobic DF reactor (Table 2).

Table 2. The type and amount of food waste that was thoroughly blended before anaerobic digestion (AD) or dark fermentation (DF) based on typical household food waste composition by Buzby et al., 2014. Food waste based on wet weight in grams.

Food Waste Type	Food Waste Amount (g)
Potato	245.7
Mixed Vegetables	245.7
Water	218.9
White Bread	132.3
Apple	95.5
Cooked Chicken	75.6
Pork	75.6
Banana	56.7
Cheese	56.7
Cooked Rice	37.8

2.2.2 Inoculum

A 30.0 L semi-continuous anaerobic digester was fed the food waste mixture at the University of Maryland, College Park, US. The digestate was used as inoculum for both experiments that required methanogens (Exp. #1-AD: RS digestion for bioenergy and Exp. #3-

AD: digestion with DF gas injection ratios for bioenergy). The inoculum in Exp. #1 had a pH at 8.28, a 0.79 ± 0.01% TS, and a 0.46 ± 0.02% VS. The inoculum in Exp. #3 had a pH at 8.33, a 1.10 ± 0.01% TS, and a 0.58 ± 0.00% VS.

The experiments that needed fermentation conditions used the contents of the 37.8 L anaerobic DF and microaerobic DF reactors in College Park, Maryland, US. The first fermentation experiment (Exp. #2-DF: RS dark fermentation for VFAs) used contents from both DF reactors while the second fermentation experiment (Exp. #4-DF: dark fermentation with varying ISRs for VFAs) only used anaerobic DF effluent. The anaerobic DF inoculum in Exp. #2 had a pH of 5.75, a 2.50 ± 0.00% TS, and a 1.80 ± 0.01% VS. The microaerobic inoculum in Exp. #2-DF had a pH of 5.67, a 2.28 ± 0.02% TS, and a 1.55 ± 0.00% VS. The anaerobic DF inoculum in Exp. #4-DF had a pH at 4.36, 1.64 ± 0.01% TS, and a 1.44 ± 0.01% VS.

2.2.3 Experimental Design

Experiment #1: Anaerobic Digestion of Residual Solids for Bioenergy Production

A biochemical methane potential (BMP) protocol, created by Owen et al. (1979) and adapted by Moody et al. (2011), was used for anaerobic incubation. The duration of the BMP test was 36 days at which time the daily CH_4 production was <1% of cumulative CH_4 production. Triplicate inoculum-only controls were incubated simultaneously to quantify the biodegradable material attributed to the inoculum and not the tested substrates, which was subtracted from the overall CH_4 production values. The TS and VS of the substrates and inoculum were tested before being loaded into the triplicate 250-mL serum bottles at a 2:1 ISR based on the VS content (Fig. 1). There were nine reactors in total: three with residual solids from anaerobic DF, three with residual solids from microaerobic DF, and three inoculum-only controls (Fig. 1). After the

triplicate BMP reactors were loaded with substrate and inoculum, nitrogen gas was sparged in the bottles for three minutes to ensure anaerobic conditions. Immediately after sparging with nitrogen gas, triplicate reactors of each treatment were capped with a rubber septum and placed in a temperature-controlled incubator at 35°C and 120 rpm. The TS, VS, chemical oxygen demand (COD), VFAs, and pH were tested pre-BMP and post-BMP. The biogas quantity and quality (CH_4 and CO_2) were tested every 2 – 3 days based on the daily biogas production.

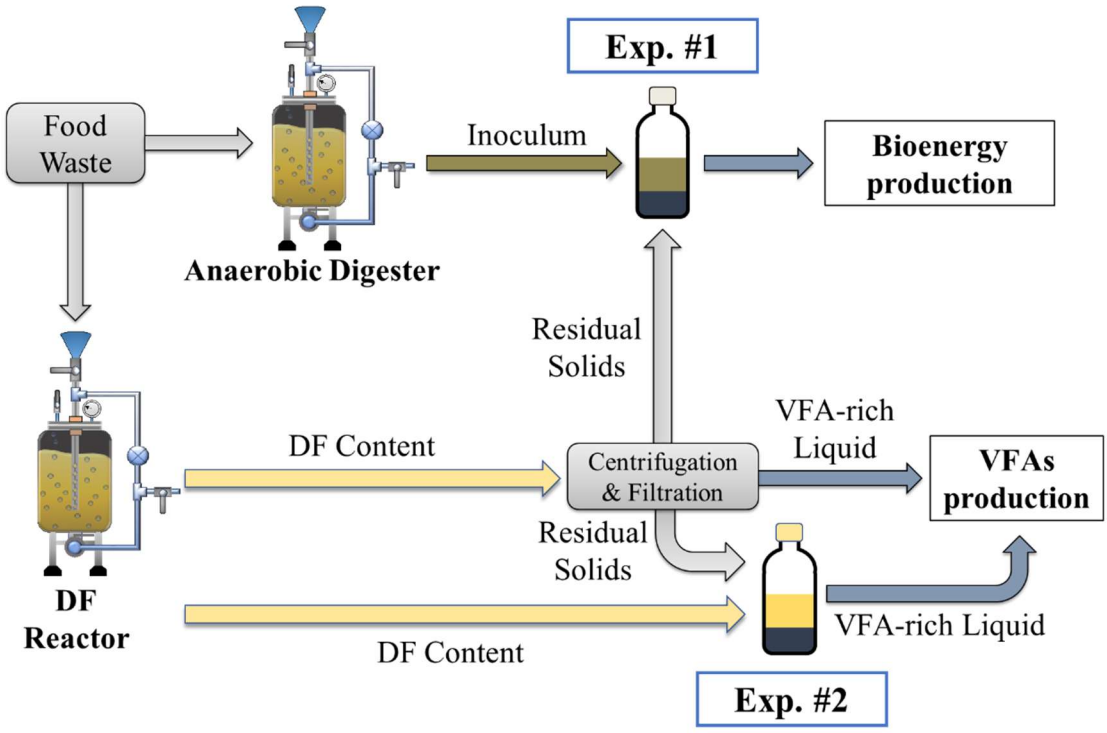

Figure 1. After dark fermentation (DF) of food waste for volatile fatty acids (VFA) production, residual solids were separated from the liquid fraction and then were anaerobically digested for bioenergy production in the form of CH_4 (Exp. #1-AD: RS digestion for bioenergy) or further fermented for additional VFA production (Exp. #2-DF: RS dark fermentation for VFAs). In Exp. #2, two separate contents from anaerobic and microaerobic DF reactors were used.

Experiment #2: Volatile Fatty Acids Production from Residual Solids Dark Fermentation

Dark fermentation batch tests followed a similar protocol to the BMP test; however, instead of AD inoculum, the inoculum conditioned for DF was used and the DF duration was 23

days (Fig. 1). The TS and VS of the two substrates and two inoculums were tested before loaded into singlet 250-mL serum bottles at a 2:1 ISR based on the VS content. The two substrates (residual solids from anaerobic and microaerobic DF) were fermented separately in each inoculum source which was from the anaerobic and microaerobic DF reactors, resulting in four treatments (Fig. 2). After each batch reactor was loaded with substrate and inoculum, nitrogen gas was sparged in the reactors for three minutes to ensure anaerobic conditions. The reactors were capped with a rubber septum immediately after nitrogen sparging and placed in a temperature-controlled incubator at 35°C and 120 rpm. The TS, VS, COD, VFAs, and pH were tested pre- and post-fermentation. The biogas quantity and quality (CH_4, CO_2, H_2) were tested every 2 – 3 days based on the daily biogas production.

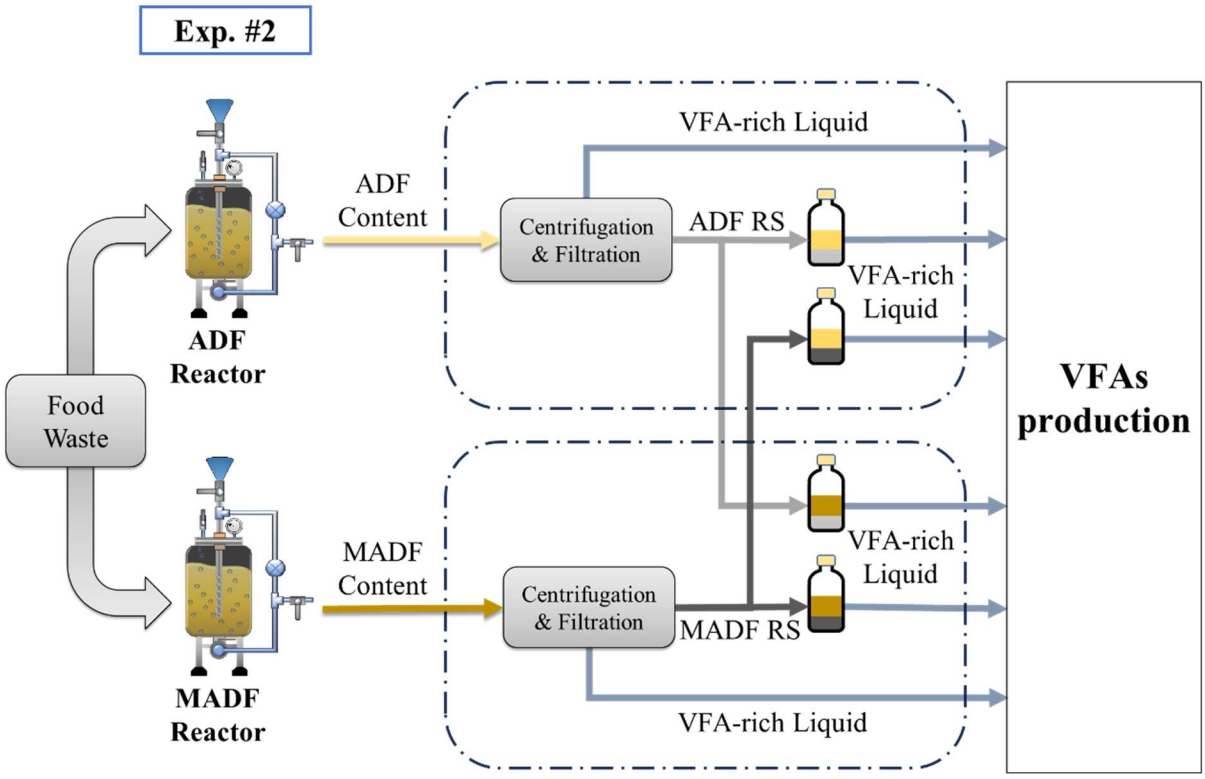

Figure 2. Food waste was processed through anaerobic dark fermentation (ADF) and microaerobic dark fermentation (MADF) for volatile fatty acids (VFA) production (Exp. #2-DF: RS dark fermentation for VFAs). The residual solids (RS) from both ADF and MADF were

separated from the liquid fraction and fermented for further VFA production in both ADF and MADF content.

Experiment #3: Integration of Dark Fermentation Gas in Anaerobic Digestion of Residual Solids and Food Waste

This experiment tested the effect of anaerobically digesting residual solids with DF gas injected into the AD reactor for increased CH_4 production (Fig. 3). The gas from the 37.8 L anaerobic DF reactor averaged at 16.3 ± 5% H_2, 50 ± 13% CO_2, and 6 ± 3% CH_4. Four ratios of H_2, CO_2, and CH_4 mixtures were tested, including DF gas directly from the 37.8 L DF reactor (Table 3). The liquid portion of the DF gas injection experiment bottles was injected with a DF gas mixture at the rates of 50 mL/day for the first nine days, 15 mL/day for six days, and 5 mL/day for the remainder of the experiment. This was to account for decreasing DF gas production in the batch experiment over time. Two treatments, one with residual solids as substrate and another with food waste as substrate, were not injected with DF gas to compare residual solids and food waste AD without DF gas injection.

The digestion vessels (500 mL with 180 mL of liquid volume) were loaded with a 2:1 ISR based on VS content. The bottles were sparged with nitrogen gas for three minutes, sealed with a rubber septum, and incubated at 35°C and 120 rpm. The TS, VS, COD, VFAs, and pH were conducted before digestion and after digestion. Biogas quantity and quality (H_2, CO_2, CH_4) were tested every day for nine days and decreased to every three days for the remainder of the 27-day digestion period.

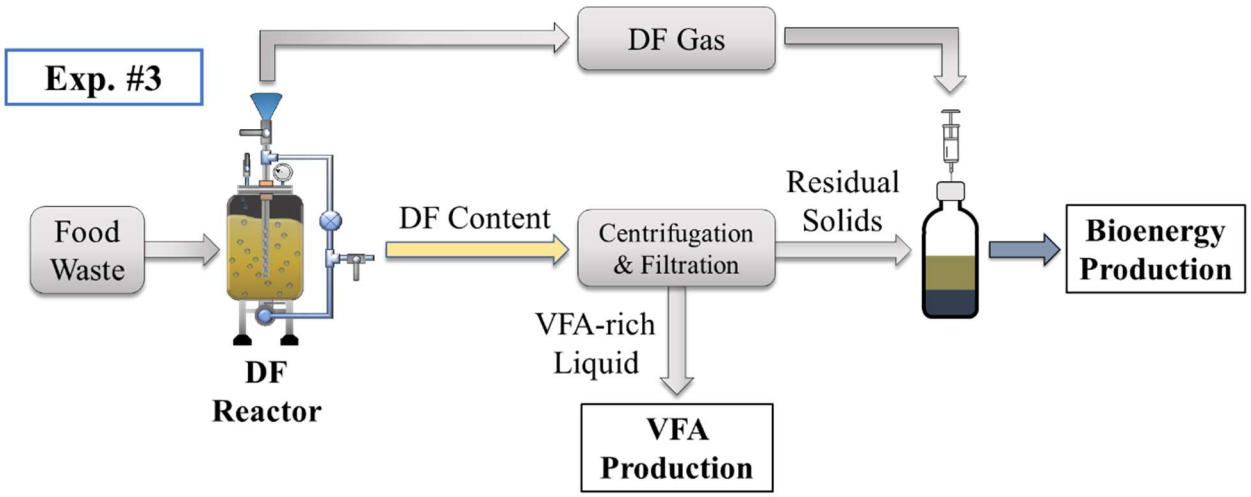

Figure 3. System design of bioenergy production from residual solids and dark fermentation (DF) gas after food waste anaerobic DF for volatile fatty acids (VFA) production (Exp. #3-AD: digestion with various DF gas ratios added for bioenergy). The DF gas was comprised of hydrogen (H_2), carbon dioxide (CO_2), and methane (CH_4).

Table 3. Treatments for anaerobic dark fermentation (DF) gas injection into the liquid portion of triplicate digestion bottles. The gas from the 37.8 L anaerobic DF reactor was analyzed on a gas chromatograph prior to each injection into the DF Gas Mixture treatment. The three other DF gas treatments (15%, 30%, and 50% H_2) were prepared in the lab from pure gas cylinders. All treatments with DF gas injections used anaerobic DF residual solids as substrate.

Treatments	DF Gas Mixture (% H_2 : % CO_2 : % CH_4)
DF Gas Mixture	16:50:6
DF Gas 15% H_2	15:80:5
DF Gas 30% H_2	30:65:5
DF Gas 50% H_2	50:50:0
Anaerobic DF Residual Solids Only	0:0:0
Food Waste Only	0:0:0
Inoculum Only (without solids)	0:0:0

Experiment #4: Dark Fermentation of Residual Solids and Food Waste for Volatile Fatty Acids Production

The DF batch experiment tested the effect of residual solids fermentation in anaerobic DF inoculum at three ISRs. The varying ISRs also resulted in varying OLRs for the semi-continuous DF reactor design (Table 4). A food waste treatment was included to compare DF of food waste

and residual solids. The substrates fermented for 15 days and the pH, VFAs, and gas (H_2, CO_2, CH_4) were analyzed every three days. The TS, VS, and COD were tested before and after the entire fermentation period.

Table 4. Design for food waste and residual solids dark fermentation (DF) for volatile fatty acids (VFAs) production (Exp. #4-DF: dark fermentation with multiple ISRs for VFAs). Inoculum to substrate ratios (ISR) based on different target organic loading rates (OLR) for different semi-continuous DF reactor designs.

Treatment	OLR (g VS/L-day)	ISR
Anaerobic DF Residual Solids	9.45	1.5:1
	7.19	2:1
	3.5	4:1
Food Waste	7.19	2:1
Inoculum Only	0	1:0

2.2.4 Analytical Testing

Biogas Quality and Quantity Testing

The biogas and DF gas were volumetrically measured using a 100 mL glass, gas-tight syringe, equilibrated to atmospheric pressure, pierced through the top of the rubber septum at room temperature (20 – 22°C). The composition of biogas and DF gas from the batch experiment bottles were analyzed for CH_4, CO_2, and H_2 using a gas chromatograph (Agilent Technologies, Inc.; Shanghai China; model 7890A) with a thermal conductivity detector at 250°C, a HP-Plot Q capillary column (Agilent J&W; US), He as the carrier gas for CH_4 and N as the carrier gas for H_2 at 8.6 mL/min, an oven operated at 60°C for 2 min and subsequently ramped at 30°C/min to 240°C.

Water Quality Testing

Each sample was tested for pH, TS, VS, and COD, within 48 h of sample collection. An Accument AB15 pH probe was used to analyze the pH. The TS and VS followed APHA

Methods APHA 2540D and 2540E (Walter, 1961). The TS was dried at 105°C until the sample weight remains within 4% of sample weight. After drying, the VS was calculated by comparing the TS weight to the sample after being incinerated at 550°C. The COD was analyzed using a HACH high-range COD digestion kit and spectrophotometry.

Volatile Fatty Acids Testing

Samples were acidified using 5.25 N sulfuric acid to pH < 2 and filtered to 0.22 μm. Acetic, propionic, butyric, and valeric acids were measured individually and as a total VFA concentrations. The samples from Exp. #1-AD (RS digestion for bioenergy) and Exp. #2-DF (RS dark fermentation for VFAs), were analyzed in a gas chromatograph (GC) and Exp. #3-AD (digestion with DF gas injection ratios for bioenergy) and Exp. #4-DF (dark fermentation with varying ISRs for VFAs), were analyzed in a high-performance liquid chromatograph (HPLC). The GC (Agilent Technologies, Inc.; Shanghai China; model 7890 A) had a flame ionization detector (FID) at 300 C, a DB-FFAP capillary column (Agilent J&W; US), He as the carrier gas at 1.80 mL/min injection temperature of 250°C and the oven operated at 100°C for 2 min and subsequently ramped at 10°C/min for a total run time of 10 min. The HPLC (Waters; US; Model QSM-R, FTN-R, 2998) used the Elution mode – Gradient: 90:10 ratio (10 mM K_2HPO_4 buffer, pH 2.0 and Acetonitrile (100)); Flow rate of 1 mL/min, column temperature at 40°C, and column C18.

2.2.5 Statistical Analysis

An analysis of variance (ANOVA) and a Tukey honest significant difference (HSD) multiple comparisons test was used to determine the significant differences in CH_4 and VFAs. The *p*-values < 0.05 were considered as significant. The reported values were averages with

standard errors (SE). All statistics were performed in StatPlus Pro 7.6.5.

2.3 Results and Discussion

2.3.1 Experiment #1: Anaerobic Digestion of Residual Solids for Bioenergy

The separated residual solids produced from anaerobic dark fermentation (ADF) conditions had a shorter lag phase for CH_4 production than residuals solids produced from microaerobic dark fermentation (MADF) conditions. The ADF residual solids produced 67.1% of its cumulative CH_4 production in 13 days while MADF solids only produced 29.5% in 13 days (p-value < 0.001). The ADF residual solids remained higher than MADF solids until Day 23 when the CH_4 production was not significantly different between the two residual solid types (p-value = 0.358). However, the total CH_4 production of the MADF residual solids was significantly higher than solids from ADF after 36 days of digestion (395 ± 8 and 430 ± 4 mL CH_4/g VS, respectively) (p-value = 0.010; Fig. 4). The average percent CH_4 in the biogas during steady state, Days 19 – 36, was 53.0 and 55.9% CH_4 for ADF and MADF residual solids, respectively. The maximum percent CH_4 in the biogas was on Day 23 at 61.9% and 67.9% CH_4 for ADF and MADF residual solids, respectively (Table 5). The shorter lag phase from the ADF residual solids indicated a shorter hydrolysis period. This shorter lag phase is indicative of the food waste being initially fermented under ADF conditions, resulting in VFAs available in a shorter timeframe but less available substrate available over time compared to the MADF conditions.

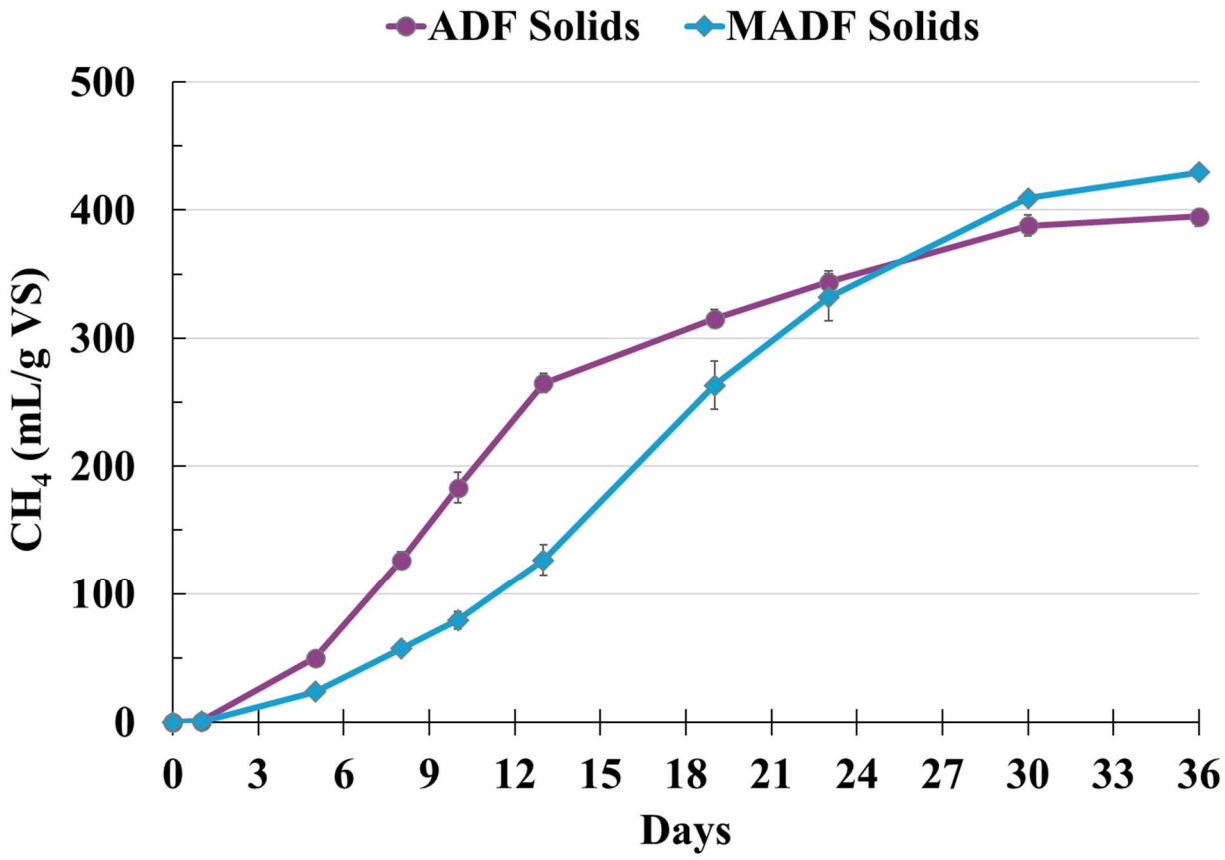

Figure 4. The cumulative methane (CH_4) production over 36 days of digestion for two substrates, anaerobic (ADF) and microaerobic (MADF) dark fermentation residual solids, normalized by gram of volatile solids (VS). Error bars represent standard error for triplicate reactors.

The TS, VS, pH, and COD were similar before and after digestion in the two treatments (Table 5). The ADF and MADF residual solids had TS reductions of 26.9% and 30.2% and VS reductions of 40.9% and 42.4%, respectively, during the AD process. The COD reductions were higher at 68.6 and 75.7%, respectively. The organic matter reductions show that the microorganisms used the residual solids from dark fermentation for CH_4 production (Meegoda et al., 2018).

Table 5. Total solids (TS), volatile solids (VS), and pH of the residual solids from anaerobic dark fermentation (ADF) and microaerobic dark fermentation (MADF) pre- and post-anaerobic digestion. The methane (CH_4) production after 36 days of digestion and average percent CH_4 in the biogas are shown with standard error from triplicate reactors.

Treatment	Pre TS (%)	Post TS (%)	Pre VS (%)	Post VS (%)	Pre pH	Post pH	Cumulative CH_4 (mL/g VS)	CH_4 (%)
ADF Residual Solids	1.03 ± 0.01	0.75 ± 0.02	0.66 ± 0.01	0.39 ± 0.02	8.27	8.22 ± 0.02	395 ± 8	53.0 ± 4.6
MADF Residual Solids	1.04 ± 0.01	0.73 ± 0.03	0.69 ± 0.01	0.39 ± 0.02	8.29	8.27 ± 0.01	430 ± 4	55.9 ± 6.0

Zhang et al. (2019) digested food waste and achieved a CH_4 yield of 593 mL/g VS after 50 days, while Parra-Orobio et al. (2018) produced less than 120 mL CH_4/g VS after 35 days of digestion of food waste. The difference between the CH_4 yields could be a result of different food waste compositions, with Zhang et al. (2019) digesting rice, meat, vegetable, and fruit, and Parra-Orobio et al. (2018) digested potato, banana peels, citrus, non-citric fruits, and herbs, which are more recalcitrant. This current study digested the remaining solids after DF of a food waste mixture containing rice, non-citrus fruits, meat, potato, and cheese and produced a maximum of 430 mL CH_4/g VS after 36 days of digestion. This was 27.5% less than Zhang et al. (2019), however, the substrates were the remaining solids after the VFAs were removed from the initial DF. The bioenergy produced from the residual solids was in addition to the primary extracted VFAs product (9.84 and 7.35 g/L VFAs for ADF and MADF, respectively). The remaining solids between ADF and MADF had similar organic composition (VS and COD), with the ADF residual solids producing the majority of its CH_4 within 13 days while the MADF residual solids had a long lag phase. Residual solids from either anaerobic or microaerobic DF can be used for bioenergy production, in addition to the VFA extracted from the primary food

waste fermentation.

2.3.2 Experiment #2: Volatile Fatty Acids Production from Residual Solids Dark Fermentation

The total amount of VFAs increased in all treatments ranging from 24.3% to 45.4%, with the residual solids from MADF fermented in MADF inoculum having the greatest VFAs increase (from 5.62 ± 0.02 to 8.18 ± 0.01 g/L VFAs) after 23 days of fermentation (Fig. 5). The residual solids from ADF that were fermented in the MADF inoculum had the highest total VFAs both pre- and post-experiment at 6.44 g/L and 8.37 g/L, respectively. The residual solids from anaerobic and microaerobic DF that were fermented in MADF inoculum had higher increases in total VFAs than when fermented in ADF inoculum. The anaerobic and microaerobic residual solids fermented in MADF inoculum had a 29.9% (6.44 ± 0.03 to 8.37 ± 0.02 g/L VFAs) and 45.4% (5.62 ± 0.02 to 8.18 ± 0.01 g/L VFAs) increase in total VFAs, respectively. The same substrates fermented in ADF inoculum increased by 24.3% (3.96 ± 0.02 to 4.93 ± 0.04 g/L VFAs) and 28.3% (3.71 ± 0.03 to 4.76 ± 0.01 g/L VFAs), respectively. There was a 41.1% difference in VFAs between the anaerobic residual solids fermented in ADF inoculum (4.93 ± 0.04 g/L VFAs) and MADF inoculum (8.37 ± 0.02 g/L VFAs). There was a 41.8% difference in VFAs between the microaerobic residual solids that were fermented in the ADF (4.76 ± 0.01 g/L VFAs) and MADF inoculum (8.18 ± 0.01 g/L VFAs). The 41.1 and 41.8% differences between the residual solids fermented in the two inoculum sources may indicate that the inoculum source contributed towards the VFA production.

The inoculum sources were from anaerobic and microaerobic DF reactors that processed food waste for VFA production. The inoculum sources were tested for VFAs before being loaded into the batch experiment bottles, however, there were no inoculum-only controls tested in this experiment. The ADF inoculum source had 6.31 g/L VFAs and the MADF inoculum source had

10.9 g/L VFAs. This 42.1% difference of total VFAs from the ADF and MADF inoculum sources most likely contributed to the 41.1 and 41.8% differences measured in the residual solids fermentation in the ADF and MADF inoculum sources. The VFAs present in the MADF inoculum (10.9 g/L VFAs) were higher than the ADF inoculum (6.31 g/L VFAs) which resulted in the treatments with MADF inoculum to contain more total VFAs after 23 days of fermentation.

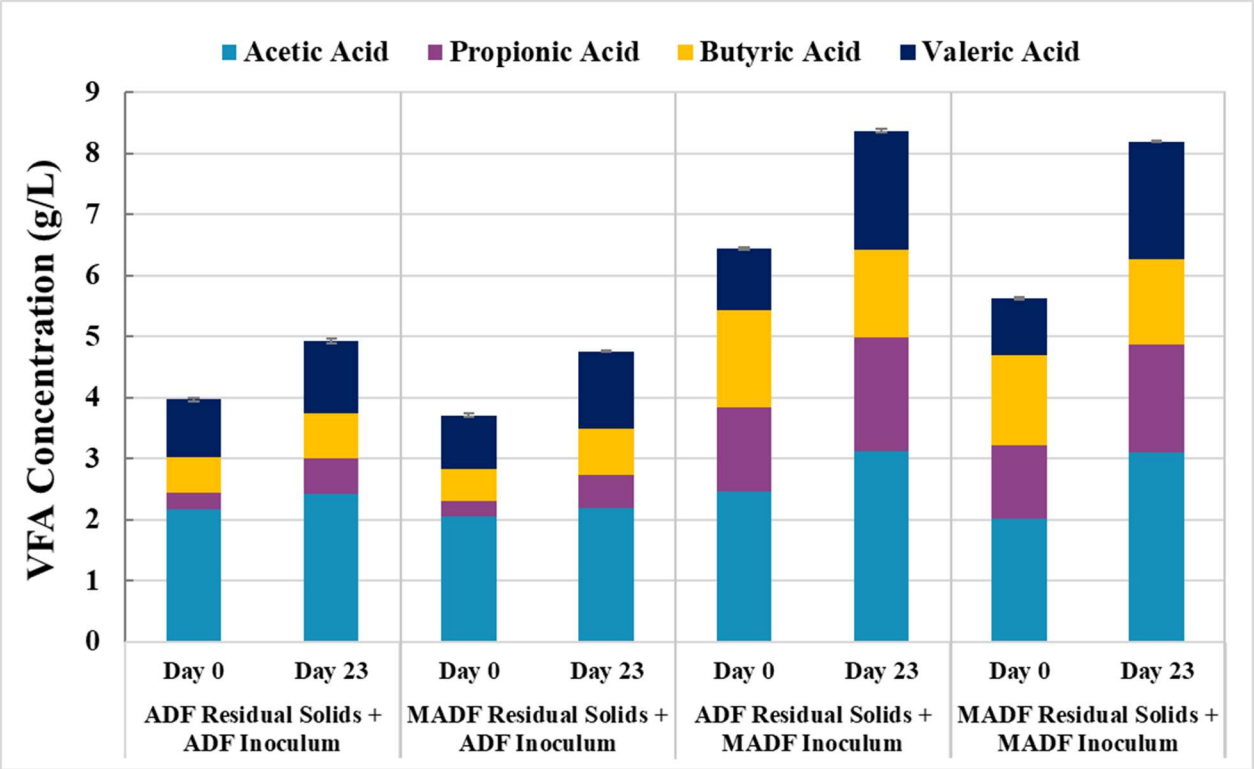

Figure 5. Volatile fatty acids (VFA) concentration of four treatments using residual solids and inoculum from anaerobic (ADF) and microaerobic dark fermentation (MADF). Standard error bars based on total VFAs from analysis triplicates.

The average pH of all four treatments was stable from pre-experiment (5.71 ± 0.06) to post-experiment (5.77 ± 0.04) (Table 6) with a pH below 6, which is favorable for fermentation conditions. All treatments had TS and VS reductions which corresponded to the increase in VFA production (Table 6; Fig. 5). The residual solids from ADF that were fermented in ADF

inoculum had the lowest decrease in VS at 9.5% (2.86 ± 0.04 to 2.69 ± 0.01%) and the lowest increase in VFAs at 24.3% (3.96 ± 0.02 to 4.93 ± 0.04 g/L VFAs). The largest increase in VFAs at 45.4% (5.62 ± 0.02 to 8.18 ± 0.01 g/L VFAs) corresponded to the largest reduction in TS and VS of 25.9% (3.01 ± 0.05 to 2.39 ± 0.01%) and 39.9% (2.21 ± 0.04 to 1.58 ± 0.01%), respectively, from the MADF residual solids that were fermented in MADF inoculum (Table 6).

Table 6. Total solids (TS), volatile solids (VS), and pH of the treatments pre- and post-fermentation. Average values were taken from analyses replicates as each treatment was a single experiment bottle. Treatments included anaerobic (ADF) and microaerobic dark fermentation (MADF) residual solids and inoculum.

Treatment	TS (%)		VS (%)		pH	
	Pre	Post	Pre	Post	Pre	Post
ADF residual solids fermented in ADF inoculum	2.86 ± 0.04	2.69 ± 0.01	2.20 ± 0.00	2.01 ± 0.01	5.64	5.78
MADF residual solids fermented in ADF inoculum	3.26 ± 0.03	2.76 ± 0.20	2.49 ± 0.04	2.05 ± 0.02	5.86	5.75
ADF residual solids fermented in MADF inoculum	2.79 ± 0.03	2.40 ± 0.01	1.97 ± 0.05	1.64 ± 0.01	5.60	5.69
MADF residual solids fermented in MADF inoculum	3.01 ± 0.05	2.39 ± 0.01	2.21 ± 0.04	1.58 ± 0.01	5.73	5.86

A previous fermentation study achieved higher VFAs with the organic fraction of municipal solid waste at varying pH levels. The organic fraction of municipal solid waste produced 12 and 10 g/L VFAs at a controlled pH 5 and an uncontrolled pH, respectively (Battista et al., 2022). This current study was similar to the Battista et al. (2022) organic fraction of municipal solid waste as the pH was stable at an average of 5.70 ± 0.04 for all treatments and had a variety of food such as rice, cheese, meat, bananas, apples, and bread. Although the stable pH and variety of starch, carbohydrates, and proteins promoted a beneficial environment for

fermentation in this current study, the highest VFAs at 12 g/L in Battista et al. (2022) was 30.3% greater than the highest VFAs achieved in this current study (8.37 g/L VFAs). The difference in VFAs could be from the fermentation of residual solids and not fresh food waste. The residual solids were the remaining solids after an initial food waste fermentation. The carbon converted into VFAs in the initial fermentation reduced the useful carbon accessible in the residual solids.

Another observation made from this current study was that the residual solids, regardless of anaerobic or microaerobic DF source, produced more VFAs when fermented in microaerobic inoculum than when fermented in anaerobic inoculum. Li et al. (2024) conducted a thorough review on microaeration in digestion and fermentation and found that aerating a food waste reactor promoted extracellular enzymes for hydrolysis—the rate-limiting step—and increased efficiency. The microaerobic inoculum could explain the high VFA production, however, none of the treatments post-fermentation had significantly different VFA concentrations (*p*-value: 0.053 – 0.996).

2.3.3 Experiment #3: Integration of Dark Fermentation Gas in Anaerobic Digestion of Residual Solids and Food Waste

The residual solids collected from ADF after separation for VFA extraction were anaerobically digested with methanogenic inoculum. Only ADF residual solids were used due to the extra energy needed to maintain a microaerobic environment being higher than the extra energy produced during AD using microaerobic inoculum. Four out of five treatments that digested ADF residual solids were injected with four different DF gas compositions (% H_2: % CO_2: % CH_4) to determine the effect of using the gas from DF to increase CH_4 yield for subsequent digestion. Food waste, without DF gas injections, was also anaerobically digested to compare the CH_4 potential of residual solids that have undergone fermentation and compared to

unfermented food waste.

Residual solids from ADF (492 ± 11 mL CH_4/g VS) and unfermented household food waste (470 ± 11 mL CH_4/g VS) without DF gas injections produced 4.4 to 8.9 times more CH_4 than the four treatments with DF gas injections (81 – 132 mL CH_4/g VS) (Fig. 6; Table 7). The ADF residual solids and regular food waste had similar CH_4 yield after the 27-day digestion (*p*-value = 0.680). The cumulative CH_4 production among the four DF gas injection treatments showed a negative relationship between the percent H_2 injected and CH_4 production (Fig. 6). The DF gas mixture extracted from the semi-continuous lab-scale DF reactor (37.8 L) had an average gas composition of 16 ± 5% H_2 50 ± 13% CO_2 and 6 ± 3% CH_4 throughout the 27-day experiment. The DF gas mixture (126 ± 15 mL CH_4/g VS) and gas injection of 15:80:5 (H_2:CO_2:CH_4) (132 ± 10 mL CH_4/g VS) produced the similar amounts of CH_4 after 27 days of digestion (*p*-value = 0.0854). These low H_2 treatments produced more CH_4 than the higher H_2 treatments of 30:65:5 H_2:CO_2:CH_4 (96 ± 0.2 mL CH_4/g VS) and 50:50:0 H_2:CO_2:CH_4 (81 ± 15 mL CH_4/g VS) (*p*-value < 0.001). The low H_2 gas injections (DF gas mixture and 15:80:5 H_2:CO_2:CH_4) were still significantly lower than the non-injection treatments (*p*-value < 0.001).

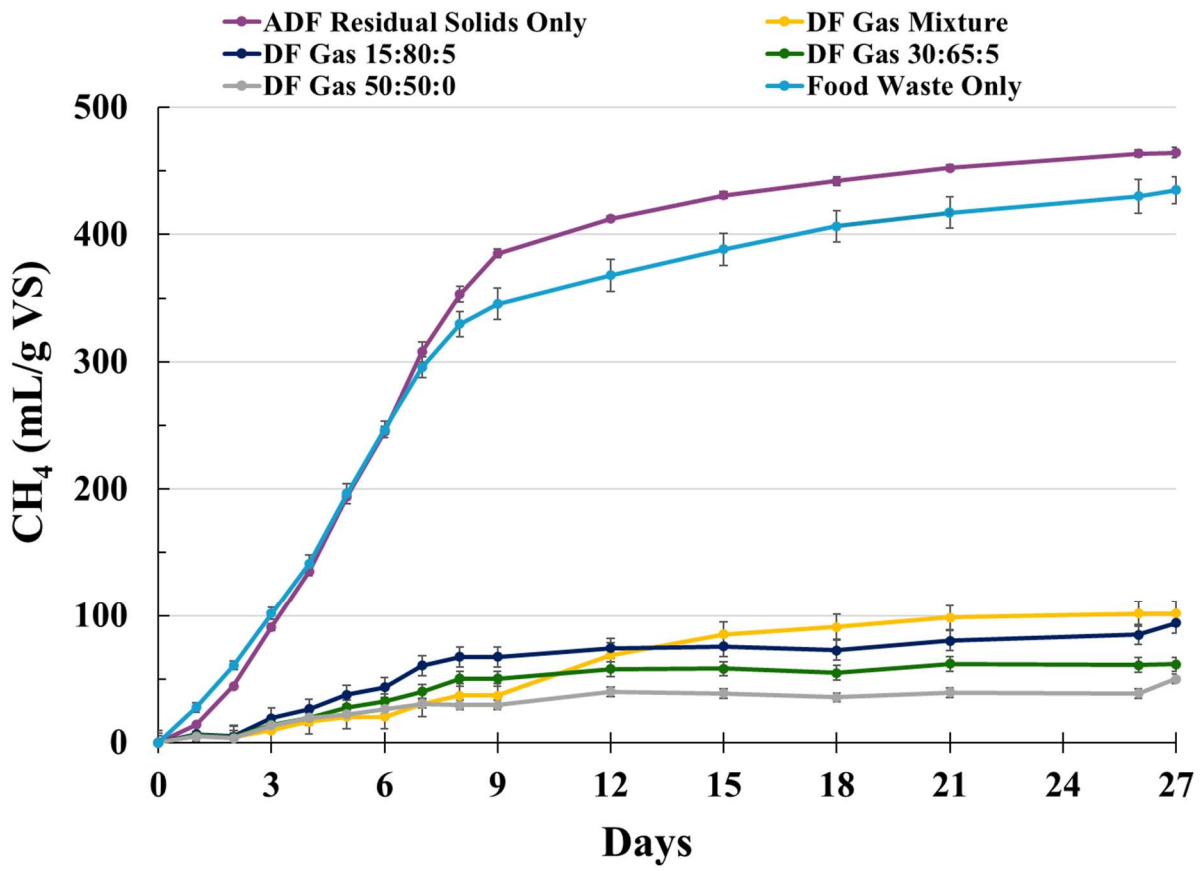

Figure 6. Cumulative methane (CH_4) production, mL per gram of volatile solids (VS), for two treatments without dark fermentation (DF) gas injections and four treatments with DF gas injections comprised of different mixtures of H_2:CO_2:CH_4 (%). All DF gas injection treatments used anaerobic dark fermentation (ADF) residual solids. Error bars based on standard error from triplicate reactors.

Table 7. The average cumulative biogas and average percent CH_4 with standard errors based on triplicate reactors. Six treatments were digested over 27 days including four dark fermentation (DF) gas injection treatments with residual solids (RS) from anaerobic dark fermentation (ADF) as substrate and a mixture of gas comprised of H_2:CO_2:CH_4. Two treatments did not have DF gas injections (ADF RS Only and Food Waste Only).

Treatment	Cumulative Biogas (mL/g VS)	Cumulative CH_4 (mL/g VS)	Average CH_4 Day 0-8 (%)	Average CH_4 Day 9-18 (%)	Average CH_4 Day 19-27 (%)
ADF RS Only	755 ± 18	492 ± 11	54.5 ± 7.4	70.8 ± 2.6	60.3 ± 0.1
DF Gas Mixture	401 ± 41	126 ± 15	21.7 ± 3.7	40 ± 0.5	36.1 ± 1
DF Gas 15:80:5	381 ± 27	132 ± 10	27.5 ± 4.5	40.6 ± 1.4	35.9 ± 0.9
DF Gas 30:65:5	274 ± 6	96 ± 0.2	28.3 ± 5	44.5 ± 0.4	40.6 ± 0.5
DF Gas 50:50:0	241 ± 34	81 ± 15	28.7 ± 5.2	50.6 ± 0.7	47.1 ± 0.9
Food Waste Only	733 ± 11	470 ± 11	54.7 ± 6.5	70.4 ± 1.4	65.1 ± 0.9

The greatest percent CH_4 in the biogas achieved for all treatments occurred on Day 9 as the non-injection treatments produced 73.7 and 78.5% CH_4 for ADF residual solids and food waste, respectively, and the injection treatments produced 39.4 to 49.5% CH_4. The CO_2 was injected into the treatments every time the gas was measured, which was one to three days depending on biogas production. The amount of CO_2 injected (50 to 80%) and H_2 (15 to 50%) throughout the experiment likely diluted the CH_4 produced in batch experiment with injections, resulting in a lower percent CH_4 (21.7 to 50.6% CH_4) over time. The ADF residual solids (54.5 – 70.8%) and food waste (54.7 – 70.4% CH_4) achieved a similar average percent CH_4 in the biogas after 27 days of digestion (p-value = 0.435; Table 7). The non-significant difference of CH_4 percentage and yield from these two substrates could be from the similar carbon compositions.

The residual solids were the remaining solids after DF of food waste and the VFA separation process. These solids were most likely food waste that did not have enough time to ferment in the 12-day HRT. Additionally, the residual solids may have undergone partial hydrolysis resulting in a higher CH_4 yield, but the non-injection ADF residual solids treatment (492 ± 11 mL CH_4/g VS) was not statistically different from non-injection food waste treatment without fermentation (470 ± 11 mL CH_4/g VS) (*p*-value = 0.680; Table 7).

Three gases (CO_2, CH_4, and H_2) were measured one to three days after each DF injection to observe the flow of the DF gas injection in the digestion process (Fig. 7). The H_2 was less than 1% of the total gas composition in the DF gas mixture, 15:80:5, and 30:65:5 (H_2:CO_2:CH_4) DF gas injection treatments. The 50:50:0 ratio of H_2:CO_2:CH_4 had an average of 1.6% H_2 in the biogas composition throughout the 27-day digestion. There was little H_2 remaining in the biogas which implied that the injected H_2 gas transformed into CH_4. The CO_2 ranged from 23.0 to 46.2% in the injection treatments and had a direct relationship with the amount CO_2 injected into the reactor. The CH_4 in the biogas was not significantly different in the DF gas mixture, 15:80:5, and 30:65:5 H_2:CO_2:CH_4 injection treatments throughout the experiment (*p*-value: 0.054 – 1.000). The CH_4 in the biogas for the 50:50:0 H_2:CO_2:CH_4 treatment was significantly higher than the DF gas mixture and the 15:80:5 H_2:CO_2:CH_4 treatments throughout the steady state (*p*-value < 0.001; Days 9 – 27; Table 7). This implied that the different ratios from 15 – 30% H_2 and 65 – 80% CO_2 injected did not impact CH_4 production. However, a 50:50 H_2:CO_2 produced a higher percent CH_4 in the biogas compared to the gas that came from a semi-continuous 37.8 L DF reactor, the DF gas mixture treatment (16:50:6 H_2:CO_2:CH_4), and a gas mixture that represented a DF reactor without gas fluctuations (15:80:5 H_2:CO_2:CH_4). The minimum ratio of H_2:CO_2 that could be injected into a digester to achieve percent CH_4 of at least 51% is 1:1

H_2:CO_2 (50:50:0 H_2:CO_2:CH_4) (Table 7).

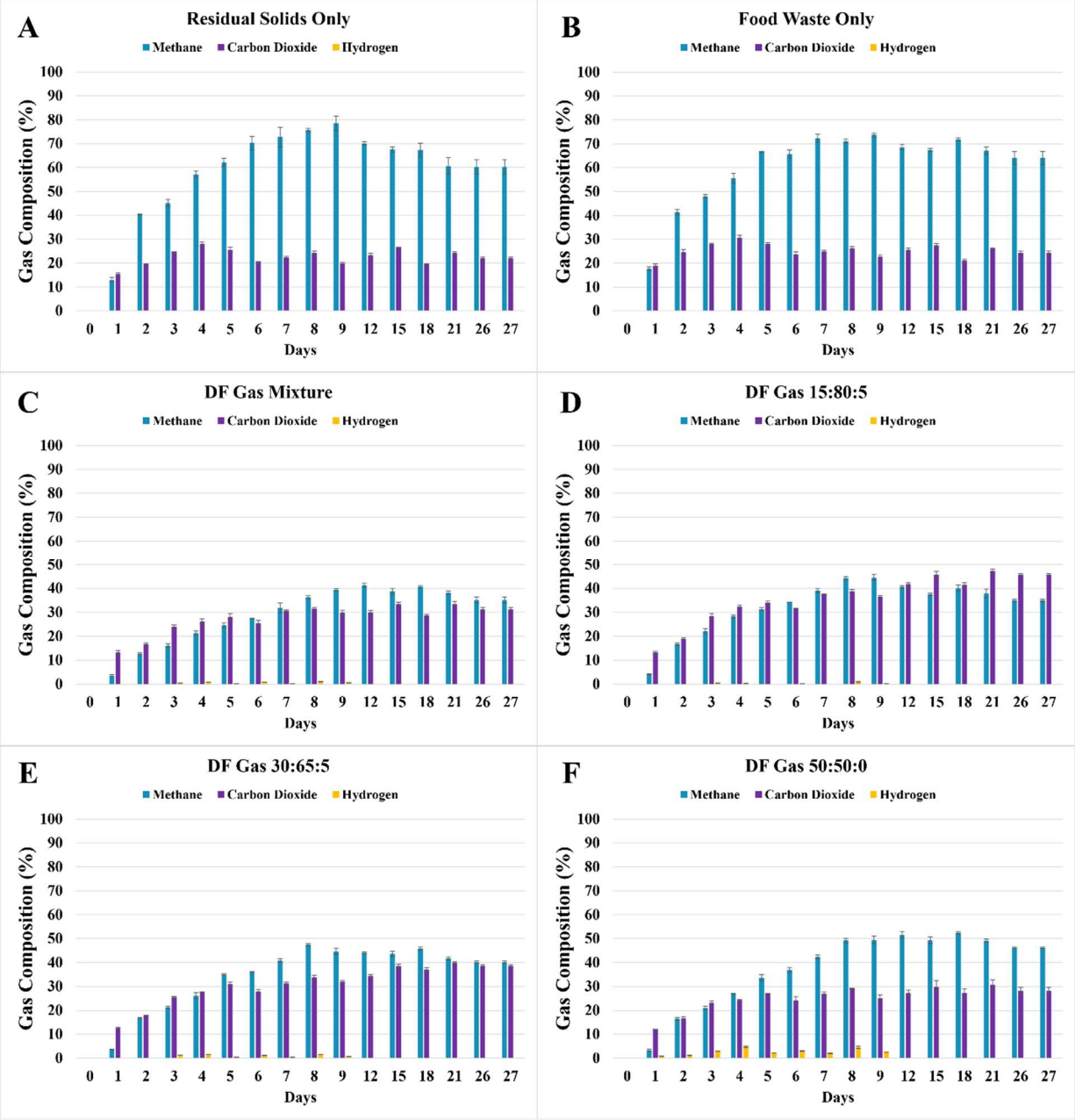

Figure 7. The composition of the biogas produced over 27 days of digestion. Anaerobic dark fermentation (ADF) residual solids (RS) were used with three ratios of H_2:CO_2:CH_4 (%) dark fermentation (DF) gas injection treatments (Fig. C – F). The RS only (Fig. A) and food waste (FW) (Fig. B) only treatments had no DF gas injections. Standard error bars were based on triplicate reactors.

The food waste treatment had the greatest VFAs reduction (84.6%) and all ADF residual solids treatments had higher total VFAs after digestion; however, the high VFA levels did not affect the pH as it remained stable, but high, at approximately 8.05 (Fig. 8). The ADF residual solids with the DF gas mixture injection had the highest VFAs (6.18 g/L) after digestion which was 79.6% significantly higher than the food waste only treatment (*p*-value = 0.026). While the large reduction in VFAs (84.6%) contributed to the high CH_4 production in the food waste treatment (470 ± 11 mL CH_4/g VS), the non-injection ADF residual solids produced 4.5% more cumulative CH_4 (492 ± 11 mL CH_4/g VS) with half the VFA reduction (41.3%; Fig. 8; Table 7). The 41.3% VFAs reduction (from 8.56 ± 1.04 to 5.03 ± 1.15 g/L VFAs) in the non-injection ADF residual solids treatment and high CH_4 production (470 ± 11 mL CH_4/g VS) was most likely due to VFAs being formed and used during the digestion process while the non-injection food waste treatment only used the existing VFAs from pre-digestion resulting in an 84.6% decrease (from 10.57 ± 0.25 to 1.72 ± 0.16 g/L VFAs).

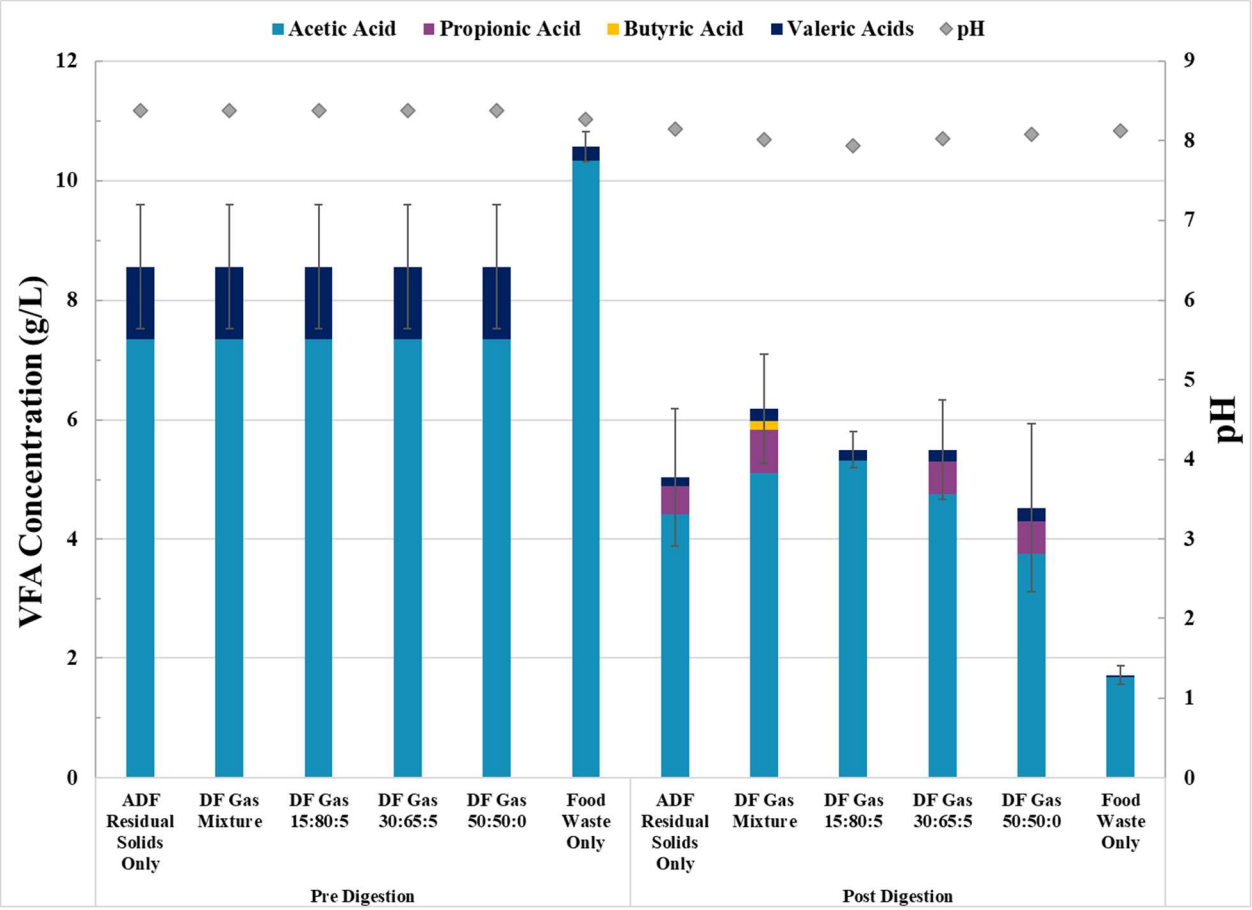

Figure 8. Total volatile fatty acids (VFA), shown as bars, and pH, shown as diamonds, for six treatments before and after 27 days of digestion. The treatments included food waste and residual solids from anaerobic dark fermentation (ADF) with and without dark fermentation (DF) gas injections at four ratios of H_2:CO_2:CH_4 (%). The standard error (SE) bars were based on triplicate reactors, with the SE bars for pH not visible due to small SE.

The TS and VS in ADF residual solids treatment, regardless of injection or non-injection, were reduced by 24.2 to 27.4% and 38.0 to 39.8%, respectively (Table 8). The food waste treatment had less TS and VS reduction at 16.9% (1.3 ± 1.1 to 1.1 ± 0.2% TS) and 27.6% (0.8 ± 1.1 to 0.5 ± 0.3% VS), respectively, but there were no significant differences of TS and VS reduction among all treatments (*p*-value: 0.640 – 1.000 for TS; *p*-value: 0.985 – 1.000 for VS). The non-injection treatments, ADF residual solids and food waste, produced similar cumulative CH_4 after digestion even though the TS and VS reduction was greater in the ADF residual solids

by 28% (1.4 ± 0.1 to 1.0 ± 0.3%) and 30% (0.9 ± 0.1 to 0.5 ± 0.0%), respectively (Table 7 and 8). The COD decreased in all treatments with an average of 42.4% in all ADF residual solids treatments, regardless of injection or non-injection, and 34.9% in the food waste treatment. The post-experiment COD was similar in all treatments ranging from 8 – 9 g/L.

Table 8. Quality analysis of percent total solids (TS) and volatile solids (VS) pre and post digestion of six treatments including residual solids from anaerobic dark fermentation (ADF), food waste, and dark fermentation (DF) gas injections comprised of H_2:CO_2:CH_4 (%). Standard error for pre digestion based on triplicates in the analysis. Post digestion standard error based on triplicate reactors. The DF gas injection treatments used ADF residual solids.

Treatment	TS (%)		VS (%)	
	Pre	Post	Pre	Post
ADF Residual Solids Only	1.4 ± 0.1	1.0 ± 0.3	0.9 ± 0.1	0.5 ± 0.0
DF Gas Mixture	1.4 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.5 ± 0.0
DF Gas 15:80:5	1.4 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	0.5 ± 0.0
DF Gas 30:65:5	1.4 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	0.5 ± 0.0
DF Gas 50:50:0	1.4 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	0.5 ± 0.0
Food Waste Only	1.3 ± 1.1	1.1 ± 0.2	0.8 ± 1.1	0.5 ± 0.3

Jo et al. (2018) conducted a study with food waste digestion over several HRTs and achieved a maximum CH_4 yield of 424 mL/g VS after 34 days of regular food waste digestion. Gao et al. (2021) achieved about 500 mL CH_4/g VS after 70 days of a batch AD system. This current study produced similar amounts at 470 ± 11 mL CH_4/g VS after 27 days of digestion. This current study completed digestion in 27 days, which was 20.6 and 61.4% shorter digestion time compared to Jo et al. (2018) and Gao et al. (2021), respectively. The shorter digestion period could be due to the food waste mixture in this study including a variety of carbohydrates, proteins, and starches while Jo et al. (2018) mainly had starches. Gao et al. (2021) tested a 1:1 ISR (based on VS) while this current study used a 2:1 ISR (based on VS) which could explain the 61.4% longer digestion period (70 days) to produce only 6% more cumulative CH_4 (500 mL

CH_4/g VS) compared to this current study (27 days; 470 ± 11 mL CH_4/g VS). Additionally, the non-injection treatments in this current study had no observed lag and produced significantly more CH_4 than injection treatments within the first five days (*p*-value = 0.048). Rafieenia et al. (2017) also did not observe a lag phase for food waste digestion and produced 241 mL CH_4/g VS in approximately 75 days, which was 64% longer (75 days) and 48.7% less cumulative CH_4 compared to this current study (27 days; 470 ± 11 mL CH_4/g VS).

The low biogas production in DF gas injection treatments in this current study could be a result of microbial inhibition from high CO_2 contributions. High CO_2 levels could acidify the pH in AD as the dissolved CO_2 increases in the inoculum, which could decrease methanogen populations as they favor a pH of 6.5 – 8 (Chen et al., 2014; Zhao et al., 2021). However, Ceron-Chafla et al. (2020) found that the microbial community may respond to high CO_2 partial pressure by reducing CH_4 production to increase a pH buffer. This could explain the stable pH at 8.05 (Fig. 7) and the low CH_4 production in this current study. Another reason for biogas inhibition in DF gas injection treatments could be from the low H_2 to high CO_2 ratios as studies recommended a 4:1 H_2:CO_2 ratio to provide enough H_2 for CH_4 production without causing high H_2 partial pressure (Wahid et al., 2019). The low H_2 to high CO_2 ratios were tested in this study to use a gas composition that represented a semi-continuous DF reactor with low H_2 to high CO_2 gas production and to determine if DF gas with low H_2 could be incorporated in AD.

The low CH_4 production could be caused by microbial inhibition in injection treatments as previous literature that studied microbial communities with H_2 and CO_2 gas injections observed low CH_4 production with low H_2 additions as well. Agneessens et al. (2017) analyzed the microbial communities in food waste AD with gas injections ranging from 2:1 to 10:1 (H_2:CO_2). High H_2 injections promoted a diverse distribution of methanogenic bacteria as

hydrogenotrophic methanogens with low affinity for H_2 would typically be outcompeted in digesters without H_2 injections, supporting the benefits of higher H_2 to lower CO_2 ratios. This current study tested lower ratios of 15:80:5, 30:65:5 and 50:50:0 of H_2:CO_2:CH_4 that could cause more competition for existing methanogens. The CO_2 injected into the reactors was in addition to the existing CO_2 present in the headspace of the reactor, which likely further increases the amount of CO_2 per H_2. Overabundance of CO_2 and low H_2 added to reduce the CO_2 to CH_4 most likely decreased enzymatic activity resulting in low CH_4 production.

Another potential reason for the low CH_4 production in DF gas injection treatments was the high level of CO_2 injected (50 – 80%) into the reactors, which likely diluted the percentage of CH_4 present in the biogas. Biogas in AD without DF gas injections from Day 9 to 27 was comprised of 60.2 – 78.4% CH_4 and 19.6 – 27.4% CO_2. The treatments with 50 – 80% CO_2 gas injections added to the CO_2 that was already present in the reactors, ranging from 25.1 – 47.2% CO_2 (Fig. 7). During Days 9 – 27, the highest CO_2 injection of 80% (15:80:5 H_2:CO_2:CH_4) resulted in a higher CO_2 (36.6 – 47.2% CO_2,) than CH_4 (34.9 – 44.5% CH_4) (Fig. 7). In comparison, the non-injection ADF residual solids treatment had an average of 67.2 – 72.2% CH_4 and 24.4 – 27.3% CO_2 during the 27-day digestion (Table 7). Another study had found that biogas produced from AD comprised of 50 – 75% CH_4 and 25 – 50% CO_2 (Anukam et al., 2019). The non-injection treatment was on the higher end of that percent CH_4 range and on the lower end of that percent CO_2 range (67.2 – 72.2% CH_4 and 24.4 – 27.3% CO_2) while the opposite was found in the injection treatments (43.1 to 64.4% CH_4 and 35.5 to 55.5% CO_2). The low percent CH_4 in the injection treatments was likely diluted by the 50 – 80% CO_2 injections.

A gas balance was calculated by subtracting the daily production of biogas from the daily gas injected for H_2, CO_2, and CH_4, individually. In the 27-day batch test, the amount of H_2 and

CO_2 present in the reactors was less than the amount injected while the reactors had more CH_4 present than the amount injected. The decrease in H_2 and CO_2, and the increase in CH_4 indicated that H_2 and CO_2 were consumed to form CH_4, based on the Sabatier reaction that states four H_2 and one CO_2 can be converted to one CH_4 (Styring et al., 2023). A limitation to this gas balance was not being able to distinguish if the gas present in the reactor was the result of all the gas injected being consumed to form CH_4 based on the Sabatier reaction or if gas was injected and not consumed, diluting the CH_4 produced from digestion. The latter may be true as the CO_2 present in the reactor was 62 – 99% of the amount injected into the reactors for all injection treatments.

The CH_4 achieved in this current study was low compared to studies with higher H_2 to lower CO_2 ratios. Agneessens et al. (2017) tested different ratios of H_2 to CO_2 injections and the lowest ratio (2:1) generated the least amount of CH_4 (76.8 ± 8.5%) while ratios containing more H_2 produced 89.0 ± 8.3% CH_4 (4:1 H_2:CO_2) and 100% CH_4 (6:1, 8:1, 10:1 H_2:CO_2). Another study injected 4:1 H_2:CO_2 over 15 days in unlabelled maize leaf AD, increasing CH_4 purity from 67 to 89% (Mulat et al., 2017). This current study only achieved an average of 50.6 ± 0.7% of CH_4 on Days 9 to 18 in the highest H_2 to CO_2 ratio at 1:1 (Table 7). Before this current study, no ratios less than 2:1 were tested for its effect on CH_4 production. The results of this study show that low H_2 to high CO_2 ratios can either inhibit or dilute CH_4 production and DF reactors with similar gas compositions should be avoided for DF gas integration in AD.

2.3.4 Experiment #4: Dark Fermentation of Residual Solids and Food Waste for Volatile Fatty Acids Production

Exp. #4-DF (dark fermentation with varying ISRs for VFAs) anaerobically fermented residual solids collected from ADF and the VFA separation process. The residual solids in Exp.

#3-AD (digestion with DF gas injection ratios for bioenergy) and Exp. #4-DF (dark fermentation with varying ISRs for VFAs) were collected at the same time to effectively compare the products from AD and DF. Five treatments were tested in Exp. #4: residual solids from ADF fermented at three ISRs (1.5:1, 2:1, 4:1), food waste fermented at 2:1 ISR, and an inoculum-only control at 1:0 ISR.

The pH of the reactors was below 4.5 during the first 3 days of the batch experiment (Table 9), thus, the pH was adjusted to 7 on Day 3 to reduce pH inhibition during fermentation. The pH of the treatments (excluding the control) increased from an average of 4.28 ± 0.05 on Day 3 to 5.48 ± 0.10 on Day 6. The pH remained stable from Day 6 to Day 15 as the average pH was 5.86 ± 0.07 at the end of the fermentation batch experiment (Table 9). The food waste ISR 2:1 treatment dropped in pH on Day 9 from 5.17 ± 0.02 to 4.99 ± 0.94 which was significantly lower than the pH of the other treatments on Day 9 (p-value < 0.001). However, the pH recovered and none of the pHs were significantly different on Day 12 (p-value: 0.441 – 1.000) or Day 15 (p-value: 0.457 – 1.000).

Table 9. The pH of four treatments using anaerobic dark fermentation (ADF) residual solids (RS) and food waste at three inoculum to substrate ratios (ISR) every three days of a 15-day fermentation period. Standard error based on triplicate reactors. There was no standard error on Day 0 as the triplicate reactors were equivalent pre-fermentation.

Treatment	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
ADF RS ISR 1.5:1	4.34	4.29 ± 0.01	5.54 ± 0.09	6.24 ± 0.74	5.95 ± 0.05	5.87 ± 0.05
ADF RS ISR 2:1	4.29	4.33 ± 0.03	5.64 ± 0.08	6.64 ± 0.07	6.10 ± 0.03	5.99 ± 0.03
ADF RS ISR 4:1	4.32	4.35 ± 0.01	5.55 ± 0.07	6.45 ± 0.07	5.94 ± 0.02	5.89 ± 0.04
Food Waste ISR 2:1	4.34	4.15 ± 0.003	5.17 ± 0.02	4.99 ± 0.94	5.68 ± 0.06	5.67 ± 0.05
Inoculum-only Control ISR 1:0	4.36	4.37 ± 0.00	5.93 ± 0.06	6.65 ± 0.04	6.10 ± 0.00	6.08 ± 0.01

The VFA production among the three ISRs with ADF residual solids and the food waste treatment were similar within each of the six days tested (p-value > 0.05). The three ISRs tested with the ADF residual solids (1.5:1, 2:1, 4:1) were not significantly different from each other on any day (p-value > 0.05). This indicated that the higher inoculum level (ISR 4:1) was not needed, as enough inoculum in the ISR 1.5:1 was shown to proceed with VFA production. The greatest amount of VFAs (28.05 ± 0.89 g/L) in this experiment was on Day 9 in the residual solids ISR 1.5:1 treatment but it was only significantly higher than the inoculum-only control that had no substrate added (p-value = 0.034). Since the VFA production between ISR 1.5:1 with residual solids and the inoculum-only control on Day 9 were the only significant results, the residual solids could be fermented in six days to achieve 26.4 ± 2.1 g/L VFAs to reduce fermentation time by nine days (from 15 days to 6 days).

The food waste and residual solids at the 2:1 ISR had similar total VFAs across all days (p-value: 0.080 – 1.00). The maximum VFAs for both food waste and ADF residual solids at ISR 2:1 was on Day 6 at 23.72 ± 1.44 and 24.73 ± 1.13 g/L VFA, respectively (p-value = 0.998; Fig. 9). The similarity between the two substrates at ISR 2:1 may be from the similar compositions of food waste and the residual solids from ADF. The residual solids were most likely unfermented food waste remaining after a 12-day HRT; therefore, the compositions (TS and VS) were similar (Table 10). A lag phase was observed as the total VFAs increased in all treatments from Day 3 to Day 6 (Fig. 9). The VFAs significantly decreased from Day 9 to Day 12 in residual solids ISR 2:1 (p-value = 0.009), ISR 1.5:1 (p- value < 0.001), ISR 4:1 (p-value < 0.001), and food waste ISR 2:1 (p-value = 0.013) possibly due to the easily degradable substrates being utilized by Day 9 and the more recalcitrant substrates remaining on Days 12 and 15.

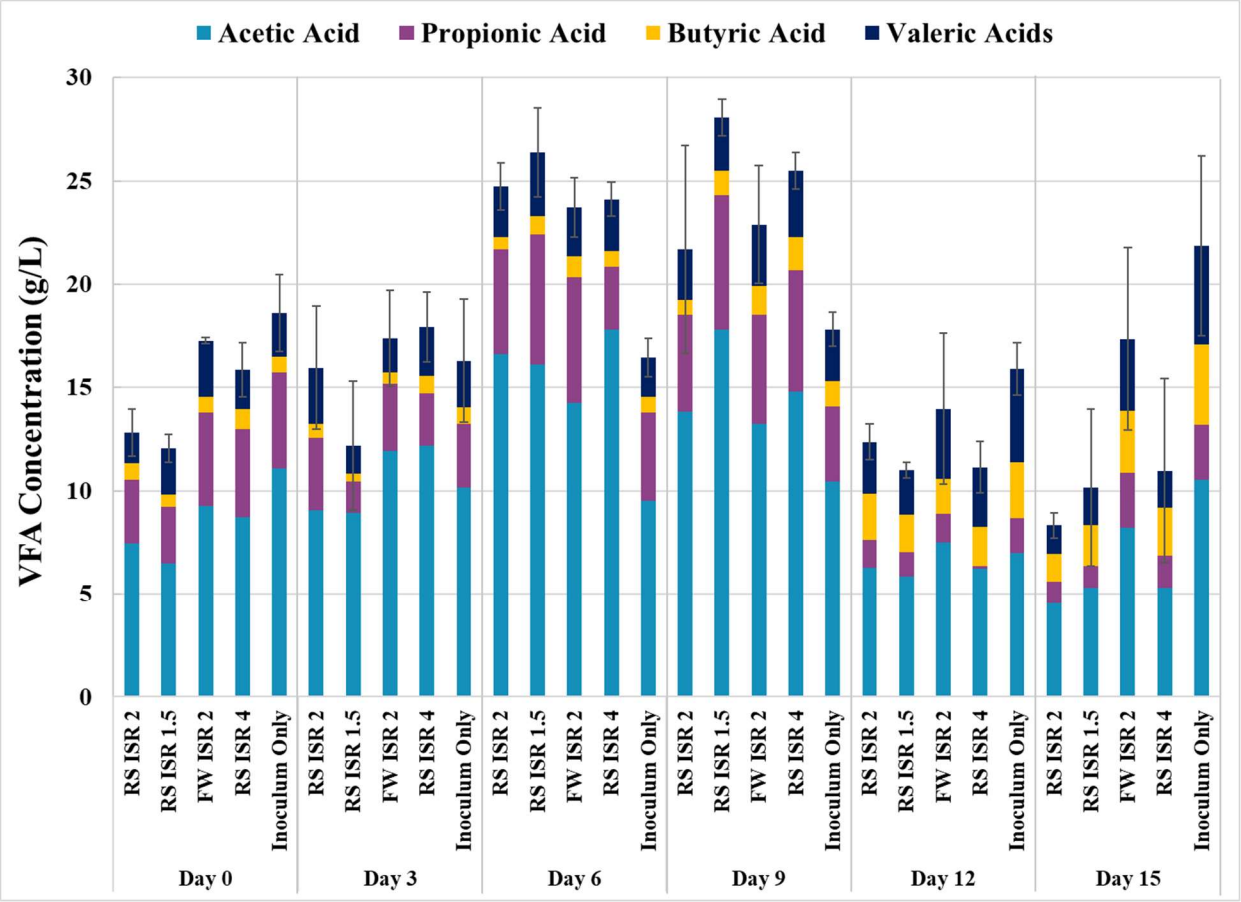

Figure 9. Total volatile fatty acids (VFA) concentration for five treatments over 15 days of fermentation. Treatments included anaerobic residual solids (RS), food waste (FW), and varying inoculum to substrate ratios (ISR) of 1.5:1, 2:1, 4:1, and an inoculum-only control. Standard error bars based on total VFA production.

The residual solids ISR 1.5:1 produced the most amount of VFAs (28.05 ± 0.89 g/L) during the 15-day fermentation but had small to no change in TS (2.7 ± 0.8 to 2.7 ± 0.1%) and VS (2.4 ± 0.5 to 2.2 ± 0.3%) (Table 9). The food waste ISR 2:1 treatment had the greatest VS reduction at 12.9% (2.3 ± 0.3 to 2.0 ± 0.1%) and had higher VFAs compared to the residual solids treatments at the end of the experiment (p-value = 0.080; Fig. 9; Table 9). The pre-fermentation COD was highest in the ADF residual solids ISR 2:1 and 1.5:1 at 49.7 g/L COD for both treatments and on Day 6 these treatments had the highest VFAs (24.73 ± 1.13 and 26.36 ±

2.15 g/L VFAs, respectively) (Fig. 9). The post-fermentation COD was greatest at 50.4 and 57.9 g/L for ADF residual solids ISR 2:1 and 1.5:1, respectively, and the VFA production was the lowest on the last day of fermentation (8.32 ± 0.62 and 10.14 ± 3.80 g/L VFAs, respectively).

Table 10. The total solids (TS) and volatile solids (VS) pre and post fermentation of four treatments including anaerobic dark fermentation (ADF) residual solids and food waste at different inoculum to substrate ratios (ISR). Pre-fermentation standard error based on analyses triplicates and post-fermentation standard error based on triplicate reactors.

Treatment	Pre TS (%)	Post TS (%)	Pre VS (%)	Post VS (%)
ADF Residual Solids ISR 1.5:1	2.7 ± 0.8	2.7 ± 0.1	2.4 ± 0.5	2.2 ± 0.3
ADF Residual Solids ISR 2:1	2.4 ± 0.2	2.6 ± 0.1	2.2 ± 0.2	2.0 ± 0.4
ADF Residual Solids ISR 4:1	2.1 ± 0.1	2.3 ± 0.5	1.8 ± 0.2	1.8 ± 0.5
Food Waste ISR 2:1	2.5 ± 0.2	2.6 ± 0.2	2.3 ± 0.3	2.0 ± 0.1
Inoculum Only Control ISR 1:0	1.8 ± 0.5	2.1 ± 0.2	1.5 ± 0.4	1.5 ± 0.3

The gas production had a maximum of 245 ± 2 mL/g VS in the ADF residual solids ISR 4:1 and the food waste ISR 2:1 produced 208 ± 7 mL/g VS which was 15% less gas. The other ADF residual solids treatments and inoculum-only produced less than 102 mL/g VS. There was no CH_4 present and marginal amounts of H_2 were produced (0.03 to 11.0 mL/g VS). The gas production rate corresponded with the VFA production as there was a high amount of VFAs on Day 9 when the gas spiked in all treatments (Fig. 9; Fig. 10). There was a six-day lag phase observed in the gas and the VFAs were high on Day 6 even though the DF gas was minimal (Fig. 10).

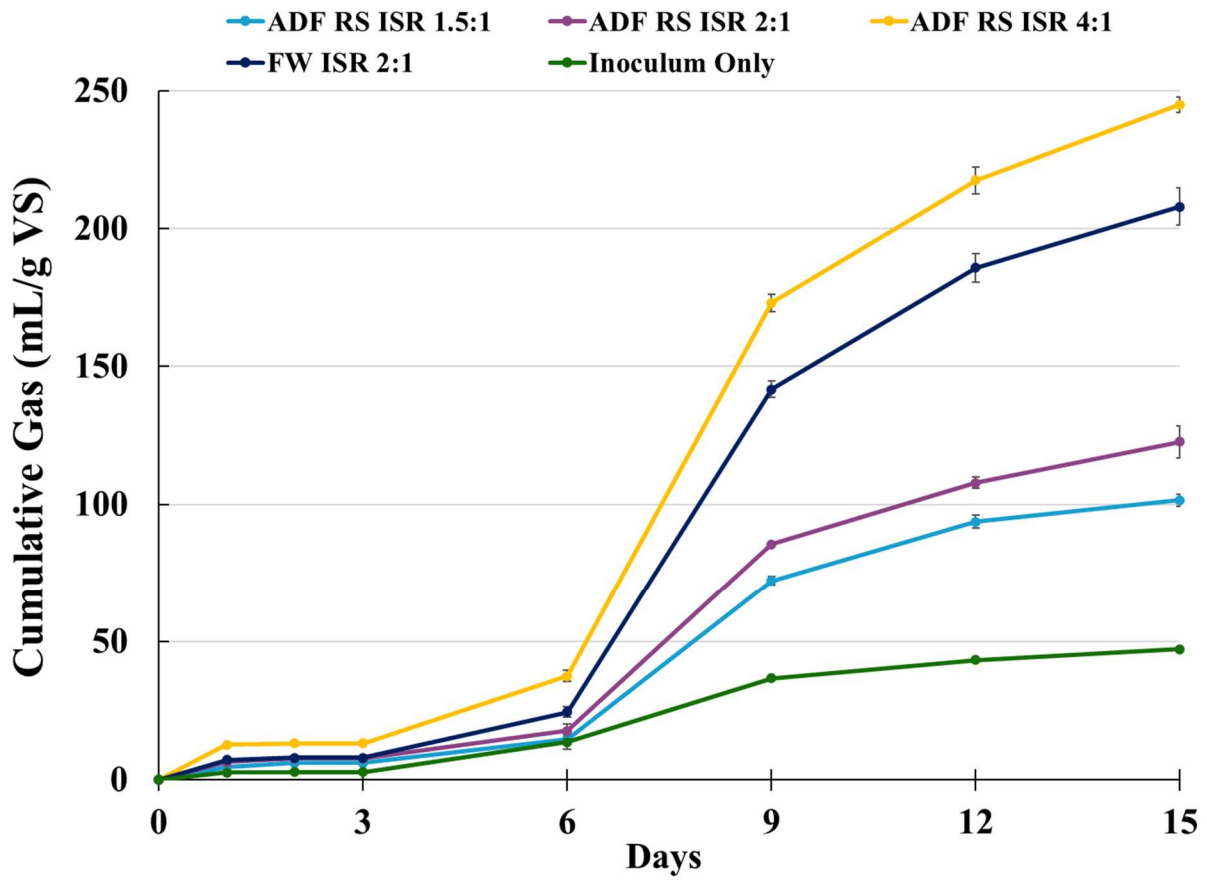

Figure 10. Cumulative dark fermentation (DF) gas production, normalized by grams of volatile solids (VS), of residual solids (RS) from anaerobic dark fermentation (ADF) and food waste (FW) at three inoculum to substrate ratios (ISR). Error bars represented the standard error from triplicate reactors.

Eryildiz et al. (2020) tested a range of pH levels from 4 to 6 in citrus food waste fermentation and there was a direct relationship between increasing pH and increasing VFA production. The highest VFAs (0.793 g VFA/g VS_{added} or 26.43 g/L VFAs) in Eryildiz et al. (2020) were achieved in 22 days at a pH of 6. Slezak et al. (2021) achieved 13.9 g/L VFAs from kitchen-based food waste in four days with a pH at 8. Grzelak et al. (2018) produced 19.5 g/L VFAs in two days with a pH at 7 and 8 with food waste DF. This current study produced 23.7 ± 1.4 and 24.7 ± 1.1 g/L VFAs in food waste ISR 2:1 and residual solids ISR 2:1 in six days, respectively, with an average pH at 5.29 ± 0.12. This current study achieved similar amounts of

VFAs compared to Eryildiz et al. (2020) but in 16 fewer days. Slezak et al. (2021) and Grzelak et al. (2018) produced 41.4% and 17.7% lower VFAs than the VFAs produced in this current study, however, those VFAs were achieved within four and two days of fermentation, respectively.

The gas production in Eryildiz et al. (2020) was 220 mL/g VS which was 10% lower and 5% higher compared to the gas production in this current study from ADF residual solids ISR 4:1 (245 ± 2 mL/g VS) and food waste ISR 2:1 (208 ± 7 mL/g VS), respectively. Eryildiz et al. (2020) did not report the composition of the gas, however, this current study mainly produced CO_2 as there was no CH_4 present and marginal amounts of H_2. Other previous studies were able to produce biohydrogen in food waste fermentation: Martinez-Mendoza et al. (2022) achieved 50.0 mL H_2/g VS in fruit and vegetable waste fermentation, Zhang et al. (2024) obtained 40.5 mL H_2/g dry weight in heat-treated sludge and food waste co-fermentation, and Ding et al. (2017) produced 34.7 mL H_2/g VS in food waste mono-fermentation. Food waste DF had the potential of biohydrogen production; however, this current study was not able to achieve that.

2.3.5 Efficiency of Residual Solids Utilization in Food Waste Dark Fermentation

Food waste DF for VFA production required the physical separation of the VFA-rich liquid and any remaining solids through centrifugation. Only the liquid portion has accessible VFAs which resulted in a by-product in the form of residual solids. This current study digested and fermented the residual solids to determine the amount of product (bioenergy and VFAs) that can be retrieved from this system in addition to the VFAs produced in the initial food waste DF for VFA production. The four experiments (Exp. #1, #2, #3, #4) determined the efficiency (mL CH_4/g VS or g VFA/g VS) of four systems: 1) residual solids AD for bioenergy production; 2) residual solids DF for VFA production; 3) residual solids AD with DF gas integration for bioenergy production; and 4) residual solids DF at various ISRs for VFA production.

The first set of experiments digested (Exp. #1-AD: RS digestion for bioenergy) and fermented (Exp. #2-DF: RS dark fermentation for VFAs) only residual solids from the VFA separation process. The initial fermentation of food waste—that the residual solids were extracted from—produced 0.41 g VFAs/g VS (9.84 g/L VFAs) for anaerobic DF and 0.32 g VFA/g VS (7.35 g/L VFAs) for microaerobic DF. The residual solids fermentation produced an additional 0.20 g VFAs/g VS (4.93 g/L VFAs) in anaerobic DF inoculum and 0.39 g VFA/g VS (8.37 g/L VFAs) in microaerobic DF inoculum. The efficiency of the initial food waste anaerobic DF (0.41 g VFA/g VS) was 0.21 g VFA/g VS higher than residual solids from anaerobic DF (0.20 g VFA/g VS). The efficiency of the initial food waste that underwent microaerobic fermentation (0.32 g VFA/g VS) was 0.07 g VFA/g VS lower to fermentation of the residual solids from microaerobic fermentation (0.39 g VFA/g VS). There was also the potential for bioenergy production from the ADF and MADF residual solids at 395 ± 8 and 430 ± 4 mL CH_4/g VS, respectively.

The second set of experiments with DF gas integration for bioenergy production (Exp. #3-AD: digestion with various DF gas ratios added for bioenergy) and DF at different ISRs (Exp. #4-DF: dark fermentation with multiple ISRs for VFAs) used residual solids from an initial anaerobic food waste DF that produced 0.82 g VFA/g VS (17.2 g/L VFAs). Exp. #4-DF produced an additional 1.46 g VFA/g VS (25.5 g/L VFAs) after nine days of residual solids fermentation at an ISR 4:1. Exp. #3-AD produced 492 ± 11 mL CH_4/g VS after 27 days of AD without DF gas integration. Food waste AD was tested in Exp. #3-AD to compare to residual solids AD and produced a maximum of 470 ± 11 mL CH_4/g VS. The residual solids, from anaerobic DF of food waste, had a 33 and 4% higher production of VFAs and CH_4 production than food waste, respectively.

The additional amount of product that could be achieved from residual solids was determined by calculating the efficiency of food waste and residual solids fermentation (g VFA/g VS) and digestion (mL CH_4/g VS). The residual solids showed the potential to produce more VFAs and CH_4 compared to food waste. The by-product (residual solids) from food waste DF to VFA production was valorized through additional bioenergy and VFA production. In conclusion, the process of food waste DF to VFA production can be increased by digesting and fermenting the by-products (residual solids).

2.4 Conclusion

This study established the impact of residual solids and food waste DF at three ISRs, and AD with DF gas integration. The results showed that residual solids remaining after food waste DF can be extracted for additional products such as CH_4 and VFAs. DF gas integration with lower H_2 to higher CO_2 ratios diluted CH_4 production and only DF reactors that could produce high H_2 should be integrated with AD. Residual solids can be refermented at an ISR of 1.5:1 for six days for maximum VFA production at a low inoculum level. These findings can be used to improve efficiency of food waste conversion to bioenergy and VFAs.

Chapter 3: Volatile Fatty Acids Production through Dark Fermentation of High Salinity Food Processing Waste

3.1 Introduction

Municipal solids waste landfills contribute to one-third of the United States' anthropogenic methane emissions which is a greenhouse gas 25 times more potent than carbon dioxide (US EPA, 2019). Food waste in landfills contributes to 58% of methane emissions annually in the United States (Walter, 1961). Other issues associated with food waste include freshwater and soil health depletion, transportation usage, and economic losses (USDA, 2021). Food waste is an abundant source of organic material with a high carbon content beneficial for volatile fatty acids (VFA) creation i.e. short carbon chains. These VFAs can be used to generate pharmaceuticals, bioenergy, and bioplastics (Agnihotri et al., 2022). The VFAs need to be broken down from large carbon chains, such as food waste, through a biological process known as dark fermentation or acidogenic fermentation. Dark fermentation is a biological waste-treatment technology that transforms organic matter into VFAs, carbon dioxide gas (CO_2), and hydrogen gas (H_2) in the absence of light and oxygen. While food waste is an abundant source of carbon, there are challenges with mono-fermentation of food waste such as acidity and high organic loads (Elbeshbishy et al., 2017). Food waste with high salinity from kitchen-waste and biodiesel by-products pose other challenges such as disruption of cellular osmolarity and less diverse microbial communities (Wang et al., 2023; L. Zhang et al., 2016). A potential solution is the co-fermentation of food waste and high salinity food processing waste.

Food waste dark fermentation for VFA production has been well-studied. Various experiments have tested different organic loadings rates and pHs, two parameters that have a large impact on fermentation. Low organic loading rates successfully produced high VFAs,

while higher loading rates inhibited the microbial community (Slezak et al., 2017; Swiatkiewicz et al., 2021). The microbes could also be inhibited by acidic pHs (below 4). Studies have shown high VFA production when the pH was manually adjusted above a pH of 6 (Grzelak et al., 2018; Slezak et al., 2017). The solutions to inhibition from high organic loads and acidic pHs require additional materials, costs, and time. Lower organic loading rates require longer storage capacity and processing time, increasing energy consumption (Sun et al., 2019). Buffers for pH increase overall costs and maintenance.

Food waste feedstocks that are high in salinity pose another issue for fermentation. The high salt can inhibit VFA production through cellular osmolarity disruption. Several studies have tested different salinity concentrations to determine the extent of its effect on VFA production. There was a negative relationship between increasing salinity and decreasing VFA production as the salt inhibited VFA production by 80 to 90% compared to regular food waste fermentation (Huang et al., 2022; N. Liu et al., 2017). Conversely, another study observed higher VFAs in high salinity fermentation, but the maximum VFAs produced was 82% less than regular food waste in other studies (Huang et al., 2022; Liu et al., 2017; Sarkar et al., 2020). Prior studies have used sodium chloride (NaCl) to evaluate the effect of salinity on VFA production, but these studies do not factor in the organic content contributing to fermentation with high salinity food processing waste.

Organic matter and salinity can be found in glycerol obtained as a by-product from biodiesel creation. Glycerol production has increased with rising biodiesel demand over the past decade (Attarbachi et al., 2023). The biodiesel manufacturing process involves high speed centrifugation that separates into two layers of methyl ester fatty acids and crude glycerol (Bagnato et al., 2017). The unprocessed glycerol contains salt and organic matter which could be

fermented for VFAs (Anitha et al., 2016). The mono-fermentation of glycerol for VFA production was challenging in prior studies (Montiel-Jarillo et al., 2021; Silva et al., 2013); however, controlled oxidation-reduction potential, direct interspecies electron transfer (DIET), and two-stage AD were tested to improve glycerol processing (Im et al., 2019; F. M. S. Silva et al., 2018; Vesga-Baron et al., 2021). Another potential solution is co-fermentation with another waste stream.

Co-fermentation with two or more substrates is a common technique with challenging substrates such as glycerol. Menezes et al. (2023) digested vinasse and glycerol which prevented mono-digestion failure of vinasse. This co-digestion could also dilute inhibitory compounds such as sulfate and provide a balance of bacteria and acetoclastic archaea (Menezes et al., 2023). Other substrates that have been co-fermented with glycerol include molasses, glucose, and banana waste (Haosagul et al., 2019; Pereyra et al., 2020; Sawasdee et al., 2019). Prior studies have not extensively studied the co-fermentation of glycerol or saline food waste and food waste mixtures.

The contribution of organic content and salinity has not been assessed in food waste and high salinity food processing waste co-fermentation. The main objective of this research was to determine the effect of varying salinity concentrations on VFA production in mono- and co-fermentation of food waste and high salinity food processing waste (derived from glycerin processing). The results from this current study could provide a better understanding of high salinity food processing waste dark fermentation and insight into VFA production which could reduce the material cost and valorize multiple organic waste streams.

The objectives of this research were to: 1) determine the effect of four salinity concentrations on VFA production in mono- and co-fermentation of food waste and high salinity

food processing waste (derived from glycerin processing); 2) analyze the effect of food waste and high salinity food processing waste co-fermentation on pH, organic content, and gas production; and 3) statistically compare mono- and co-fermentation of food waste and high salinity food processing waste using analysis of variance (ANOVA) tests.

3.2 Materials and Methods

3.2.1 Substrate and Inoculum

High salinity food processing waste (HSFW) consisted of settled glycerin sludge from vegetable oil processing into biodiesel provided by an industry partner in Ohio, US and refrigerated at 2.7ºC upon collection. The HSFW had a sodium (Na) concentration of 85.6 g/L, which is equivalent to 214 g/L sodium chloride (NaCl) based on 0.400 g Na in 1 g NaCl (McLean et al., 2023). The food waste (FW) was a lab-prepared mixture adapted from the household FW characterization by the US Department of Agriculture and had salt concentration of 0.0092 g/L Na, which was equivalent to 0.023 g/L NaCl (Table 2; Buzby et al., 2014). Based on the Standard Methods (Walter, 1961), the average total solids (TS) and volatiles solids (VS) concentration of the HSFW were 43.3 ± 0.60% and 20.7 ± 0.46%, respectively. The average TS and VS concentration of the FW was 21.6 ± 0.29% and 20.4 ± 0.28%, respectively. The HSFW was a liquid, and the FW was a solid paste, therefore, the FW was mixed with water based on a 2.5 g VS/L-day organic loading rate (OLR) to create a liquid substrate. This OLR was chosen to emulate two semi-continuous, lab-scale (37.8 L) dark fermentation reactors located at College Park, MD, US which were maintained at a 2.5 g VS/L-day OLR at mesophilic conditions (35°C) with the pH adjusted to 7 using 10% KOH once a week, and 12-day hydraulic retention time (HRT). One reactor operated under anaerobic conditions (810 days) while the other was

microaerobic (739 days), which involved sparging with 0.5 L O_2/min for one hour, twice a week. The microaerobic condition was tested to improve VFA production by increasing hydrolysis efficiency and microbial diversity (Cao et al., 2022; Nguyen et al., 2019; Wang et al., 2020). The microbes required for the semi-continuous experiment were sourced from a 1:1 mixture (by volume) of content from the anaerobic and microaerobic dark fermentation reactors. Both inoculum sources were incubated with FW at a salinity level of 0.023 g/L NaCl FW, which was 99.9% lower than the concentration of the HSFW (214 g/L NaCl) substrate.

3.2.2 Experimental Design

The semi-continuous experiment tested three ratios of the FW and water mixture to HSFW (by volume) including FW-only and HSFW-only as controls (Table 11). The 0.5 L reactors followed a 12-day HRT for five consecutive periods. Every 3 – 4 days, the digestate was collected followed by adding freshly prepared substrate into the dark fermentation reactors. The dark fermentation samples were analyzed for pH and VFAs every 3 – 4 days. At the end of each HRT, the TS, VS, chemical oxygen demand (COD), soluble chemical oxygen demand (sCOD), and dark fermentation gas quality and quantity (H_2, CH_4, CO_2) were tested. The pH of the reactor contents that fell below a pH of 7 were adjusted to 7 once per week using 10% KOH.

Table 11. Experimental design for food waste (FW) and high salinity food processing waste (HSFW) dark fermentation in triplicate semi-continuous reactors. The total volume of the reactors (0.4 L) was removed and replaced with fresh substrate over a 12-day HRT. Each treatment had different organic loading rates (OLR) and sodium concentrations (g/L Na).

Treatment	Ratio (FW:HSFW)	HSFW (mL)	FW and Water Mixture (mL)	Total OLR (g VS/L-day)	Na (g/L)
FW Only	1:0	0	400	2.5	0.0
Blend of FW and HSFW	3:1	100	300	6.3	21.4
	1:1	200	200	10.1	42.8
	1:3	300	100	13.9	64.2
HSFW Only	0:1	400	0	17.8	85.6

3.2.3 Analytical Testing

Dark Fermentation Gas Quality and Quantity Testing

The dark fermentation gas was collected in 0.5 L Tedlar sample bags (with polypropylene fitting) attached to the 0.5 L reactor bottles. The gas bags were volumetrically measured using a plastic 60 mL syringe and tested for CH_4, CO_2, and H_2 using a gas chromatograph (Agilent Technologies, Inc.; Shanghai China; model 7890A) with a thermal conductivity detector at 250°C, a HP-Plot Q capillary column (Agilent J&W; US), He as the carrier gas for CH_4 and N as the carrier gas for H_2 at 8.6 mL/min, an oven operated at 60°C for 2 min and subsequently ramped at 30°C/min to 240°C. The dark fermentation gas quantity and quality were analyzed depending on gas production.

Water Quality Testing

All triplicate reactors were tested within 48 h of sample collection. An Accument AB15 pH probe was used to analyze the pH. The TS and VS were measured following APHA Methods APHA 2540D and 2540E (Walter, 1961). The TS analysis involved pipetting 10 mL of liquid sample into a pre-dried (at 550°C) porcelain crucible and then dried at 105°C until the weight

remained constant. The VS analysis placed the dried TS crucibles in a 550°C furnace oven until a constant weight was achieved. The COD and sCOD were analyzed using a Hach Method 8000 high-range COD digestion kit and a Hach DR 5000 spectrophotometer (Hach Company, Loveland, Colorado, US). The sample for sCOD was filtered to 0.45 µm before used in the COD digestion kit. Sodium (Na) was tested at AgroLab Inc. (Harrington, DE, US) using the CAT method.

Volatile Fatty Acids Testing

Acetic, propionic, butyric, iso-valeric, and valeric acids were measured after acidified to a pH < 2 with 5.25 N sulfuric acid and filtered to 0.22 µm. The treatments with HSFW were diluted with a 1:1 dilution factor for VFAs analysis due to high salt content. The selected VFAs were analyzed through a high-performance liquid chromatograph (HPLC) (Waters; US; Model QSM-R, FTN-R, 2998), Elution mode - Gradient: 90:10 ratio (10 mM K_2HPO_4 buffer, pH 2.0 and Acetonitrile (100)); Flow rate of 1 mL/min, column temperature at 40°C, and column C18.

3.2.4 Statistical Analysis

The VFAs (g/L) and pH production were tested for significant differences (p-value < 0.05) in StatPlus Pro 7.6.5. An analysis of variance (ANOVA) and a Tukey honest significant difference (HSD) multiple comparisons tests were selected to determine if there were significant differences.

3.3 Results and Discussion

3.3.1 Effect of Organic Loading Rates (OLR) and Salinity Concentrations on VFA production

There was an initial lag phase in the first HRT for the total VFA production, but a steady state was observed in the remaining four 12-day HRTs (Fig. 11). After the initial lag phase in the first HRT, the HSFW-only treatment produced the most amount of VFAs compared to the co-fermentation of HSFW and FW, and FW-only treatments with an average of 32.45 ± 0.55 g/L VFAs for the remaining four HRTs (Fig. 11). The maximum VFAs produced in the 62-day semi-continuous fermentation was 36.04 ± 0.54 g/L VFAs on Day 51 in the HSFW-only treatment. The maximum VFAs in the HSFW-only treatment was 25.4 to 74.2% higher than the other four treatments of FW and HSFW co-fermentation and FW mono-fermentation. The direct relationship between increasing HSFW loading and increasing VFA production reflected the biodegradability and fermentation stability of HSFW as a substrate.

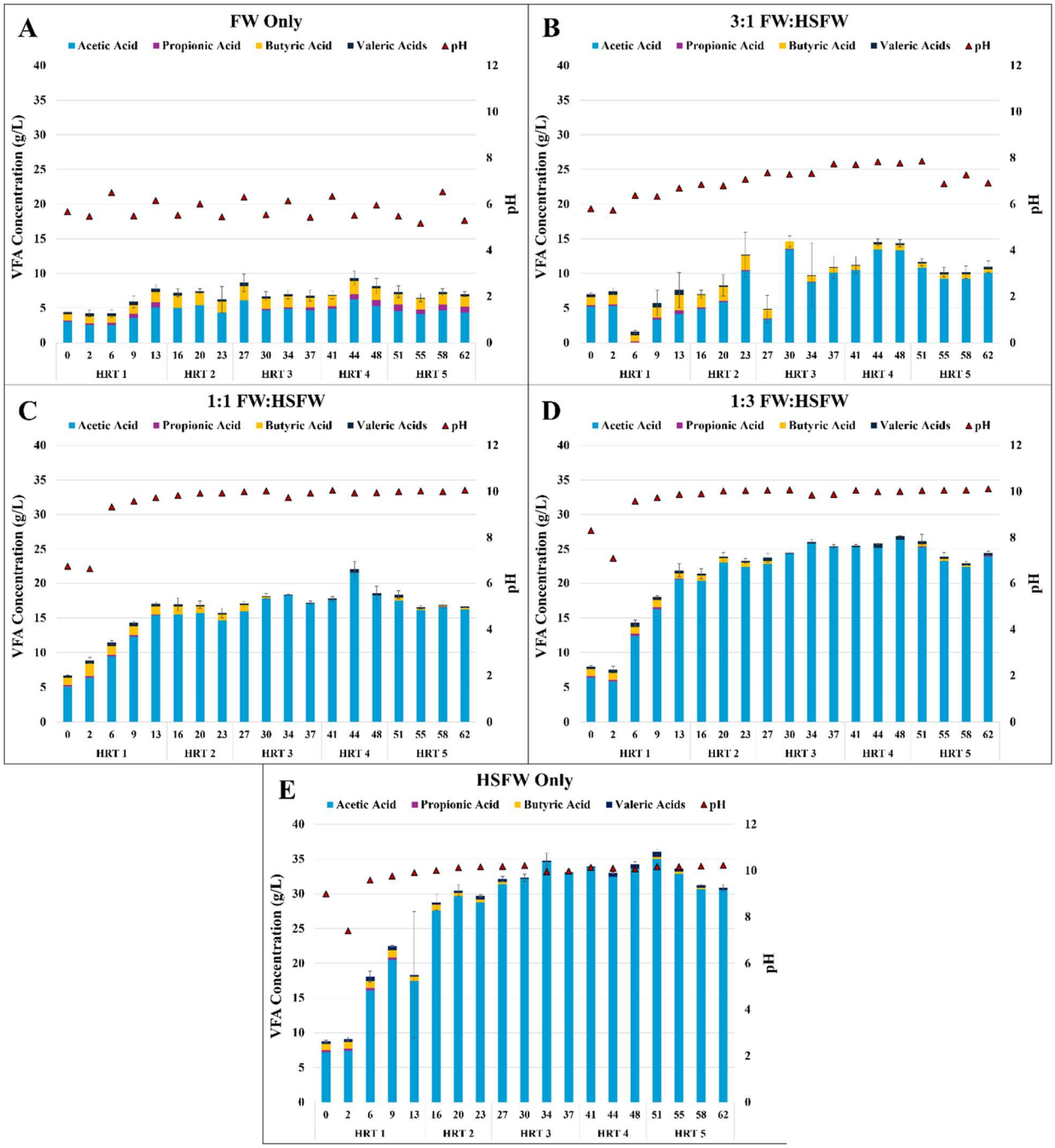

Figure 11. The composition of volatile fatty acids (VFA), shown as bars, and pH, shown as triangles, over five hydraulic retention times (HRT) of 12 days from five treatments including food waste (FW) (Fig. A), high salinity food processing waste (HSFW) (Fig. B), and three ratios of FW to HSFW by volume (Fig. C – E). The standard error (SE) bars for VFAs were based on the total VFA production from triplicate reactors, and the SE bars for pH were based on triplicate reactors, but were not visible due to small SE.

A statistically significant relationship was observed between increasing HSFW loading and increasing total VFA production (p-value < 0.001; Fig. 11). This may correspond to the OLR of the treatments rather than the salinity. The HSFW contained 20.7 ± 0.46% VS as well as salinity (85.6 g/L Na) and only one of these parameters could be fixed. The goal of the semi-continuous experiment was to determine the effect of salinity on VFA production in FW and HSFW dark fermentation. As the salinity concentration increased from adding more HSFW, the OLR increased as well.

The HSFW-only treatment (85.6 g/L Na) had an OLR at 17.8 g VS/L-day while the FW-only treatment (0.0 g/L Na) had a 2.5 g VS/L-day OLR. The 86.0% difference between OLRs had a drastic effect on the VFA production. The VFA production in the HSFW-only treatment was significantly higher than all other treatments in HRTs 2 - 5 (p-value < 0.001; Fig. 11). The FW-only and 3:1 FW:HSFW had statistically similar VFAs at 6.78 – 8.66 g/L VFAs and 4.90 – 11.21 g/L VFAs, respectively, on six out of the 19 sampling days which meant that increasing the OLR by 152% from 2.5 to 6.3 g VS/L-day in the FW-only and 3:1 FW:HSFW treatment did not significantly impact VFA production (p-value > 0.05). There was no negative effect on increasing the OLR to 10.1, 13.9, and 17.8 g VS/L-day in the 1:1, 1:3 FW:HSFW treatments, and HSFW-only treatments, respectively, as the total VFA production significantly increased as OLR increased (p-value < 0.001; Fig. 11).

After the initial lag phase in HRT 1, the VFA production in each treatment was consistent from HRT 2 – 5 (Fig. 11). The five treatments had average total VFAs from HRT 2 – 5 of 32.45 ± 0.54, 24.58 ± 0.39, 17.65 ± 0.4, 10.79 ± 0.75, and 7.33 ± 0.22 g/L VFAs for HSFW-only, 1:3 FW:HSFW, 1:1 FW:HSFW, 3:1 FW:HSFW, and FW-only, respectively. The standard errors (SE) for the VFA concentrations in all treatments from HRT 2 – 5 ranged from 0.23 – 0.75, with

the 3:1 FW:HSFW treatment having the greatest SE and FW-only treatment having the lowest SE. The consistency of the VFA production in all treatments demonstrated the reliable performance of FW and HSFW mono- and co-fermentation systems.

Swiatkiewicz et al. (2021) completed a similar study with semi-continuous FW dark fermentation at two OLR, two HRTs, and a controlled pH at 7. The highest VFA production (24.8 g/L) was achieved at the higher OLR (5.0 g VS/L-day) and the longer HRT (10 days). The authors suggested that the longer HRT provided microorganisms enough time to process the large amount of organic material loaded into the reactor. The lower OLR at 2.5 g VS/L-day reached a maximum VFA production of 9.0 g/L in the 10-day HRT. This current study achieved 0.03% higher VFA concentration (9.29 ± 1.01 g/L) than Swiatkiewicz et al. (2021) at the same OLR (2.5 g VS/L-day) and a 12-day HRT in the FW-only treatment. The 3:1 FW:HSFW treatment had a 6.3 g VS/L-day OLR and produced 14.60 ± 0.78 g/L VFAs which was 41.1% less than in 5.0 g VS/L-day treatment in Swiatkiewicz et al. (2021) at 24.8 g/L VFAs. This current study produced 22.10 ± 1.11 g/L VFAs at the 10.9 g VS/L-day OLR and Swiatkiewicz et al. (2021) achieved 24.8 g/L VFAs at 5.0 g VS/L-day which was 10.9% higher VFA production at a 54.1% lower OLR than this current study, indicating a higher efficiency in Swiatkiewicz et al. (2021). The maximum VFAs achieved in this current study (36.04 ± 0.54 g/L) was 31.2% higher than the maximum VFAs in Swiatkiewicz et al. (2021) (24.8 g/L VFAs) at a 71.9% higher OLR (17.8 g VS/L-day).

Slezak et al. (2017) fermented kitchen-based FW and found that an OLR of 48.2 g VS/L-day and an HRT at 4 days could produce 9.81 g/L VFAs which was 8.25% higher VFAs and 89.6% lower OLR in Swiatkiewicz et al. (2021) (9.0 g/L VFAs and 5.0 g VS/L-day OLR). Slezak et al. (2017) observed a direct relationship between increasing VFA production and

increasing OLR from 4.13 to 48.2 g VS/L-day. Slezak et al. (2021) improved VFA production (13.9 g/L) by decreasing the OLR to 25.5 g VS/L-day at a 4-day HRT but this was 61% lower than the maximum VFA produced in this current study (36.04 ± 0.54 g/L).

Wainaina et al. (2020) tested a 34-day semi-continuous FW fermentation at four OLRs of 4, 6, 8, and 10 g VS/L-day. The semi-continuous system had a 15-day lag phase which was three days longer than this current study at a 12-day lag phase. The results showed that increasing OLR improved VFAs concentration (g/L) and the average VFAs after the lag phase was 36.99 ± 1.68 g/L from Days 16 – 34 at 10 g VS/L-day. The lower OLRs at 4 and 6 g VS/L-day had average VFAs concentrations at 11.44 ± 1.09 and 16.04 ± 0.84 g/L which was 69.1 and 56.6% lower than the 10 g VS/L-day, respectively. In comparison to this current study, the highest VFAs were achieved at 36.04 ± 0.54 g/L in a 12-day HRT and 17.8 g VS/L-day and was 2.6% lower than Wainaina et al. (2020) which produced 36.99 ± 1.68 g/L at a 10-day HRT and 10 g VS/L-day OLR.

This current study observed a direct relationship between increasing VFA production and increasing OLR from 2.5 to 17.8 g VS/L-day and 12-day HRT with the highest VFA production of 36.04 ± 0.54 g/L at 17.8 g VS/L-day in the HSFW-only treatment. Previous literature showed the same trend at OLRs from 2.5 to 48.2 g VS/L-day and HRTs at 4 to 10 days in FW fermentation (Slezak et al., 2017; Swiatkiewicz et al., 2021; Wainaina et al., 2020). The VFA production from HSFW mono-fermentation in this current study and the mono-fermentation of FW in previous literature indicated a similar biodegradability of two carbon-rich substrates for VFA production.

3.3.2 Effect of Salinity on VFA Production

The salinity concentrations of this semi-continuous fermentation of FW and HSFW were adjusted to determine the effect of salinity on VFA production. As previously mentioned, HSFW has organic content and salinity. When the salinity was adjusted to test how salinity affects VFA production, the OLR was consequentially adjusted as well. This resulted in a wide range of OLRs from 2.5 to 17.8 g VS/L-day. The VFAs were normalized by g VS to negate the variability from OLRs and to observe the direct effect of salinity (Fig. 13).

When normalizing by g VS, the FW-only treatment produced the most VFAs in HRTs 2 – 5 (0.65 – 1.24 g VFA/g VS) while the salinity treatments produced 37.9 – 70.8% less VFAs per g VS compared to no salinity (0.19 – 0.77 g VFA/g VS) (p-value: 0.746 – 1.000; Fig. 13). The VFAs produced, normalized by g VS, were consistent in each HRT for the five treatments as the SE ranged from 0.01 – 0.16 (Fig, 13). The wide range of salinity concentrations from 21.4 to 85.6 g/L Na did not significantly influence VFA production efficiency (p-value > 0.05). However, it was hypothesized that elevated salinity would inhibit VFA production based on current literature, which was not observed in this current study.

Huang et al. (2022) assessed the anaerobic fermentation of synthetic kitchen wastewater with six different salinity levels ranging from 0 to 20 g/L NaCl. The batch experiment concluded that low-salinity treatments reached at least 80% of the VFAs of regular food waste in three days, while high-salinity treatments only produced 43% of the VFAs of regular FW. Liu et al. (2017) conducted a similar study using a synthetic food waste mixture fermented at four salinity levels of 3, 6, 9, and 12 g/L for five days. The highest salinity of 12 g/L produced below 5 g/L VFAs while lower salt concentrations of 3, 6, and 9 g/L produced 36.2, 33.4, and 22.7 g/L VFAs, respectively. Conversely, Sarkar et al. (2020) produced the greatest amount of VFAs in the

second highest salinity concentration (40 g/L) at 6.6 g/L after 48 hours of fermentation; lower salinity concentrations produced similar VFAs at 6.1 to 6.5 g/L. The results from Sarkar et al. (2020) produced 82% less VFAs than presented in Liu et al. (2017) which had a maximum of 36.2 g/L VFAs at a 3 g/L Na.

He et al. (2019) tested a wider range of NaCl concentrations from 10 g/L to 70 g/L in FW dark fermentation. The results showed benefits in VFA production from NaCl addition up to 10 g/L and inhibition with higher concentrations, similar to Liu et al. (2017). The maximum VFAs produced were 31.53 g/L at the 10 g/L NaCl after 15 days of fermentation. The highest salinity concentration at 70 g/L NaCl produced 22% less VFAs than 10 g/L Na, however, the overall VFAs was still high at 24.65 g/L after 19 days of fermentation. The HSFW-only treatment in this current study had 85.6 g/L Na and produced 19% more VFAs at 30.48 ± 0.83 g/L on Day 20 of the semi-continuous fermentation compared to He et al. (2019). The HSFW-only treatment achieved a maximum of 36.04 ± 0.54 g/L VFAs on Day 34, which was 29.1% higher than the highest salinity concentration (70 g/L NaCl) in He et al. (2019). The extreme salinity from HSFW mono-fermentation, in this current study, did not negatively affect VFA production compared to co-fermentation of FW and HSFW.

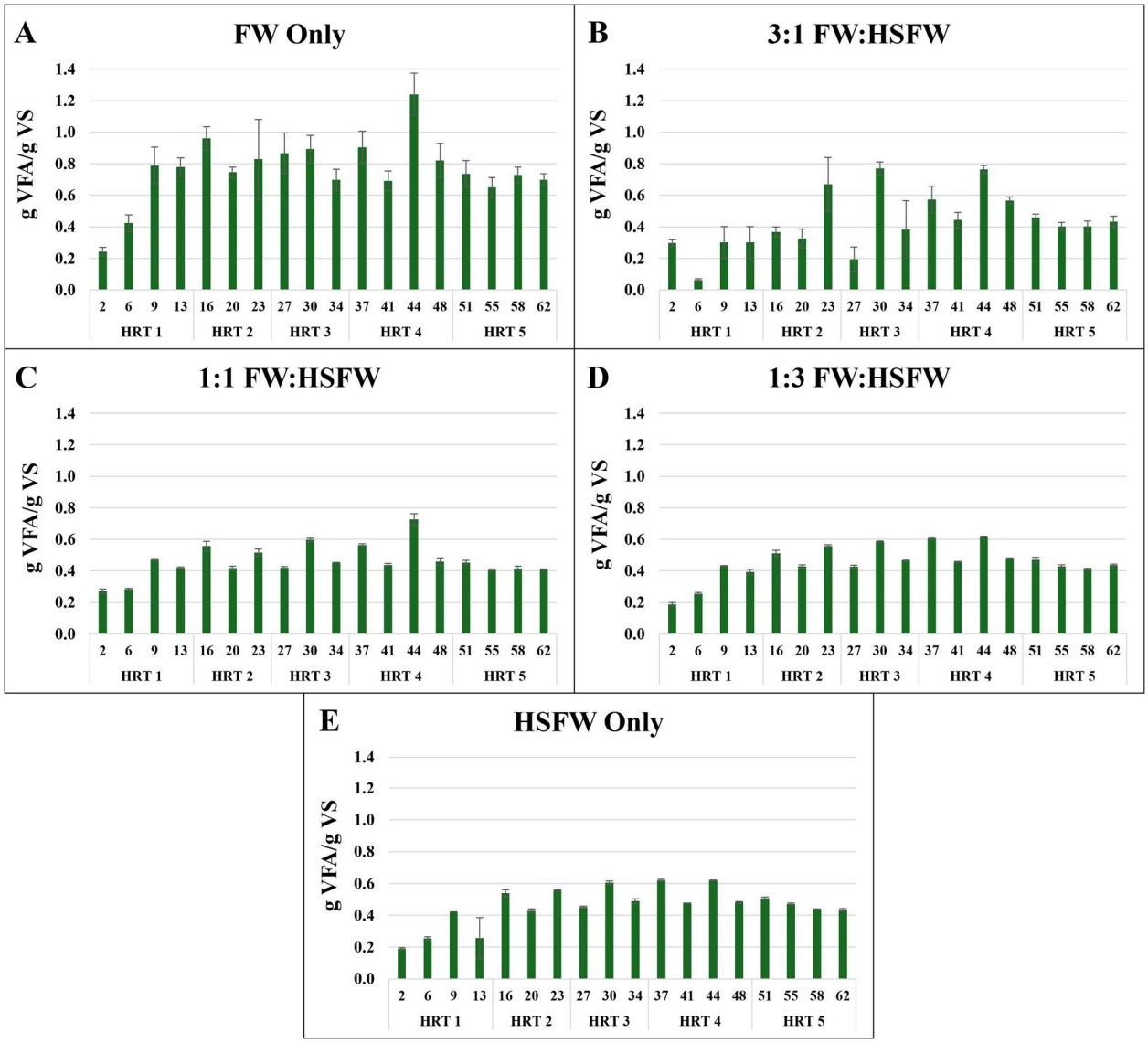

Figure 12. The total volatile fatty acids (VFA) normalized by grams of volatile solids (VS) to measure the efficiency of converting food waste (FW) to VFAs. The g VFA/g VS for the five treatments from five 12-day hydraulic retention period (HRT). The treatments include FW (Fig. A), high salinity food processing waste (HSFW) (Fig. B) and three ratios by volume of FW to HSFW (Fig. C – E). Standard error (SE) bars based on triplicate reactors.

3.3.3 Effect of Salinity on VFA Composition

The composition of VFAs in the higher salinity treatments were predominantly acetic and valeric acids while lower salinity treatments produced acetic, butyric, and valeric acids. The 3:1 FW:HSFW produced trace amounts of propionic acid and all other treatments did not produce

any propionic acid (Fig. 12). There was an observed decrease in butyric acid in salinity treatments through HRTs 2 – 5. Throughout the HRTs, the diversity of VFAs decreased in treatments with HSFW while it increased with the FW-only treatment as propionic acid increased from 0.00 ± 0.00 in the second HRT to 0.64 ± 0.15 g/L in the fourth HRT. The high salt environment most likely reduced microbial diversity which could explain the decrease in the amount of different VFAs.

He et al. (2019) found that butyric acid decreased, and propionic acid increased with higher salinity in batch FW dark fermentation. The authors suggested that there was a shift from butyric acidogenic fermentation to propionic, however, more than 50% of total VFAs were comprised of acetic acid in most treatments. This current study also observed a decrease in butyric acid as salinity increased but there was no propionic acid found in any salinity treatments throughout HRT 2 – 5 (Fig. 12).

He et al. (2019) observed a decrease in typical acidogenic bacteria, *Bacteroidetes* phylum, and an increase in *Actinobacteria* phylum, a class of bacteria typically found in coastal salt marsh soils. He et al. (2019) concluded that *Bacteroidetes* phylum was not adapted to salty conditions and the *Actinobacteria* was suitable for high salinity conditions. Li et al. (2021) found a similar trend of decreasing microbial diversity with increasing salinity due to the microorganism death due to osmotic pressure; the *Actinobacteria* phylum grew with the high salinity treatments. The high VFA production found in the HSFW mono-fermentation in this current study could be explained by the microbial communities adapting to the saline environment.

3.3.4 Effect of pH on VFA Production

The pH was directly related to the pH of the substrate added to each reactor; the pH of the FW and water mixture was acidic at 5.83 ± 0.06 and the pH of the HSFW was alkaline at 9.85 ± 0.24 (Table 12). The pH increased with HSFW loading and was consistent within each treatment throughout the five HRTs (Table 12). The alkalinity of the HSFW substrate acted as a pH buffer for the FW and water substrate. Dark fermentation of FW tends to decrease in pH due to the acidity of the substrate and increasing VFA production. It is common industrial practice to use alkaline buffers such as calcium hydroxide ($Ca(OH)_2$) and sodium hydroxide (NaOH) to prevent inhibition from acidic conditions which can account for 15% of total costs (Pau et al., 2022). This current study adjusted the reactors that were lower than a pH of 7 back to a pH of 7 every week to prevent acidic inhibition as a neutral pH has been successful in previous studies (Hussain et al., 2017; Lu et al., 2020; Swiatkiewicz et al., 2021; L. Zhang et al., 2020). The FW-only treatment required pH adjustment every week and the 3:1 FW:HSFW was adjusted two times during the nine-week experiment. The FW-only treatment used over 95% more potassium hydroxide (KOH) to reach a neutral pH compared to the 3:1 FW:HSFW treatment. The 1:1, 1:3 FW:HSFW, and HSFW-only treatments did not need any pH adjustments as the pH did not drop below 7 (Table 12). The high VFA production and lack of pH adjustments would decrease costs on an industrial scale in FW and HSFW co-fermentation.

While the range of pH tested across the five treatments varied greatly (5.76 – 10.12) , the change in pH over time within each substrate was low, with the SE of these values ranging from 0.02 to 0.12 using 14 sampling points over HRT 2 – 5. The HSFW-only, 1:3 FW:HSFW, and 1:1 FW:HSFW mixtures had the highest pH values and lowest SE of the values (10.12 ± 0.02, 10.01 ± 0.02 , and 9.95 ± 0.02, respectively), while the FW-only and 3:1 FW:HSFW had the lower pH

values and slightly higher SE of these values (5.76 ± 0.12 and 7.33 ± 0.11, respectively). The low SE demonstrated the effectiveness of HSFW as a pH buffer for FW dark fermentation for VFA production. The high pH (9 – 10) and low variation over four HRTs showed the inconsequential effect of high acids production through VFAs during HSFW fermentation. There was no need to adjust the pH during any time of the experiment when HSFW was added at any ratio. Conversely, the FW-only treatment had to be adjusted to a pH at 7 once a week to not fall below pH 4.5, with the average pH value (5.76 ± 0.12) based on these pH adjustments. The day after the fresh FW substrate was added into the FW-only semi-continuous reactors, the pH ranged from 4.69 to 5.18. Three days after KOH additions to raise the pH, the pH ranged from 5.95 to 6.53, as shown in Figure 11. The high VFAs produced from the HSFW in addition to eliminating the need to add KOH to the reactors is a large benefit for its inclusion.

It was expected that the alkalinity of the HSFW would inhibit VFA production as acidogenic bacteria typically thrive in slightly acidic or neutral conditions. Conversely, Cheah et al. (2019) reported that alkaline conditions of 9.5 to 10 improved VFA production compared to the acidic semi-continuous reactors. The acetic acid was the primary VFA present (91%) in the alkaline conditions. Ma et al. (2019) also found acetic acid to be the primary component in alkaline conditions (pH 9 – 11), however, the overall VFA production was minimal at 5 and 10 g/L VFAs. This current study produced more VFAs in the alkaline treatments. The 1:1, 1:3 FW:HSFW, and HSFW-only treatments had similar pHs (p-value > 0.05) and all treatments with HSFW had significantly higher pH than the FW-only treatment (p-value < 0.001). The alkalinity of the HSFW provided conditions that produced high acetic acid concentrations without the need to adjust pH using KOH.

Table 12. The average pH of the five treatments in each 12-day hydraulic retention time (HRT). The treatments include food waste (FW), high salinity food processing waste (HSFW), and three ratios of FW to HSFW by volume. The average and standard error were based on the three or four sampling days per HRT.

Treatment	pH HRT 1	pH HRT 2	pH HRT 3	pH HRT 4	pH HRT 5
FW Only	5.86 ± 0.20	5.65 ± 0.17	5.85 ± 0.21	5.93 ± 0.23	10.19 ± 0.01
3:1	6.19 ± 0.18	6.90 ± 0.08	7.42 ± 0.10	7.77 ± 0.03	10.06 ± 0.01
1:1	8.41 ± 0.69	9.89 ± 0.03	9.92 ± 0.06	9.97 ± 0.03	10.01 ± 0.01
1:3	8.92 ± 0.53	9.98 ± 0.04	9.96 ± 0.05	10.01 ± 0.01	7.23 ± 0.22
HSFW Only	9.13 ± 0.46	10.09 ± 0.04	10.08 ± 0.06	10.10 ± 0.02	5.62 ± 0.31

3.3.5 Gas Quality, Total Solids (TS), Volatile Solids (VS), and Chemical Oxygen Demand (COD)

In the first HRT, the dark fermentation gas production had an inverse relationship with the VFA production (Fig. 14). The FW-only treatment produced 1354 ± 665 mL gas but only produced an average of 5.32 ± 0.70 g/L VFAs in HRT 1. The HSFW-only produced 47.0% more VFAs (15.35 ± 2.73 g/L) but 93.4% less gas production (90 ± 19 mL) in HRT 1 (p-value < 0.001). The gas production in the FW-only treatment was significantly higher than the other four treatments in the first HRT (p-value: 0.000 – 0.016), but then the gas production significantly decreased in the FW-only treatment from HRT 1 to HRT 2 (p-value < 0.001) and none of the treatments were significantly different in HRTs 2 – 5 (p-value: 0.643 – 1.000). While there was a significant relationship between increasing VFA production and increasing HSFW loading (Fig. 11), the biogas production did not increase with increasing VFA production.

The composition of the dark fermentation gas was mainly N_2. The FW-only and 3:1 FW:HSFW had 30 – 40% CO_2 and the other three treatments (1:1 FW:HSFW, 1:3 FW:HSFW, HSFW-only) had negligible amounts of CO_2, CH_4, and H_2. There have been studies that show atmospheric N_2 replacing the H_2 that leaks from gas bags due to the small particle size of H_2 (Barghash et al., 2022; Boshagh & Rostami, 2020; Guerrero-Sodric et al., 2023). As a result,

potential leakage from the gas bags in this semi-continuous experiment should be considered.

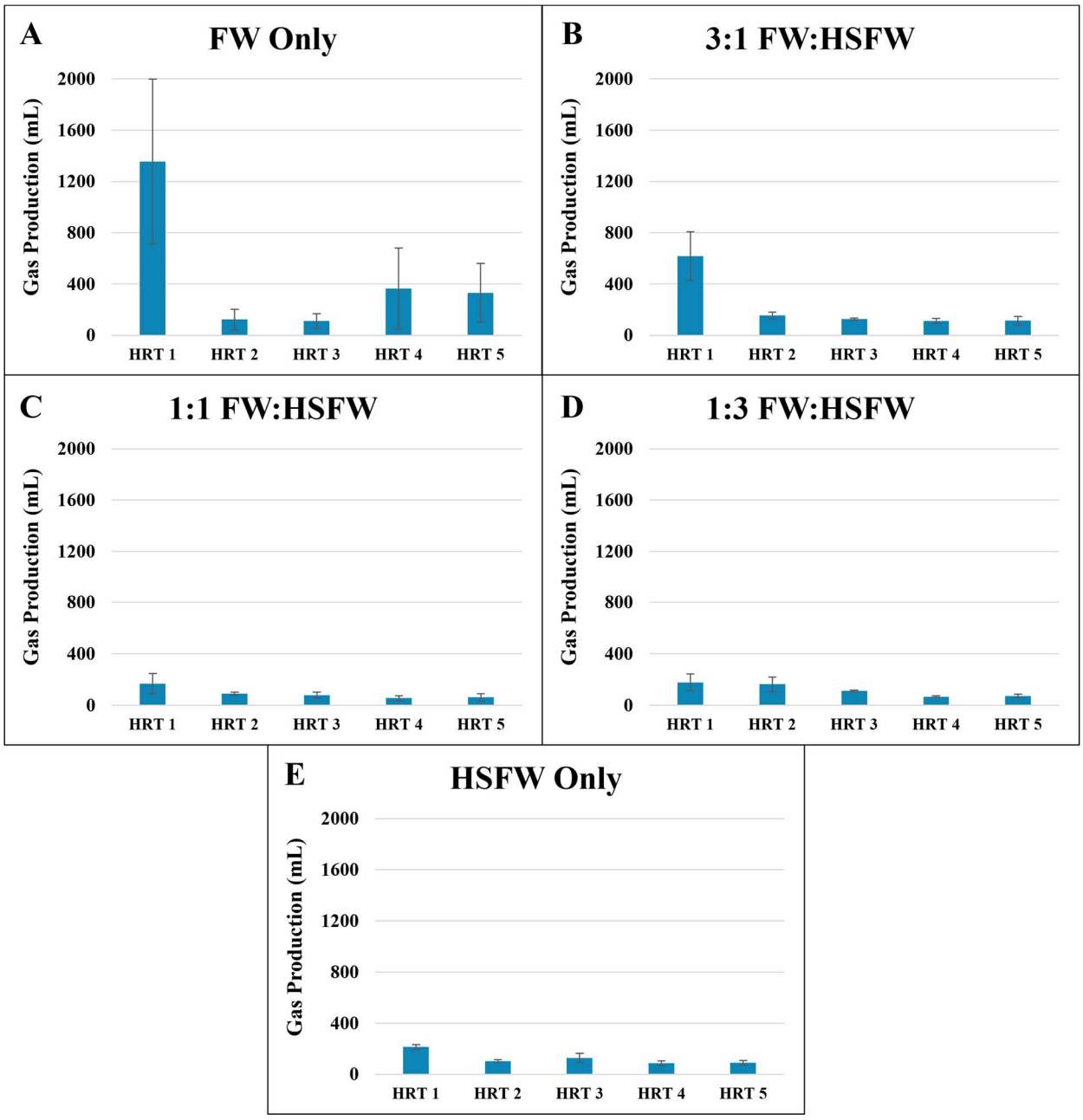

Figure 13. Total dark fermentation gas production for each 12-day hydraulic retention time (HRT). The treatments included food waste (FW) only (Fig. A), high salinity food processing waste (HSFW) only (Fig. B), and three ratios of those two substrates, by volume (FW to HSFW) (Fig. C – E). Standard error (SE) bars based on the three or four sampling days per HRT.

The COD and sCOD were greatest in the HSFW-only treatment and lowest in the FW-only treatment in all five HRTs, which corresponded to the high VFA production in the HSFW mono-fermentation (32.45 ± 0.55 g/L VFAs) and the low VFA production in the FW mono-fermentation (7.33 ± 0.23 g/L VFAs) in HRTs 2 – 5 (Fig. 15; Fig. 11). There was a general trend of increasing COD and sCOD with greater HSFW loading through HRT 3, after which point the COD and sCOD stayed consistent or even decreased, with the largest decreases seen in HRT 5 for the 3:1 FW: HSFW and HSFW-only. This could indicate that fermentation microbes adjusted to the high organic loading with higher ratios of HSFW inclusion over time and were able to maintain consistent degradation of organic matter over time, with no buildup of COD observed.

The large, significant decrease in COD concentrations in the 3:1 FW:HSFW treatment from 244 ± 3 g/L COD in HRT 4 (Day 48) to 62 ± 15 g/L COD in HRT 5 (Day 58) (p-value < 0.001) corresponded with a 29% decrease in VFA production (14.36 ± 0.55 g/L in HRT 4 to 10.18 ± 0.88 g/L in HRT 5). However, when the COD increased to 205 ± 12 g/L COD in the post-experiment analysis (Day 62) (p-value < 0.001), the VFAs remained at 10.98 ± 0.86 g/L VFAs. This shows that while there was some volatility in organic concentrations in this treatment, the VFA values mainly consistent levels over time (Figure 12).

The FW-only treatment had the lowest initial COD and sCOD among the five HRTs (36 ± 1 g/L COD and 15 ± 0 g/L sCOD) (p-value: 0.368 –1.000), which corresponded with the low initial VFAs levels (4.42 ± 0.01 g/L VFAs) (Fig. 11). Overall, a direct relationship was shown between the organic concentration (COD and sCOD) and the VFA production in all of the treatments (Fig. 15; Fig. 11), with high organic concentration and loading leading to higher concentrations of VFA being produced.

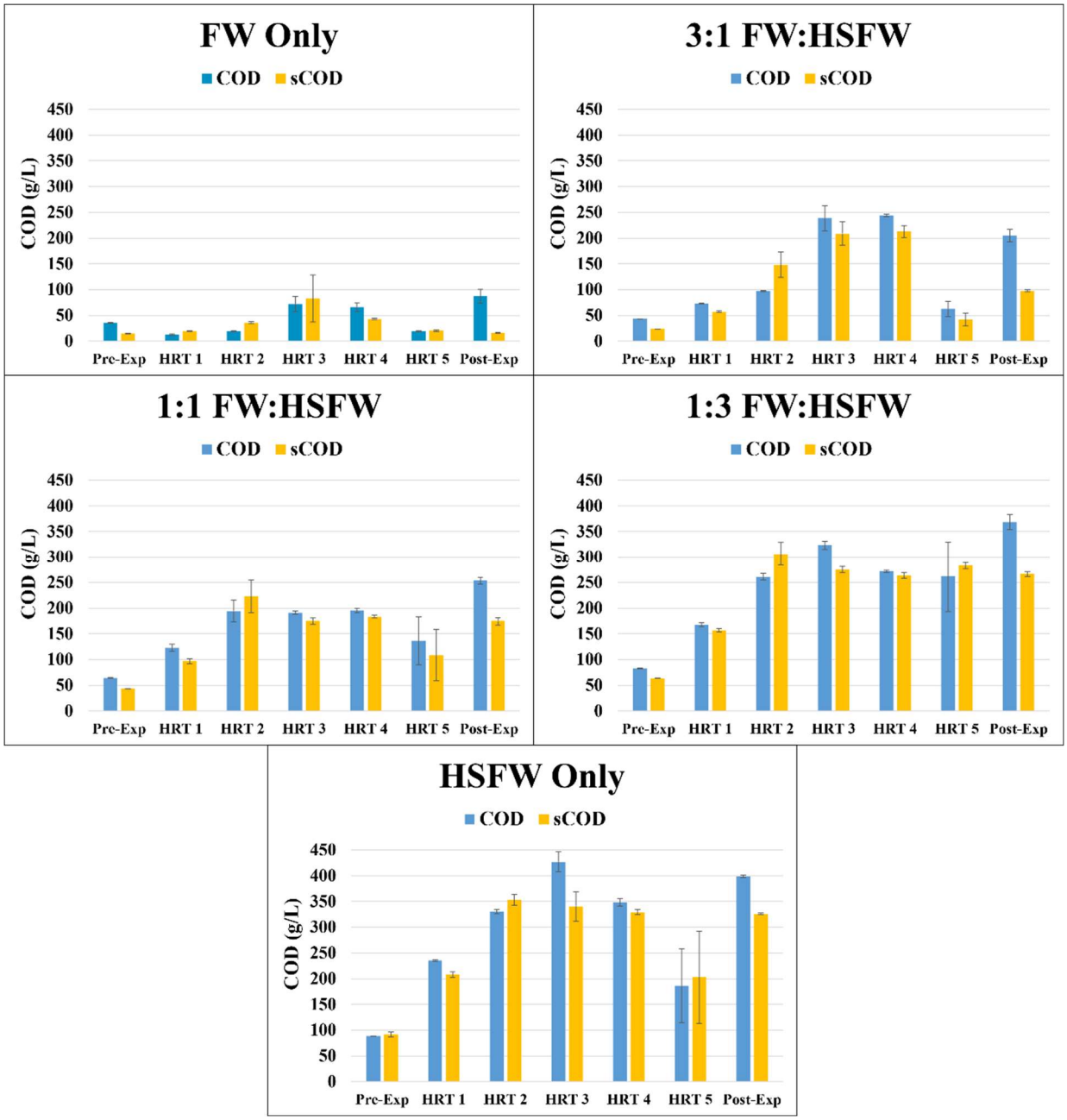

Figure 14. The chemical oxygen demand (COD) and soluble chemical oxygen demand (sCOD) (g/L) of the five ratios, by volume, of food waste (FW) to high salinity food processing waste (HSFW) over four hydraulic retention periods (HRT) of 12 days. Standard error (SE) bars based on the three or four sampling days per HRT.

The organics measured through the COD and sCOD had a similar trend to the organics measured through VS (Table 13). A steady state was achieved during HRT 2 – 5, which was shown in the VS (%). The VS increased from pre-experiment to end of HRT 1 (12 days) by 73,

85, 62, 68% in the 3:1, 1:1, 1:3 FW:HSFW, and HSFW-only treatments, respectively (Table 13). The FW-only treatment decreased by 17% and remained low throughout the five HRTs. In the steady state, the VS content had a direct relationship with the OLR of each treatment.

Table 13. Volatile solids (%) and standard error of five treatments throughout five hydraulic retention times (HRT) of 12 days. The treatments included high salinity food processing waste (HSFW), food waste (FW), and three mixtures by volume of FW to HSFW. The standard error was based on triplicate reactors.

Treatment	Pre-Exp	HRT 1	HRT 2	HRT 3	HRT 4	HRT 5	Post-Exp
FW	1.4 ± 0.2	1.2 ± 0.9	1.0 ± 1.4	1.1 ± 3.0	0.9 ± 1.3	20.5 ± 0.8	21.2 ± 1.5
3:1	1.5 ± 0.5	5.5 ± 2.7	6.0 ± 4.3	6.7 ± 6.1	6.5 ± 2.2	17.0 ± 1.7	17.5 ± 1.3
1:1	2.5 ± 0.3	16.4 ± 67.0	11.9 ± 4.9	11.8 ± 1.2	11.6 ± 4.4	15.0 ± 14.7	13.6 ± 3.8
1:3	4.9 ± 2.3	13.0 ± 1.9	15.5 ± 3.8	16.5 ± 1.2	16.9 ± 2.6	6.8 ± 8.4	7.6 ± 5.1
HSFW	5.1 ± 0.6	15.9 ± 1.3	24.2 ± 50.7	20.7 ± 4.3	20.4 ± 1.7	2.4 ± 0.2	2.2 ± 1.8

3.4 Conclusions

A semi-continuous experiment of FW and HSFW mono- and co-fermentation was tested to determine the effect of salinity from HSFW on VFA production. The highest VFA production of 36.04 ± 0.54 g/L was achieved after five 12-day HRTs of HSFW mono-fermentation (17.8 g VS/L-day; 85.6 g/L Na). There was no negative effect of increasing salinity (21.4 to 85.6 g/L Na) on overall VFA production when normalized by organic loading (VS) in co-fermentation and HSFW mono-fermentation. The non-salinity treatment had a higher efficiency when normalized by organic loading (VS), but it produced far less VFAs overall (9.29 ± 1.01 g/L VFAs). The VFA composition for salinity treatments included acetic and valeric acids while the

non-salinity treatment had increasing propionic acid over the five HRTs. The results from this study suggest that mono-fermentation of HSFW (derived from glycerin processing waste) is beneficial for acetic acid production and is not inhibited by high organic loads or extreme salinity. This research could increase the industrial processing of HSFW for VFA production and prevent an abundant organic waste product from entering municipal solid waste landfills.

Chapter 4: Conclusions

4.1 Overall Conclusions

This research expanded the understanding of AD and DF of three organic wastes: food waste, HSFW, and residual solids extracted from food waste DF. The effect of digesting and fermenting remaining solids after VFA separation was explored to cultivate a better understanding of how DF of food waste for VFA production can be increased through additional VFAs or bioenergy production. The knowledge on HSFW mono- and co-fermentation with food waste was increased as well. This research redirected and valorized three waste streams from landfills to bioenergy and high-value products.

The food waste DF process for VFA production resulted in a carbon-rich residual solids by-product that could be further utilized for bioenergy and VFA production. There were two DF systems tested: anaerobic and microaerobic. The VFA production of the residual solids extracted from these two systems was more successful in microaerobic DF (8.37 ± 0.02 g/L VFAs) than anaerobic DF (4.93 ± 0.04 g/L VFAs). This differs from the VFA results of the initial food waste fermentation, where the residual solids were collected from, as the anaerobic DF system produced 9.84 g/L VFAs compared to 7.35 g/L VFAs from the microaerobic DF system. The solids remaining after microaerobic DF could have undergone further hydrolysis which is the rate-limiting step in DF and makes smaller carbon chains available for VFA production. Additionally, three ISRs for residual solids fermentation were tested (1.5:1, 2:1, 4:1) to determine the optimal amount of substrate required for high VFA production. All three ISRs had similar VFA production which indicated that only a small amount of inoculum was required (1.5:1 ISR) to produce a high amount of VFAs (28.05 ± 0.89 g/L) within nine days of fermentation.

Bioenergy was also produced from these residual solids. Bioenergy production from the residual solids of both the anaerobic and microaerobic DF systems were similar at 395 ± 8 and 430 ± 4 mL CH_4/g VS, respectively. However, the additional energy requirements needed to maintain the microaerobic environment were greater than the extra energy produced, hence the microaerobic treatment had lower net bioenergy production compared to the anaerobic treatment. This research found that the residual solids extracted from food waste DF had the same CH_4 potential (492 ± 11 mL CH_4/g VS) compared to unfermented food waste (470 ± 11 mL CH_4/g VS). The overall production of bioenergy and additional VFAs was increased by undergoing a secondary fermentation cycle with the residual solids extracted from food waste DF.

In addition to the AD of food waste and residual solids from VFAs separation, the implementation of DF gas was tested to determine its effect on bioenergy production. A lab-scale DF reactor that produced a low amount of H_2 and a high amount of CO_2 was coupled with the AD of food waste and residual solids from VFAs separation. A unique aspect of this study was that a low H_2 to high CO_2 ratio was tested while most of the literature has tested high H_2 to low CO_2 ratios. This low H_2 to high CO_2 ratio was selected to determine how to valorize the gas from a DF reactor with a low H_2 production that otherwise could not be used for direct energy consumption.

There were limitations to injecting DF gas that contained a low H_2 to high CO_2 ratio because it either inhibited or diluted the CH_4 produced from residual solids AD. The microbes could have been inhibited because the DF gas injections tested were ratios of 1:1, 1:2, 1:3, and 1:5 of H_2:CO_2 but CH_4 is formed from a 4:1 H_2 to CO_2 ratio. There was not enough H_2 present in the DF gas injection for the microbes to reduce CO_2 into CH_4. Alternatively, the CH_4 produced could have been diluted from the high amount of CO_2 gas injected into the digestion reactors as

the organic content of the substrate (VS and COD) decreased over the digestion period, but CH_4 production was limited. The incorporation of DF gas with low H_2 and high CO_2 was not useful for bioenergy production from residual solids AD; however, the knowledge on how to utilize DF gas from a system with a low H_2 and high CO_2 was expanded.

The mono- and co-fermentation of food waste and HSFW was evaluated to determine how food waste with high salinity could be fermented without microbial inhibition from osmotic cellular destruction. The results were unique compared to most literature because acidogenic bacteria, which produce VFAs, survive in acidic to neutral pHs but the highest VFA production achieved in this study (36.04 ± 0.54 g/L VFAs) was at an alkaline pH at 9 – 10. A few articles suggested that high VFA production in an alkaline pH could be due to the microbial communities present in the reactor.

Another finding in this study was that the HSFW mono-fermentation produced significantly more VFAs than food waste mono-fermentation, even though it was expected that the extreme salinity at 85.6 g/L Na in the HSFW mono-fermentation would inhibit VFA production. The OLR was a main contributor to this as the HSFW mono-fermentation (17.8 g VS/L-day) was much higher than food waste mono-fermentation (2.5 g VS/L-day). When the VFAs were normalized by organic loading (g VS) to determine the effect of salinity, the food waste mono-fermentation did have a higher g VFAs/g VS than HSFW mono-fermentation, however it was not statistically significant. The VFA production from the co-fermentation of these two substrates had a direct relationship with HSFW loading. When normalized by g VS, the VFA production in all the co-fermentation ratios by volume (1:3, 1:1, 3:1 of food waste to HSFW) were similar to HSFW mono-fermentation and were lower than food waste mono-fermentation, yet not significantly different.

An increase in salinity with co-fermentation was expected to inhibit VFA production but the opposite was found in this study. This could be due to the microbial communities adapting to the salinity over time, favoring microbes that can produce VFAs in high saline environments. A limitation to this study was that a microbial community analysis was not conducted as it could give insight into the high VFA production with increasing salinity. The implications of food waste and HSFW mono- and co-fermentation showed how two organic waste streams can be valorized for high-value products (VFAs) that can be converted into bioenergy and bioplastics.

There is a variety of food waste that enters landfills such as fruits, vegetables, meats, dairy products, and seafood. These food wastes contain different proteins, carbohydrates, and lipids that influence the AD and DF process. There needs to be a thorough understanding of how each type of food waste responds to AD and DF systems to effectively produce bioenergy and high-value products. The residual solids by-product from VFA separation in food waste DF was found to contain useful carbon that can be converted into further VFAs and bioenergy which has not been studied in the literature, indicating the need for more research on streamlining DF system efficiency to produce bioenergy and VFAs.

Literature stated low success of HSFW mono-fermentation for VFA production, but in this current study, the HSFW mono-fermentation was significantly more successful than food waste mono-fermentation at VFA production. The results from this study emphasize the need for more research into semi-continuous and continuous DF of HSFW, to determine how VFAs can be produced from this abundant organic, but saline, waste.

The role of AD and DF in processing waste and reducing CH_4 emissions is critical to combatting the global warming crisis. The organic waste material that enters landfills and the global dependency on fossil fuels for energy, causes the CH_4 emissions that contribute to global

warming. The bioconversion of organic matter to bioenergy and high-value products can redirect organic waste from landfills and reduce the dependency on CH_4-emitting energy sources.

4.2 Future Work

A microbial community analysis will be completed by a collaborator for Chapter 3, but this analysis is outside the scope of this current study. This microbial community analysis will provide insight into why the VFA production was successful in extreme salinity and alkaline conditions. Previous literature has found that in extreme salinity conditions, the microbial community changes from typical acidogenic bacteria to bacteria typically found in coastal salt marsh soils (He et al., 2019). Cheah et al. (2019) and Ma et al. (2019) found that certain bacteria that produce mainly acetic acid in alkaline condition were present in the fermentation process. The microbial community analysis will help to determine if the high VFA production in HSFW mono-fermentation was a result of the microbial community adjusting to extreme salinity and alkaline conditions, as observed in He et al. (2019), Cheah et al. (2019), and Ma et al. (2019). Additionally, the microbial community influences the types of VFAs produced. For example, the FW-only treatment in this current study increased propionic acid over the five HRTs, while HSFW mono- and co-fermentation had no propionic acid production. This could be a result of certain microbial species present in the FW-only treatment that prefer acidic (pH of 5 – 6) and no salinity environments rather than the HSFW mono- and co-fermentation that had alkaline (pH of 9 – 10) and extreme salinity environments (21.4 – 85.6 g/L Na). Based on current literature, microbial communities have a significant role in VFA production during FW fermentation. Future work will complete a microbial community analysis to grasp a full understanding of FW and HSFW mono- and co-fermentation for VFA production.

In Chapter 2, the efficiencies of four systems that digested and fermented residual solids

from VFA separation and FW were determined based on a mL CH_4/g VS and g VFA/g VS calculations, respectively. These calculations provided insight into the how efficient it was to convert organic content (VS) into CH_4 or VFAs, however, the calculations only focused on the efficiency of individual systems (residual solids or food waste AD or DF) and did not consider the entire system (food waste DF compared to residual solids AD or residual solids DF). This current study determined the bioenergy and VFA potential of residual solids, and the carbon conversion of those residual solids to either CH_4 or VFAs, but the carbon conversion from the different FW waste conversion strategies was outside the scope of this thesis. Our collaborators who focused on the carbon conversion analyses have shown that the carbon conversion efficiency of the original system that only fermented FW for VFA production (PHA production) could be increased the most by refermenting the residual solids.

Residual solids were found to have similar VFA potential with FW, therefore, incorporating residual solids in FW fermentation to PHA production would be beneficial. The extraction of residual solids through centrifugation and physical separation with a scoopula can be time consuming and labor intensive. An in-line filter system could be a potential solution that can be explored in future pilot-scale operations that go from food waste through fermentation to bioplastic production. The in-line filter could be placed at the outflow of a fermentation reactor, allowing filtered VFA-rich liquid to exit the fermenter and leave the remaining solids in the reactor. The remaining solids would then have additional time to ferment in the reactor for further VFA production. The configuration of this system would leave the residual solids directly in the reactor which should increase the VFA output of the system.

Appendices

Appendix A: Supplementary Information for Anaerobic Digestion and Dark Fermentation of Food Waste and Residual Solids

Table A1. Total volatile fatty acids (VFA) production from anaerobic dark fermentation (ADF) and microaerobic dark fermentation (MADF) residual solids and inoculum pre- (Day 0) and post-experiment (Day 23). Average and standard error were based on VFA analysis triplicate.

Treatment	Pre-Experiment	Post-Experiment
ADF residual solids fermented in ADF inoculum	3.96 ± 0.02	4.93 ± 0.04
MADF residual solids fermented in ADF inoculum	3.71 ± 0.03	4.76 ± 0.01
ADF residual solids fermented in MADF inoculum	6.44 ± 0.03	8.37 ± 0.02
MADF residual solids fermented in MADF inoculum	5.62 ± 0.02	8.18 ± 0.01

Table A2. Average of four types of volatile fatty acids (g/L) for four treatments pre and post 23 days of fermentation. Treatments included anaerobic dark fermentation (ADF) and microaerobic dark fermentation (MADF) residual solids and inoculum. The standard error was based on analyses triplicates.

Treatment	Acetic Acid (g/L)		Propionic Acid (g/L)		Butyric Acid (g/L)		Valeric Acid (g/L)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ADF residual solids fermented in ADF inoculum	2.17 ± 0.01	2.43 ± 0.01	0.28 ± 0.00	0.57 ± 0.01	0.57 ± 0.01	0.74 ± 0.02	0.94 ± 0.01	1.18 ± 0.01
MADF residual solids fermented in ADF inoculum	2.05 ± 0.01	2.19 ± 0.01	0.25 ± 0.001	0.53 ± 0.00	0.53 ± 0.01	0.76 ± 0.00	0.88 ± 0.01	1.20 ± 0.00
ADF residual solids fermented in MADF inoculum	2.46 ± 0.02	3.12 ± 0.01	1.38 ± 0.00	1.86 ± 0.01	1.60 ± 0.01	1.44 ± 0.01	1.00 ± 0.01	1.95 ± 0.01
MADF residual solids fermented in MADF inoculum	2.02 ± 0.00	3.11 ± 0.00	1.20 ± 0.00	1.75 ± 0.00	1.47 ± 0.01	1.40 ± 0.00	0.93 ± 0.01	1.93 ± 0.01

Table A3. Total volatile fatty acids (VFA), pH, and SE of six treatments after 27 days of digestion. The treatments included food waste, anaerobic dark fermentation (ADF) residual solids, and dark fermentation (DF) gas injections with ADF residual solids. Pre digestion standard errors were based on analyses triplicates and post digestion standard errors were based on triplicate reactors.

Treatment	Total VFAs (g/L)		pH	
	Pre	Post	Pre	Post
ADF Residual Solids Only	8.56 ± 1.04	5.03 ± 1.15	8.38	8.15 ± 0.04
DF Gas Mixture	8.56 ± 1.04	6.18 ± 0.92	8.38	8.01 ± 0.02
DF Gas 15:80:5	8.56 ± 1.04	5.49 ± 0.30	8.38	7.93 ± 0.01
DF Gas 30:65:5	8.56 ± 1.04	5.49 ± 0.83	8.38	8.02 ± 0.01
DF Gas 50:50:0	8.56 ± 1.04	4.53 ± 1.40	8.38	8.08 ± 0.01
Food Waste Only	10.57 ± 0.25	1.72 ± 0.16	8.27	8.12 ± 0.06

Table A4. Total volatile fatty acids production (g/L) over a 15-day dark fermentation of food waste (FW) and anaerobic dark fermentation residual solids (RS) at various inoculum to substrate ratios (ISR). Standard errors were based on triplicate reactors.

Treatment	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
RS ISR 2:1	12.8 ± 1.1	15.9 ± 2.9	24.7 ± 1.1	21.7 ± 5.0	12.4 ± 0.9	8.3 ± 0.6
RS ISR 1.5:1	12.1 ± 0.7	12.2 ± 3.2	26.4 ± 2.1	28.1 ± 0.9	11.0 ± 0.4	10.1 ± 3.8
RS ISR 4:1	15.8 ± 1.3	17.9 ± 1.7	24.1 ± 0.8	25.5 ± 0.9	11.1 ± 1.2	10.9 ± 4.4
FW ISR 2:1	17.2 ± 0.1	17.4 ± 2.3	23.7 ± 1.4	22.9 ± 2.9	13.9 ± 3.7	17.4 ± 4.4
Inoculum Only	18.5 ± 1.8	16.2 ± 2.9	16.4 ± 0.9	17.9 ± 0.8	15.9 ± 1.3	21.8 ± 4.3

Appendix B: Supplementary Information for Volatile Fatty Acids Production through High salinity food processing waste Dark Fermentation

Table B1. The average ± SE volatile fatty acids (VFA) production from four 12-day hydraulic retention periods (HRTs 2 – 5). The average excludes the first HRT as it was a lag phase. Treatments included high salinity food processing waste (HSFW), food waste (FW), and three ratios of FW to HSFW, by volume.

Treatment	Acetic Acid (g/L)	Propionic Acid (g/L)	Butyric Acid (g/L)	Valeric Acids (g/L)	Total VFAs (g/L)
FW Only	31.87 ± 0.58	0 ± 0	0.19 ± 0.06	0.38 ± 0.04	32.45 ± 0.54
3:1	24 ± 0.44	0.01 ± 0.01	0.23 ± 0.07	0.32 ± 0.03	24.58 ± 0.39
1:1	17.03 ± 0.45	0 ± 0	0.35 ± 0.11	0.25 ± 0.02	17.65 ± 0.4
1:3	9.54 ± 0.82	0.05 ± 0.01	1.01 ± 0.14	0.18 ± 0.03	10.79 ± 0.75
HSFW Only	4.96 ± 0.16	0.43 ± 0.09	1.62 ± 0.05	0.31 ± 0.02	7.33 ± 0.22

Table B2. There were five 12-day hydraulic retention times (HRT) resulting in 62 days of semi-continuous dark fermentation of food waste (FW), high salinity food processing waste (HSFW), and three ratios of the two substrates by volume (FW to HSFW). The HRT and Day on when the highest VFAs were achieved for each treatment. Standard errors were based on triplicate reactors.

Treatment	HRT	Day	Maximum Total VFAs (g/L)
FW Only	4	44	9.29 ± 1.01
3:1	3	30	14.60 ± 0.78
1:1	4	44	22.10 ± 1.11
1:3	4	48	26.88 ± 0.07
HSFW Only	3	51	36.04 ± 0.54

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