ENHANCING CARBON EFFICIENCY OF ANAEROBIC DIGESTION THROUGH FORMATE METHANOGENESIS

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ABSTRACT

Anaerobic digestion is an important technology for waste treatment and renewable energy production. To pursue avenues of improvement in digester technology and foster a circular bioeconomy, this study investigated the impact of activated carbon, formic acid, and sodium bicarbonate addition on the performance, parameters, and ecology of bench-scale anaerobic reactors. The study found that the addition of formate in combination with activated carbon was able to significantly increase the production of biogas from digestate collected from the South Campus Anaerobic Digester (SCAD). Reactors treated with formic acid also showed evidence of superior formic acid utilization, with formic treated reactors having similar or lesser formic acid concentration than non-formic treated reactors. Additionally, formic acid treated reactors showed lower concentrations of volatile fatty acids (VFAs), including propionic and butyric acid, indicating enhanced reactor performance. DNA analysis revealed an increase in formate-scavenging methanogens and syntrophic bacteria. Thes analyses suggests that the formate treatment led to enhanced syntrophic interactions among the microbial consortia. Following these promising results, a life-cycle analysis (LCA) and were conducted on a hypothetical two-stage treatment system utilizing electrocatalysis to convert waste CO_2 into formic acid for treatment of a secondary digester. The mass balance and LCA suggest that formate-treated AD has the potential to greatly enhance energy efficiency, carbon utilization, and environmental outcomes of the AD process.

To Jennifer and Petra Grivins
I couldn't have made it without you

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
ADREC	Anaerobic Digestion Research and Education Center
CH_4	Methane
CHP	Combined Heat and Power
CO_2	Carbon Dioxide
CO_2-e	Carbon Dioxide Equivalent
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DI	Deionized
DIET	Direct Interspecies Electron Transfer
FU	Functional Unit
GHG	Greenhouse Gas
GWP	Global Warming Potential
IET	Interspecies Electron Transfer
HRT	Hydraulic Retention Time
H_2S	Hydrogen Sulfide
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
MSU	Michigan State University
NMDS	Non-Metric Multidimensional Scale

SCAD	South Campus Anaerobic Digester
TEA	Techno-Economic Analysis
TS	Total Solids
VFA	Volatile Fatty Acid
VS	Volatile Solids
WEP	Water Eutrophication Potential

CHAPTER 1: INTRODUCTION

1.1 Significance

Anaerobic Digestion (AD) is a waste treatment, energy generation, and waste-to-resources technology implemented at many dairy farms and water treatment facilities. AD takes in organic wastes, particularly animal manure and food waste, and converts their organic solids into biogas, a mixture of methane (CH_4) and carbon dioxide (CO_2). This provides a dual benefit of treating organic waste and producing renewable natural gas (RNG). As of June 2024, there were 400 manure-based AD systems operating in the United States (US EPA, 2024). In 2023, US manure-based AD operations avoided 14.8 million tonnes CO_2-e GHG emissions and generated 3.9 million MWh energy equivalent. However, the technology still faces barriers to a wider potential implementation. Although there were 400 operations in the US, the EPA AgSTAR program estimated that in June 2024 there were 8,000 large dairy and hog operations where an AD operation would be technically viable (US EPA, 2024), meaning that just 5% of the nation's potential manure-based operations were active. One barrier to the wider implementation of AD operations is the lack of economic viability for electricity and RNG for many small to medium operations. The natural gas market is often unavailable to small and medium facilities; either due to the cost of biogas upgrading systems, which are prohibitively expensive for their smaller scale; or other complications, particularly in places like the US where there is a lack of incentives and support for RNG markets (Edwards et al., 2015). For this reason, it is often the best option for AD operations to generate electricity, using it first to offset their own energy costs before selling excess to the grid (Hjort-Gregersen et al., 2011). Even with this approach, the

payback period for new digester operations based on sale of electricity to the grid remains lengthy. It is therefore desirable to create innovations in AD operation technology that can increase economic viability, and therefore implementation, through an increase in electrical yields or the production of valuable co-products.

A potential improvement in AD is greater potential GHG emission offsets and carbon utilization. The CO_2 released from AD biogas activities is biogenic; because it's produced from plant material, it will be recaptured in the next crop growing cycle. Therefore, this gas is part of the short-term carbon cycle and isn't considered as contributing to GHG emissions. However, if the CO_2 were to be captured and reused, improving its carbon efficiency, the AD operation could potentially reach net negative carbon emissions by replacing additional fossil gas usage without contributing to GHG emissions. One use for CO_2 is conversion into formic acid via electrocatalysis, which is both a valuable co-product of digestion and a promising candidate for digester addition.

A relatively new avenue of anaerobic digestion research is the effect of formate and bicarbonate addition on digester microbial cultures. Formic acid has been shown to have a positive impact on digester performance in terms of biogas yield (Li et al., 2024). This is thought to occur through the enhancement of interspecies electron transfer (IET), which improves the consumption of intermediary products, specifically volatile fatty acids (VFAs) and enhances methanation. Combining the concepts of CO_2 to formic acid conversion and formic acid enhancement of AD performance, it becomes clear that a study to evaluate the impact of formate addition and the potential of an operation which converts waste CO_2 to

formic acid for digester treatment is valuable to the improvement of the environmental, technical, and economic viability of AD as a technology.

1.2 Goals and Objectives

The primary aim of this thesis is to assess a potential system for recycling CO_2 through catalytic conversion to formate. This innovative approach seeks to enhance the efficiency and sustainability of anaerobic digestion (AD) processes. The specific objectives supporting this goal are:

- **Evaluate the impact of formate and bicarbonate addition on lab-scale bioreactors:** This involves conducting controlled experiments to determine how these additives influence the performance and efficiency of AD systems. The focus will be on key metrics such as biogas production, volatile fatty acid (VFA) concentrations, and microbial community dynamics.
- **Design a process flow for a potential formate-enhanced anaerobic digestion (AD) biorefinery:** This objective aims to create a detailed blueprint for integrating formate enhancement into existing AD systems. The process flow will outline the necessary steps, equipment, and operational parameters required to implement this technology at a larger scale.
- **Conduct a life-cycle assessment of the proposed formate-enhanced AD biorefinery:** This involves evaluating the environmental impact of the proposed biorefinery. The life-cycle assessment will provide a comprehensive evaluation of the environmental impacts from cradle to grave.

1.3 LITERATURE REVIEW

1.3.1 Anaerobic Digestion

Anaerobic Digestion (AD) is a well-characterized method of waste treatment and energy production that has been in use for decades. It is a process in which organic wastes are converted into biogas through the cooperation of different microbes using syntrophy. Syntrophy is the process by which the products of one microbe's metabolism are used as the nutrients of another (Marietou, 2021). There are four steps involved in AD: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

Hydrolysis is the process by which large molecules are broken down into smaller components that may be accessed by other microorganisms. Hydrolytic bacteria convert carbohydrates into sugars, lipids into long-chain fatty acids, and proteins into amino acids. Hydrolysis can be a rate-limiting step in the AD process (Meegoda et al., 2018).

The products of hydrolysis can be diffused through the membranes of acidogenic microbes in the second step of AD, acidogenesis. These microbes produce volatile fatty acids (VFAs) such as acetic, formic, propionic, and butyric acid. Acetic and formic acid can be utilized directly by methanogens. However, butyric and propionic acid must be converted into acetic acid through acetogenesis (F. Shen et al., 2018). Acidogenesis has been observed to be the fastest step in AD. It is important to consider this fast rate with regards to reactor stability, as VFA acidification is a common cause of reactor failure (Meegoda et al., 2018). VFA acidification is the accumulation of fatty acids in the digester to an extreme extent that causes the metabolism of microbes in the environment to fail.

Acetogenesis is the process of converting larger VFAs into acetate. (Meegoda et al., 2018) Larger VFAs in this case means VFAs with a longer carbon chain than acetate (i.e. propionate, butyrate, and valerate). This step is considered rate-limiting in the overall process due to the high Gibbs free energy of VFA oxidation, especially propionate (Mu et al., 2023). To become favorable, the propionate reaction must be coupled with a reaction that consumes hydrogen. Therefore, a successful reactor should pair propionate degradation with the conversion of H_2 to CH_4, aiming to keep the partial pressure of H_2 in the reactor below 10^{-4} atm (Mu et al., 2023).

Methanogenesis is the final step of AD, where biogas is produced by a class of obligate anaerobic archaea. These microbes can produce CH_4 primarily from acetate or hydrogen (Meegoda et al., 2018). Formate has also been observed as a possible source for methanogenesis (Belay et al., 1986).

1.3.2 Formate Addition

A major challenge in the process of anaerobic digestion is that the oxidation of fatty acids to acetate, hydrogen, and formate, i.e., acetogenesis, is thermodynamically unfavorable unless the concentration of those products is extremely low. However, the overall process of digestion can be made thermodynamically favorable due to the process of interspecies electron transfer (IET). The metabolism of fermentative organisms effectively produces surplus electrons due to reduction; meanwhile, methanogens require additional electrons and thus serve as the electron sink (L. Shen et al., 2016). To accomplish this, H_2 and formate serve as electron shuttles. An organism can release its electrons for use by other species using enzymes called hydrogenases, if using hydrogen,

or formate dehydrogenases, if using formate as the carrier. These enzymes take protons or CO_2, along with electrons, and convert them to H_2 and formate respectively. These compounds are then transferred to methanogenic organisms, which metabolize them to produce CH_4 (L. Shen et al., 2016). Using IET to couple these reactions results in an overall set of thermodynamically favorable reactions. Table 1.3.1 summarizes the reactions involved in AD and their respective Gibb's Free Energy values.

Table 1.3.1. Anaerobic Digestion Substrate Reactions (Zhang et al., 2023)

Substrate	Reaction	$\Delta G°'$(kJ/mol)
Propionate	$CH_3CH_2COOH + H_2O \rightarrow 2CH_3COOH + 3H_2 + CO_2$	+76.1
Butyrate	$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$	+48.1
Lactate	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	-4.2
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	+9.6
Formate	$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$	-130
Acetate	$CH_3COOH \rightarrow CH_4 + CO2$	-33
Hydrogen	$4H_2 + CO_2 \text{ -> } CH_4 + 2H_2O$	-135

Research suggests that reactors with formate added demonstrate increased CH_4 production. At concentrations ranging from 5 mM to 30 mM of formate, CH_4 yield from propionate was shown to be enhanced (Li et al., 2024).

1.3.3 Bicarbonate Addition

Food waste has a low C/N ratio compared to other digester feedstocks (Akindele & Sartaj, 2018). This can result in the rapid buildup of VFAs in digesters where food waste is a significant component (Gao et al., 2020). Because digesters require both neutral and stable pH due to the sensitivity of culture microbes, rapid VFA accumulation can cause digester failure (Deublein & Steinhauser, 2011). In order to counter fluctuating pH due to changes in VFA concentration, digesters require a source of alkalinity to serve as a pH buffer (Valença et al., 2021). Sodium bicarbonate ($NaHCO_3$) has been identified as an effective buffer in AD cultures, improving biogas quality, culture stability, and CH_4 yield (Valença et al., 2021). In addition to its buffering capability, bicarbonate is also a possible carbon source. It has been suggested that bicarbonate may be used as an external carbon source for biomass growth (Mokashi et al., 2016). This could make bicarbonate an effective carbon capture route through carbon storage via biomass.

1.3.4 Activated Carbon Addition

Activated carbon (AC) is a conductive material that has been shown to enhance biogas yields in AD (Tiwari et al., 2021). Conductive materials have been suggested to facilitate direct interspecies electron transfer (DIET) (Feng et al., 2023). The addition of carbonaceous materials has also been shown to shorten or eliminate reactor start-up times (Xu et al., 2021).

1.3.5 Catalytic Hydrogenation of CO_2

One reason that formate is interesting as a digester additive is that it can be made from CO_2. Using a catalyst, CO_2 may be hydrogenated to formic acid. In the general CO_2 to formate pathway, CO_2 is adsorbed on the cathode of the catalyst surface. This forms a radical CO_2 intermediate, which is a negative CO_2 ion. The exact intermediate step that follows depends on whether CO_2 bound to the catalyst with a carbon atom or an oxygen atom. Whether the reduction product that follows this step is protonated depends on the pH of the reaction medium. Finally, formic acid is formed via desorption (Ma et al., 2024).

Commonly, this is accomplished using an acidic solution to put the CO_2 into an aqueous phase, and a metal catalyst, such as ruthenium or rhodium, to catalyze the reaction (Moret et al., 2014).

1.3.6 Other Applications of Formic Acid

Even in the case that formic acid/formate proves non-useful as an AD enhancer, it is still a useful chemical for industrial applications and renewable energy systems. Notably, formic acid is dense in hydrogen, with an H_2 density of 53 g L^{-1} (Joó, 2008). This property, which is the primary reason formate is useful for methanation, also makes it a promising candidate for hydrogen storage in fuel cells. Formic acid has the added benefit of being a non-volatile liquid at ambient conditions. This mitigates many of the risks associated with the use, storage, and transportation of H_2 gas.

1.3.7 Life Cycle Assessment

Life cycle assessment (LCA) evaluates a product or process's environmental impact based on its entire life cycle. It includes such factors as manufacturing, distribution, use,

reuse, raw material extraction, recycling, and product or process end-of-life. LCA integrates the management of the framework, impact assessment, and data quality. The term “cradle-to-grave”, often used to describe LCA, refers to this complete accounting of all steps in the product or process’s lifetime (Dwilaksono, 2022, Odey et al., 2021). This more holistic method of environmental assessment, where all steps are considered together, makes LCA more robust compared to other metrics of environmental impact, such as water footprint or carbon footprint, which consider only a single dimension of environmental impact. LCAs are able to consider trade-offs between different steps and factors, which isn’t possible in less holistic methods of evaluation (Odey et al., 2021).

The United Nations Environment Programme (UNEP), Society of Environmental Toxicology and Chemistry (SETAC) Life Cycle Initiative, International Reference Life Cycle Database System (ILCD), and others have promoted the use of LCA in pursuit of economically viable and sustainable societies (Odey et al., 2021).

LCA is divided into four phases: goal and scope definition, life cycle inventory analysis, life cycle impact assessment, and life cycle interpretation (Reap et al., 2008).

CHAPTER 2: MATERIALS AND METHODS

2.1 Bench-Scale Formate Digesters

To evaluate the effect of formate and bicarbonate addition on a potential digester, a bench-scale experiment was conducted. Five conditions were tested, using digestate from SCAD as a feedstock. Each condition was tested with a biological duplicate. An overview of the operating conditions is shown in Table 2.1.1.

Table 2.1. Experimental Conditions

Reactor	Digestate	Activated Carbon	Bicarbonate	Formate
Control (C)	40 mL	No	No (5 mL DI water)	No (5 mL DI water)
Activated Carbon (A)	40 mL	4% w/v at start-up	No (5 mL DI water)	No (5 mL DI water)
Bicarbonate (B)	40 mL	4% w/v at start-up	5 mL solution, total conc. 0.5 g/L	No (5 mL DI water)
Formate (F)	40 mL	4% w/v at start-up	No (5 mL DI water)	5 mL solution, total conc. 0.1 g/L
Bicarbonate + Formate (CF)	40 mL	4% w/v at start-up	5 mL solution, total conc. 0.5 g/L	5 mL solution, total conc. 0.1 g/L

Reactors were fed Monday, Wednesday, and Friday. The working volume of the reactors was 500 mL. Feedstock was a mixture of 40 mL of digestate, 5 mL of formate solution, and 5 mL of DI (deionized) water for the Formate reactors; 40 mL of digestate 5 mL of bicarbonate solution, and 5 mL of DI water for the Bicarbonate reactors; 40 mL of digestate, 5 mL of bicarbonate solution, and 5 mL of formate solution for the Bicarbonate/Formate reactors; and 40 mL of digestate and 10 mL of DI water for the

Control and Activated Carbon reactors. Figure 2.1.1 presents a graphical representation of these feeding conditions.

The formate solution was prepared using a stock solution of formic acid that was diluted to a 1 g/L concentration. The bicarbonate solution was prepared using dry sodium bicarbonate stirred into DI water. Reactors that were treated with activated carbon were given 20 g (4% w/v) activated carbon powder at the beginning of the experiment and were not treated further with activated carbon.

The reactors were operated with an HRT of 20 days for 4 HRTs, totaling 80 days. Reactors were operated at 50 C, which is in the thermophilic range. This range was chosen to reduce start-up time, and because thermophilic digestion works well at low solids content (Yu et al., 2017). Relative to a digester fed directly with manure and food waste, these digestate-fed digesters had low solids content.

Table 2.1.2 lists key properties of the digestate used to feed the reactors. Composition of the digestate was measured on 5/17/2021.

Table 2.2. Properties of Feedstock Digestate

Measurement	Value	Units
Total Solids	40.81	g/L
Volatile Solids	29.29	g/L
COD	50800	mg/L
pH	8.22	-

2.2 Bench-Scale Analysis

2.2.1 Total and Volatile Solids (TS/VS)

Total and Volatile Solid (TS/VS) analysis was performed to give information on the organic and inorganic solid content and moisture content of samples. TS/VS analysis was based on Hach Methods 8276 and 8271. Drying time for TS samples was increased to overnight, rather than 6 hours. Furnace time for VS samples was increased to 6 hours, rather than one hour. These changes help to ensure complete drying and combustion considering the higher solid content of digestate as compared to wastewater, which the methods were developed for. Samples were mixed via manual shaking.

Materials for this test included digestate samples taken from the reactors (approximately 10 mL per test), ceramic crucibles or glass beakers (2 per test), analytical balance, oven, furnace, desiccator, desiccant, 10 mL micropipette, 10 mL micropipette tips, and a marker and whiteboard (for labeling purposes).

2.2.2 Chemical Oxygen Demand

Chemical Oxygen Demand (COD) is used as a measure of organic pollutants in wastewater and is equivalent to the amount of oxygen needed to reduce compounds within the sample. Higher COD corresponds to a higher concentration of organics. COD testing was performed according to Hach method 8000. Sample COD concentrations were consistently high enough that they required dilution to fall within the test range.

Materials for COD testing included digestate samples, DI water, heated reactor, HACH spectrophotometer 5000, HACH COD test vials, delicate wipes, 1 mL micropipette and tips, and markers.

2.2.3 pH

Correct pH is critical for digester health and proper operation. pH values close to neutral are best, with the ideal range lying between 6.4 and 8.2. The pH probe used was periodically calibrated using standards with pH values of 4.0, 7.0, and 10.0. The probe was rinsed with DI water and cleaned with a delicate wipe after calibration and between each use. The pH probe was left in a storage solution when not in use.

Materials and equipment for pH measurement were digestate samples, the pH meter and probe, DI water, calibration standard solutions at pH 4.0, 7.0, and 10.0, delicate wipes, and beakers.

2.3 Gas Production and Quality

A modified shaker system, depicted in Figure 2.3.1, was used to maintain reactor temperature and monitor biogas production. In this gas collection system, each reactor is connected via two needles and a tube to a water bottle. This water bottle is then connected to an empty bottle, which is open to the atmosphere, via a tube open on one end and with a long needle on the other. As biogas is produced in the reactor, it pushes down on the water in the water bottle. This causes the water to be displaced and flow into the empty bottle at the same volumetric rate that gas is produced. Gas production was measured by measuring the displaced water volume with a graduated cylinder.

Figure 2.3.1. Biogas Volume Measurement System

The required materials for this measurement were two bottles, two caps and rubber stoppers, two short needles, one long needles and two tubes per reactor, in addition to a Thermo-Scientific heated shaker, a graduated cylinder, and tap water.

Gas composition was determined using gas chromatography using an SRI Instruments 8610C gas chromatographer with an equipped thermal conductivity detector (TCD). The GC program used measures only nitrogen, CO_2, CH_4, and hydrogen sulfide. To measure gas composition, 5 mL of gas were injected into the GC, and the resulting values were recorded. Because the bacteria do not produce any nitrogen gas, and hydrogen

sulfide is not significant in the overall composition, the gas percentages can be adjusted to find the true CH_4/ CO_2 ratio of the gas using Equation 2.3.1.

$$Adusted\ \%CH4 = \frac{\%\ CH4}{\%\ CH4\ +\ \%CO2} * 100$$

Equation 2.3.1. Biogas Quality

2.4 VFA Analysis

To prepare samples for VFA analysis, digestate was diluted to 1/10th concentration before being centrifuged for five minutes at 6000 rpm. The samples were then passed through 0.22-micron filters before being stored for further analysis.

The primary VFA of concern in this analysis is formic acid. To measure the quantity of formic acid, nuclear-magnetic resonance spectrometry (NMR) was used. To prepare the NMR samples, a solution of 4 mg TSP-d4 in 100 mL of D_2O was prepared. Each sample was prepared in an NMR tube with 550 µL of sample and 50 µL of TSP solution. Samples were analyzed using the 600 MHz Bruker Avance NEO located in room B8 of the MSU Chemistry Building. Materials required for NMR analysis of formic acid include plastic and glass vials, micropipettes, 0.22-micron filters, 50-mL vials, centrifuge, NMR tubes, TSP-d4, D_2O, and a 600 MHz Bruker Avance NEO.

Gas chromatography was used to measure acetic, propionic, butyric, and valeric acid concentrations. For this analysis, a Shimadzu GC-2010 with an Agilent Technologies capillary column and a Shimadzu flame ionization detector (FID) was used. The test was conducted under isothermal conditions.

2.5 DNA Analysis

The first step in DNA analysis was extraction of microbial DNA. This was accomplished with the use of a QIAGEN DNeasy Powersoil Pro Kit, using QIAGEN method HB-2494-003. This method includes the required materials.

After the DNA was extracted, the DNA samples were stored at -80 C while awaiting analysis. DNA amplification was performed according to the methods detailed by Xu et al., 2025. The samples were multiplied via PCR (Polymerase Chain Reaction) to increase the concentration of DNA. The PCR used the universal primers; Pro 341 F (5'-CCTACGGGNBGCASCAG-3') for the forward primer and Pro 805 R (3'-GACTACNVGGGTATCTAATCC-5') for the reverse primer. The amplification target was the 16S rRNA gene in the V3-V4 region. A reaction solution containing 12.5 µL of GoTaq Green Master Mix, 0.5 µL of forward primer, 0.5 µL of reverse primer, 1 µL of extracted DNA, and 10.5 µL of DNase- and RNase-free water (total 25 µL) was used for PCR. An Eppendorf Mastercycler Pro Thermal Cycler Sample validity was confirmed using gel electrophoresis.

The DNA samples were then submitted to the Michigan State University Research Technology Support Facility Genomics Core for sequencing on an Illumina MiSeq flow cell (v2) using a 500-cycle reagent kit. The methods and technical documents can be found at the RTSF Genomics Core website at https://rtsf.natsci.msu.edu/genomics/.

2.6 Statistical Analysis

The recording processing of raw data was performed using Microsoft Excel. Statistical analysis was performed using R statistical software. The correct statistical test

to use is based on the normality and variance of the data (Emerson, Emilia Maria, 2024). Table 2.6.1 summarizes the different available statistical tests.

Table 2.3. Statistical Test Methods (adapted from Emerson, 2024)

Test	t-test or ANOVA	Assumed Normal Distribution?	Assumed Equal Variance?
Mann-Whitney	t-test	No	-
Welch's	t-test	Yes	No
Standard	t-test	Yes	Yes
Kruskal-Wallis	ANOVA	No	-
Brown-Forsythe and Welch	ANOVA	Yes	No
Ordinary	ANOVA	Yes	Yes

This study was conducted using biological duplicates; therefore, n=2. It is impossible to determine normality with the preferred statistical test for small sample sizes, the Shapiro-Wilk test, which requires at least 3 data points. It is then necessary to make assumptions about the data. Ordinary and Kruskal-Wallis ANOVA tests were both conducted, providing results both for and against the assumption of normally distributed data. Equal variance was tested using a Bartlett's test. Following ANOVA testing, a Tukey's Honest Significant Difference test conducted. The HSD test determines which specific means are significantly different from one another.

For DNA analysis, the R packages Vegan, MASS, Phyloseq, and ggplot2 were used to analyze taxonomic data. Mnova software (Mestrelab Research) was used to analyze the output of the NMR analysis of formic acid.

2.7 Mass And Energy Balance Analysis

Mass and energy balance analysis were conducted based on the laboratory data generated from the lab-scale digester experiment. The mass and energy balances are based on a system in which the effluent from the main digester is used as the feedstock for a secondary digester, matching the conditions of the lab-scale study. The energy inputs for the digestion operation include both thermal energy (W_{heat}, kWh-e/year), to maintain the digestion temperature, and electricity energy ($W_{electricity}$, kWh-e/year), to operate auxiliary equipment such as pumps, mixers, and the control unit. The energy inputs were estimated using the Equation 2.7.1 and Equation 2.7.2, which were modified from a previous study (Bustamante & Liao, 2017).

$$W_{heat} = m \times C_p \times (T_R - T_O) \times (1 + 20\%) \times 0.0002778$$

Equation 2.7.1. Estimated Required Heat Input

$$W_{electricity} = m \times 0.00788$$

Equation 2.7.2. Estimated Required Electricity Input

In these equations, m is the amount of the wet weight of the feedstock per year (kg); C_p is the heat capacity of the wet feedstock (4.12 kJ/(kg K)); T_R is the reactor temperature, which was 313 K for the main digester and 323 K for the formate-enhanced digester, respectively; T_O is the temperature of the wet feedstock, which was 313 K, the temperature of the filtrate from the previous digester; 20% is the percentage of the additional heat

needed to maintain the digestion temperature; 0.0002778 is the conversion factor of KJ to kWh; and 0.00788 is the average electricity demand of the MSU SCAD digester operation (kWh/kg wet feedstock).

2.8 Life Cycle Impact Assessment (LCIA)

The life cycle assessment (LCA) methodology was developed to quantify and compare the environmental impacts associated with a formate-enhanced anaerobic digester and two control configurations, AD with activated carbon and AD alone, based on the mass and energy balance. Two environmental impact categories were assessed: Global Warming Potential (GWP), expressed in CO_2-equivalents (g CO_2-e), and Water Eutrophication Potential (WEP), expressed in nitrogen equivalents (g N-e).

Greenhouse gas emissions were calculated based on CH_4 and nitrous oxide (N_2O) emissions from land application of digestate. The emission factors for land application were 0.001 g CH_4/g volatile solids (VS) and 0.005 g N_2O/g total nitrogen (TN) in the digestate. Global warming potentials were taken as 25 g CO_2-e/g CH_4 and 265 g CO_2-e/g N_2O (US EPA, 2016). Electricity usage was evaluated based on system energy demand and its associated GWP. A GWP of 491 g CO_2-e/kWh-e was used for natural gas-based electricity generation, consistent with U.S. EPA emission factors. Eutrophication impacts were assessed based on total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD) in the effluent. Characterization factors were 0.9864 g N-e/kg TN, 7.29 g N-e/kg TP, and 0.05 g N-e/kg COD.

In alignment with standard LCA practice, CO_2 emissions from the degradation of biogenic materials in the feedstock and effluent were not included in the GWP

calculations. These biogenic CO_2 emissions are considered to be offset by atmospheric CO_2 capture through plant regrowth due to the biological origin of the feedstock. The key parameters and impact factors used in the analysis are summarized in Table 2.4.

Table 2.4. Parameters of Life Cycle Assessment

	Value	Unit	Source
CH_4 emission of digestate – land application	0.001	g CH_4/g VS in the waste	(Owen & Silver, 2015)
GWP of CH_4	25	g CO_2/g CH_4/	(Turnbull, 2004)
N_2O emission of digestate – land application	0.005	g N_2O/g TN in the waste	(US EPA, 2016)
GWP of N_2O	265	g CO_2-e/g N_2O	(US EPA, 2016)
GWP of natural gas energy	491	g/kWh-e	(US EPA, 2016)
Water eutrophication potential (WEP) of TN	0.9864	g N-e/kg TN in the waste	(US EPA, 2016)
Water eutrophication potential (WEP) of TP	7.29	g N-e/kg TP in the waste	(US EPA, 2016)
Water eutrophication potential (WEP) of COD	0.05	g N-e/kg COD in the waste	(US EPA, 2016)

CHAPTER 3: FORMATE-ENHANCED AD

3.1 Gas Production

Figure 3.1.1 shows the total gas production for each reactor over the course of the experiment. This number is the entire biogas volume, including both CH_4 and CO_2. The data presented is for the individual reactors.

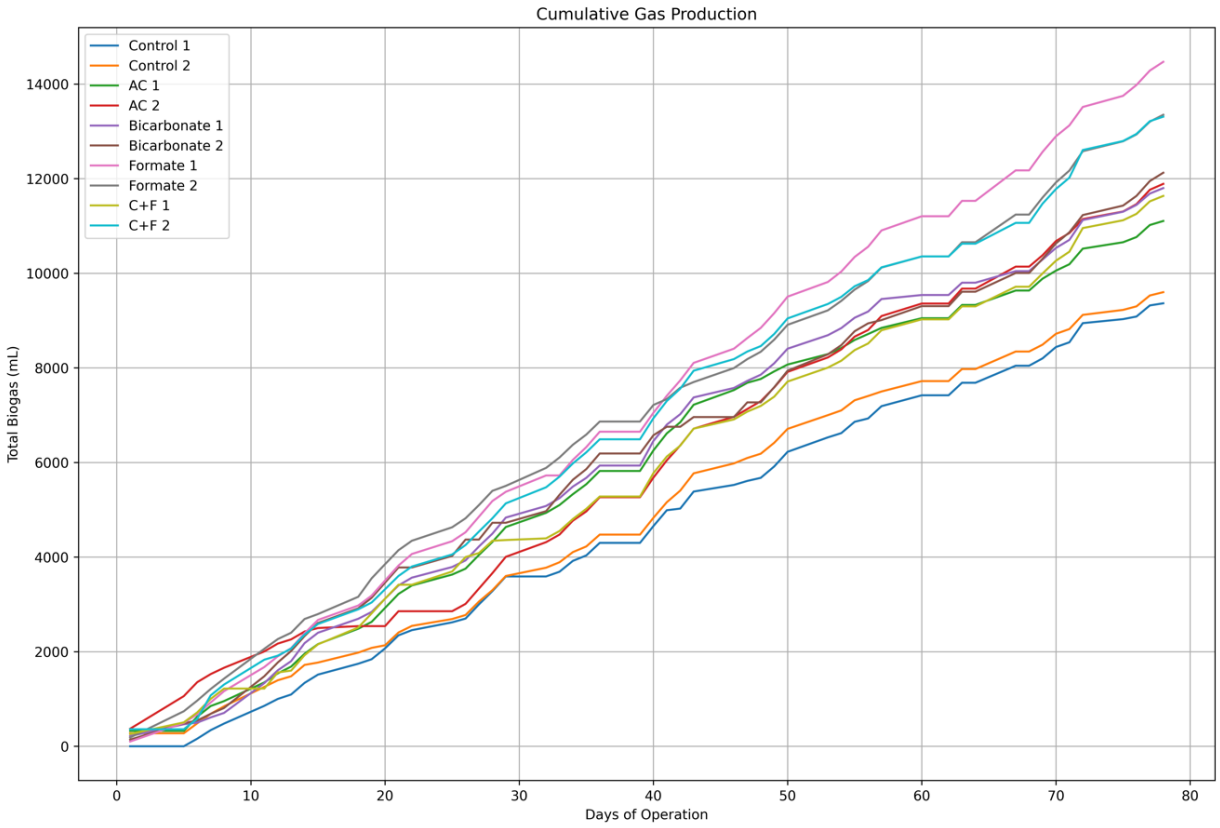

Figure 3.1.1. Cumulative Biogas Production

An ANOVA analysis of the gas production data indicated that there were significant differences between the mean gas production of different reactor conditions (a = 0.05). A following Tukey's HSD test indicated means between which a significant difference occurred.

With a = 0.05, entries with adjusted p-values below 0.05 qualify as significantly different from the compared mean. There is a significant difference between the formate reactors and the control reactors, as well as a significant difference between the bicarbonate/formate and control reactors. There is not a significant difference between other reactors, including between control and activated carbon reactors or formate and formate/bicarbonate reactors. Taken together, these factors suggest that formate was the determining factor in whether a reactor would produce more biogas. Activated carbon did not produce significantly more biogas on its own, and the addition of bicarbonate with formate did not show a significant increase in production either. Production was in fact lower, but not to a statistically significant degree.

A Kruskal-Wallis test on total gas production did not reveal a significant difference between the means. However, this may be because non-parametric tests require larger sample sizes to achieve the same power as parametric tests.

Figure 3.1.2 shows the average daily CH_4 production rate for each set of reactors in each HRT. In this figure, Ctrl-AD is Control, Ctrl-AC is Activated Carbon, R1 is Bicarbonate, R2 is Formate, and R3 is Bicarbonate and Formate. The data is the average of all data points for both replicates in the respective HRT.

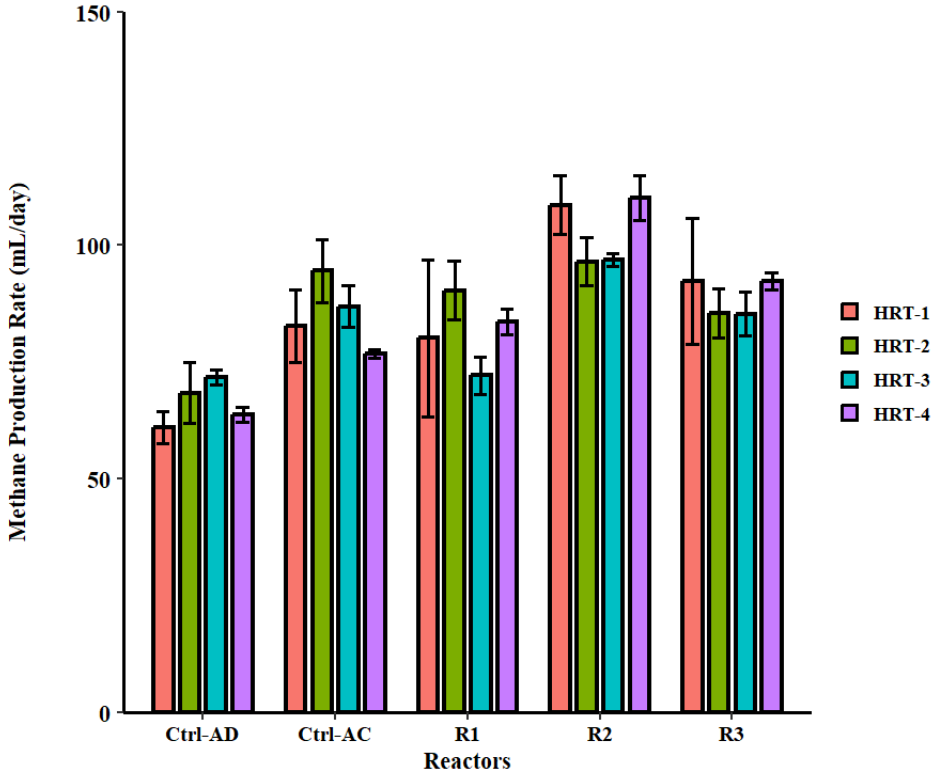

Figure 3.1.2. Daily Methane Production per Reactor and HRT

The CH_4 production of the formate-enhanced reactors is notably higher than that of other reactors across all HRTs. The difference is particularly notable in the fourth, stable HRT. The bicarbonate/formate reactor had CH_4 production between formate reactors and bicarbonate reactors, suggesting that bicarbonate was detrimental to biogas production during formate treatment.

Gas production numbers were not normalized to added volatile solids. This approach was chosen because all reactors received the same feedstock, and therefore the same volatile solids amount. In the case of the formate-added reactors, the total extra solids added was considered to be negligeable in the overall experimental results.

3.2 Gas Quality

The average gas quality of each reactor is shown in Figure 3.2.1. The quality is given in terms of the decimal fraction of the biogas that is CH_4, with 1 indicating that the gas is 100% CH_4. The data is the average of all data points for both replicates in the respective HRT.

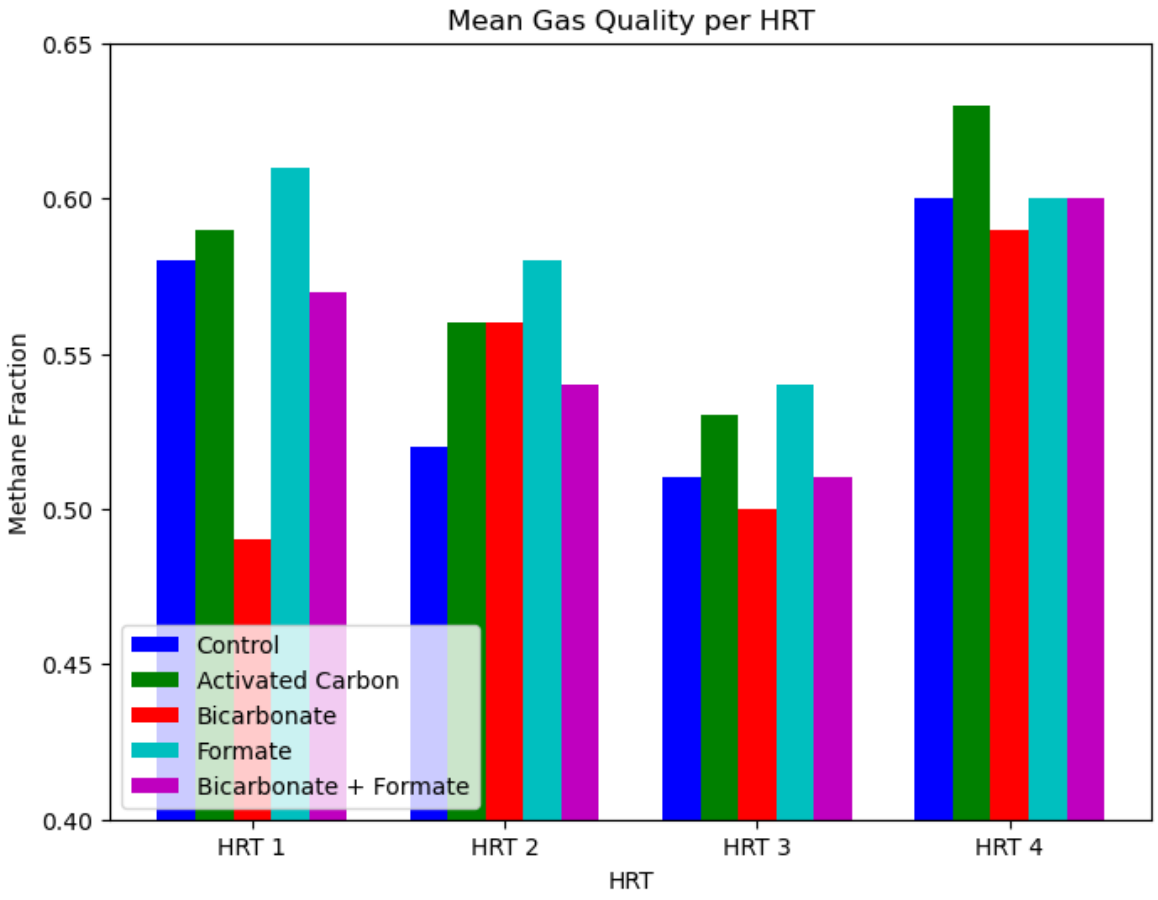

Figure 3.2.1. Mean Gas Quality Per HRT

An ANOVA test indicated that there were significant differences between means, with an extremely low p-value (p = 1.65x10^-5). A following Tukey's HSD test indicated that there were significant differences between most mean groups. Notably, there is no significant difference between formate and activated carbon reactors, nor between control

and carbonate-formate reactors. There was a significant difference between control and bicarbonate reactors, with bicarbonate reactors being worse. These results indicate that formate addition did not significantly improve biogas quality. The results also indicate that there is a significant negative impact on biogas quality exerted by bicarbonate addition.

3.3 pH, TSVS, and COD

3.3.1 Chemical Oxygen Demand

Figure 3.3.1 shows the mean COD values in mg/L for each reactor in each HRT. The data is the average of all data points for both replicates in the respective HRT. The dilution factor was then applied; reported values are undiluted COD.

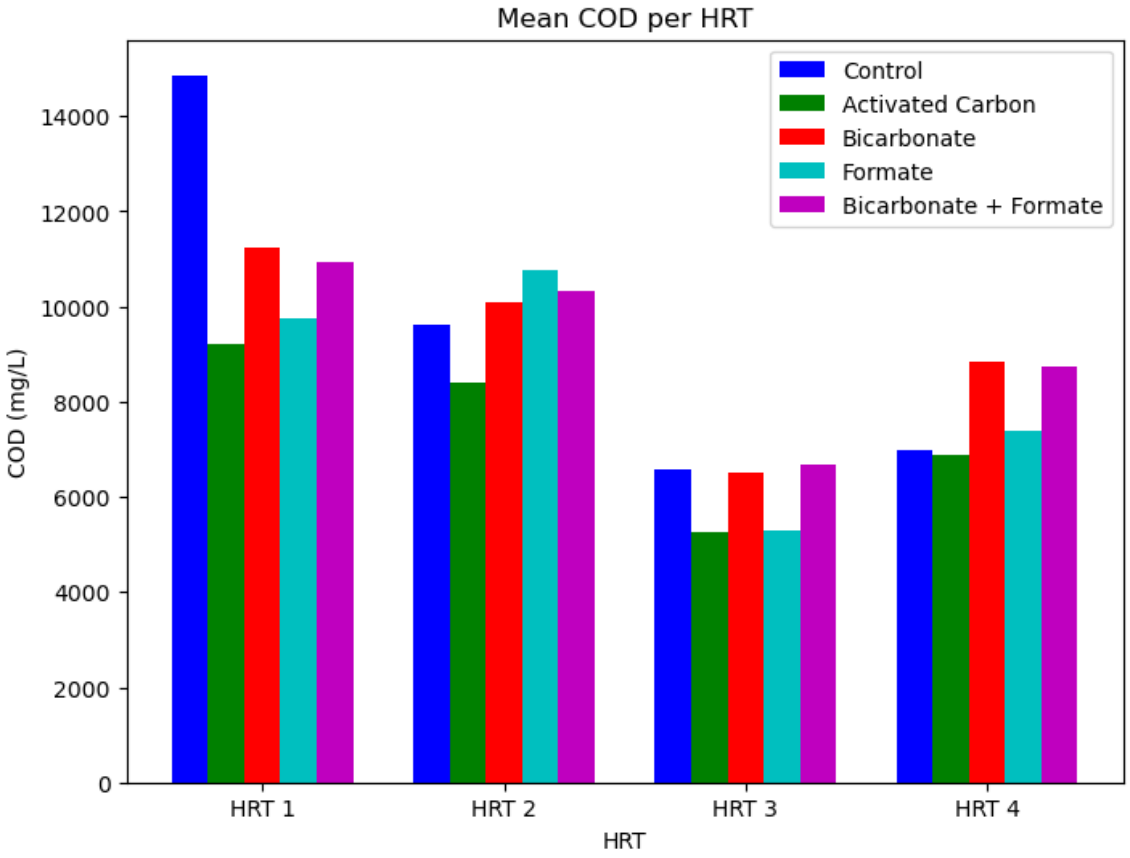

Figure 3.3.1. Mean COD per HRT

COD is an indicator of the amount of digestible solids available in the digester culture. All the reactors were fed with the same feedstock, so variations in the COD indicate differences in the digestion. Higher COD suggests that less of the organic solids available were consumed, while lower numbers indicate higher usage. Overall, COD destruction increases in the first and second HRTs, appearing to stabilize in the third and fourth. In the fourth HRT, reactors that were treated with bicarbonate show higher COD values, indicating that less COD reduction was achieved in those reactors.

Statistical tests were conducted on the fourth HRT, with the average value of all measurements for a particular reactor taken in that period used as the value for that reactor. The Kruskal-Wallis test did not indicate a significant difference between means. The ordinary ANOVA on COD did indicate a significant difference. A following Tukey's HSD test indicated that significant differences between means existed between all means except for Control-Activated Carbon and Bicarbonate/Formate-Bicarbonate. This indicates that bicarbonate addition is associated with a significant increase in COD, which in turn indicates a lower level of COD destruction and therefore a decrease in reactor efficiency. The formate reactors also showed a significant increase in COD as compared to the activated carbon reactors, indicating that COD destruction was decreased when either formate or bicarbonate was added.

3.3.2 pH

Figure 3.3.2 displays the mean pH measured for each reactor condition in each HRT. The data is the average of all data points for both replicates in the respective HRT.

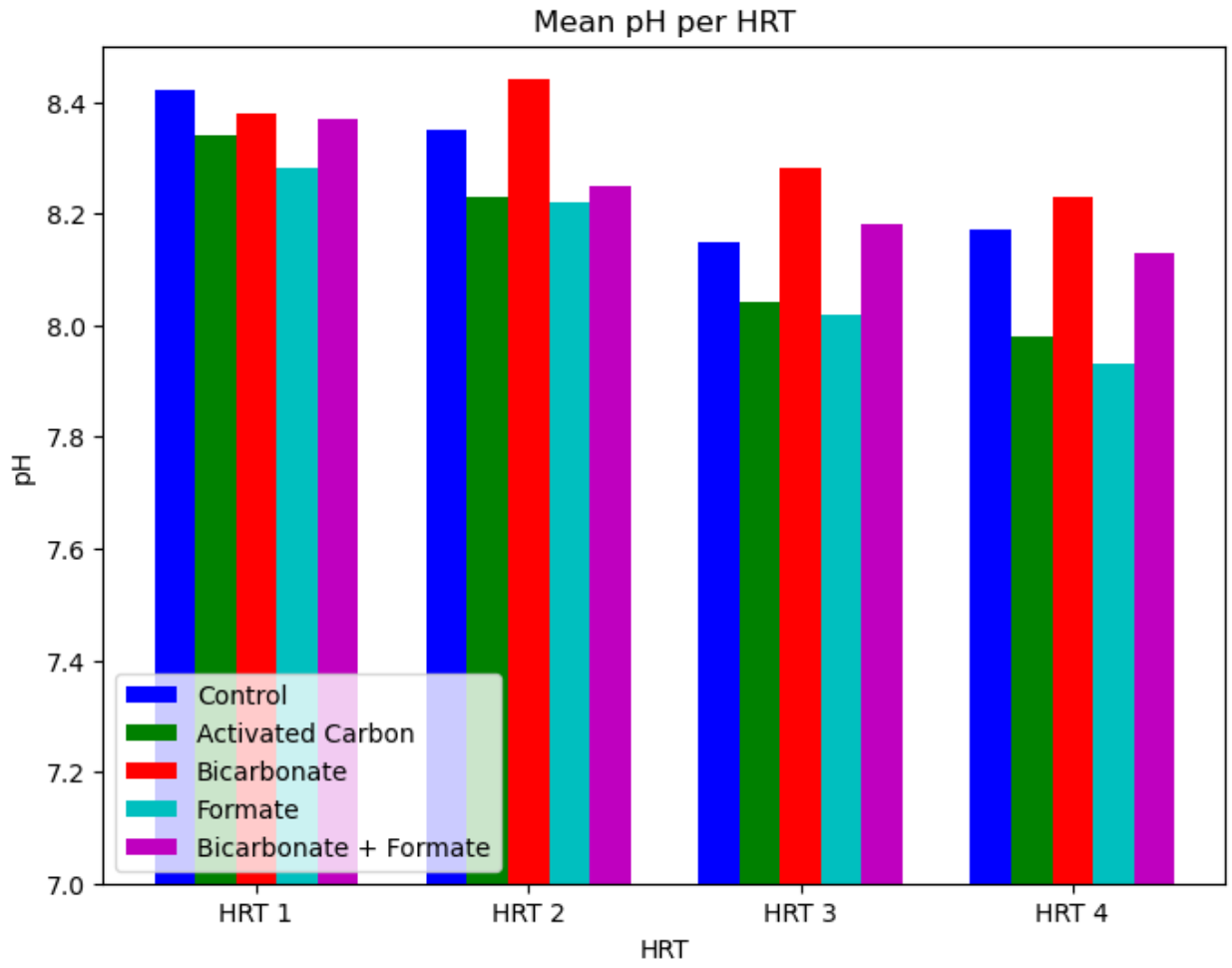

Figure 3.3.2. Mean pH per HRT

pH remained within the expected range for healthy digester operation. Every pH measurement was above 7.0, indicating some alkalinity in all reactors. The pH in the Activated Carbon and Formate reactors shows a trend of decreasing over the course of the experiment as compared to the other reactors.

A Kruskal-Wallis test did not indicate any significant difference between means; however, the ordinary ANOVA test did indicate a significant difference between means. In particular, the activated carbon and formic acid treated reactors were shown to have a significantly lower pH compared to the control reactor or either reactor that received

bicarbonate. The higher pH of the bicarbonate reactors is expected, as bicarbonate serves as a buffer to stabilize pH. However, in this case, it seems that it was not necessary to have that buffer, as none of the other reactors acidified to a problematic degree. In fact, the buffer may have inhibited the reactor activity by bringing it further outside of the ideal pH range for methanogenic activity.

3.3.3 Total and Volatile Solids

Figure 3.3.3 shows the mean total solids per HRT in g/L concnetration. The data is the average of all data points for both replicates in the respective HRT. Figure 3.3.4 is the equivalent figure for volatile solids. Both measurements are discussed together, as they follow similar trends and the differences in total solids are largely attributable to changes in volatile solids, as the degradation of volatile solids is the primary way in which the microbial culture can influence total solids.

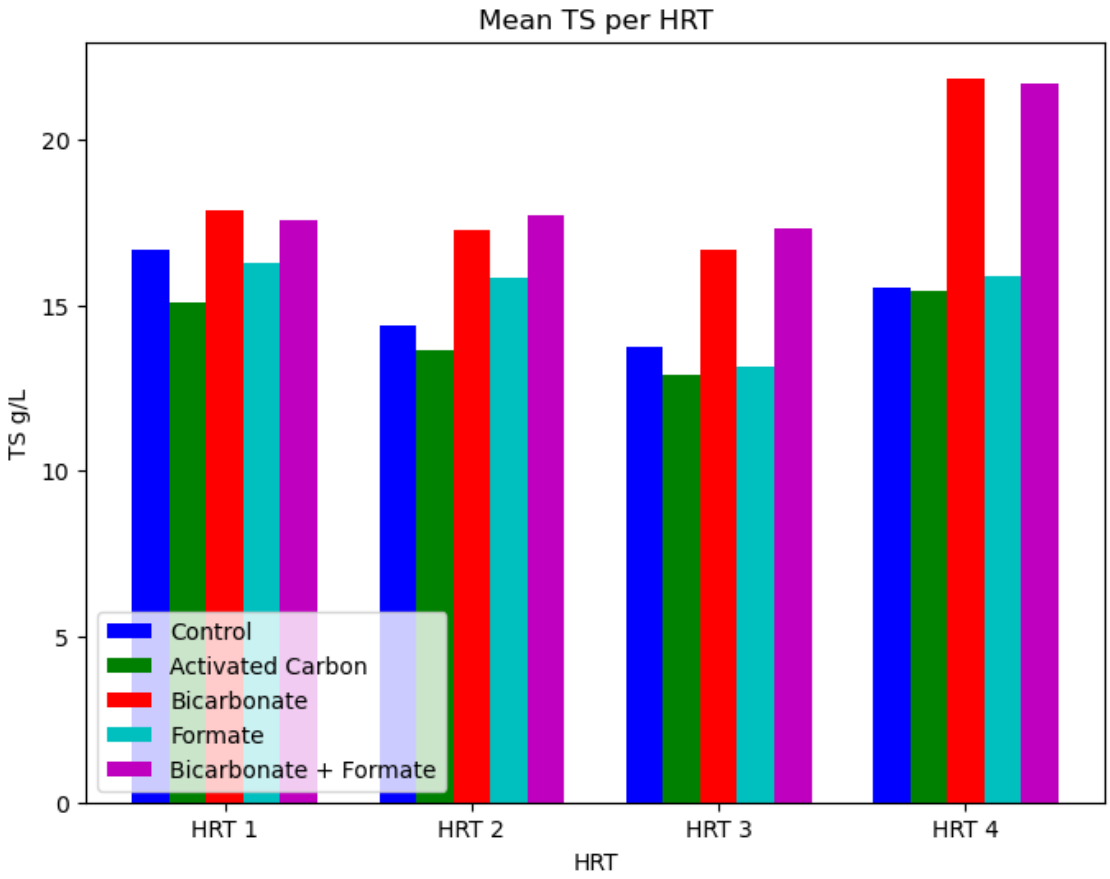

Figure 3.3.3. Mean Total Solids per HRT

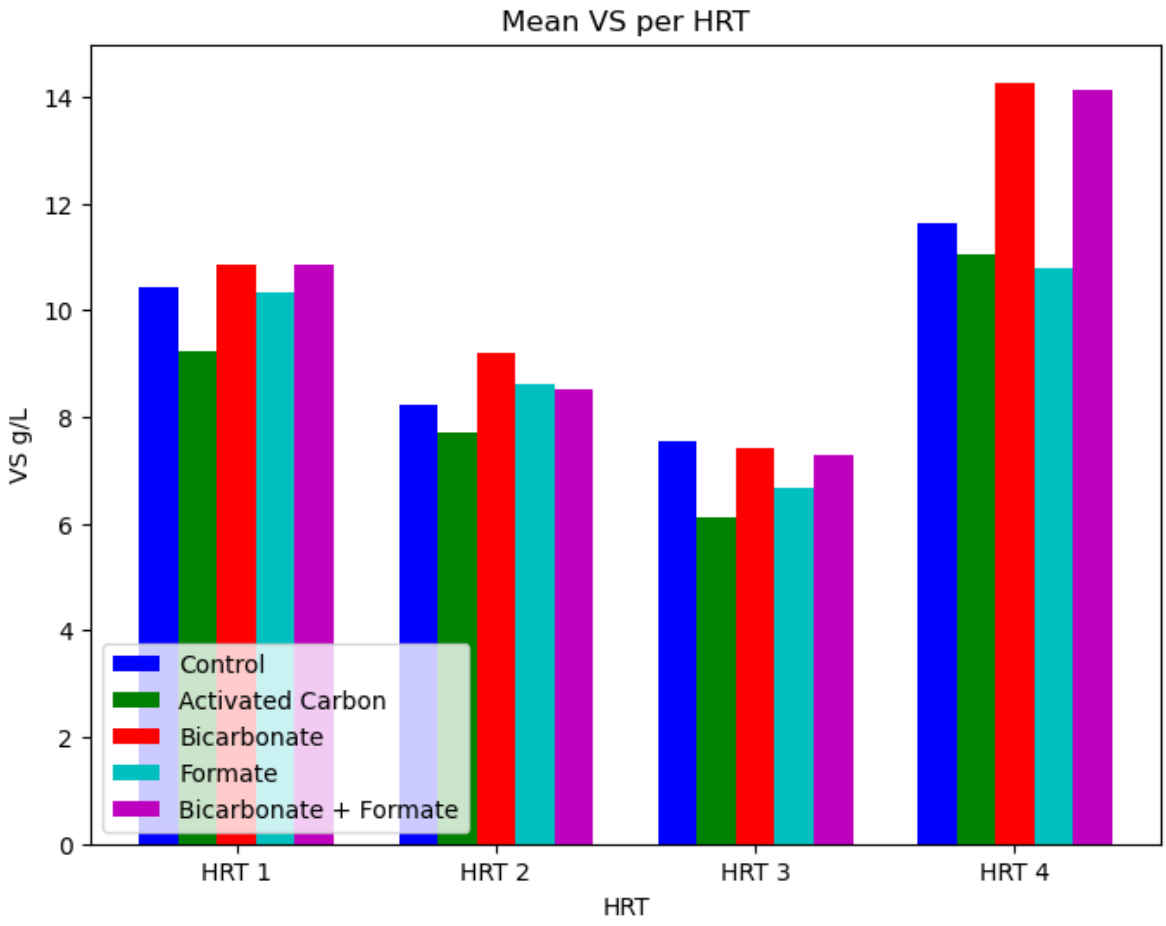

Figure 3.3.4. Mean Volatile Solids per HRT

Total and volatile solids indicate the amount of material in the digester. Most total solids are volatile solids, as this is the fraction of solids that is organic and can be degraded or burned, which makes up most of the solids in the feedstock. Greater solids concentration indicates lower performance, as biogas is produced from the degradation of volatile solids and all digesters were fed with the same feedstock with the same solids concentration.

A Kruskal-Wallis test did not detect a significant difference in either total or volatile solids between reactors; an ordinary ANOVA detected a significant difference in both. Significant differences were found between both bicarbonate-added conditions and the

non-bicarbonate added conditions, with bicarbonate-added reactors showing significantly higher concentrations of total and volatile solids. This indicates a decrease in the metabolism of organic materials in the bicarbonate treated reactors.

3.4 Volatile Fatty Acids

3.5 Formic Acid

The addition of formate was shown to have significant impacts on biogas production and quality. The average concentration of formic acid is shown at each HRT in Figure 3.5.1. For VFA data, values are the average for samples from that HRT, but from one replicate each.

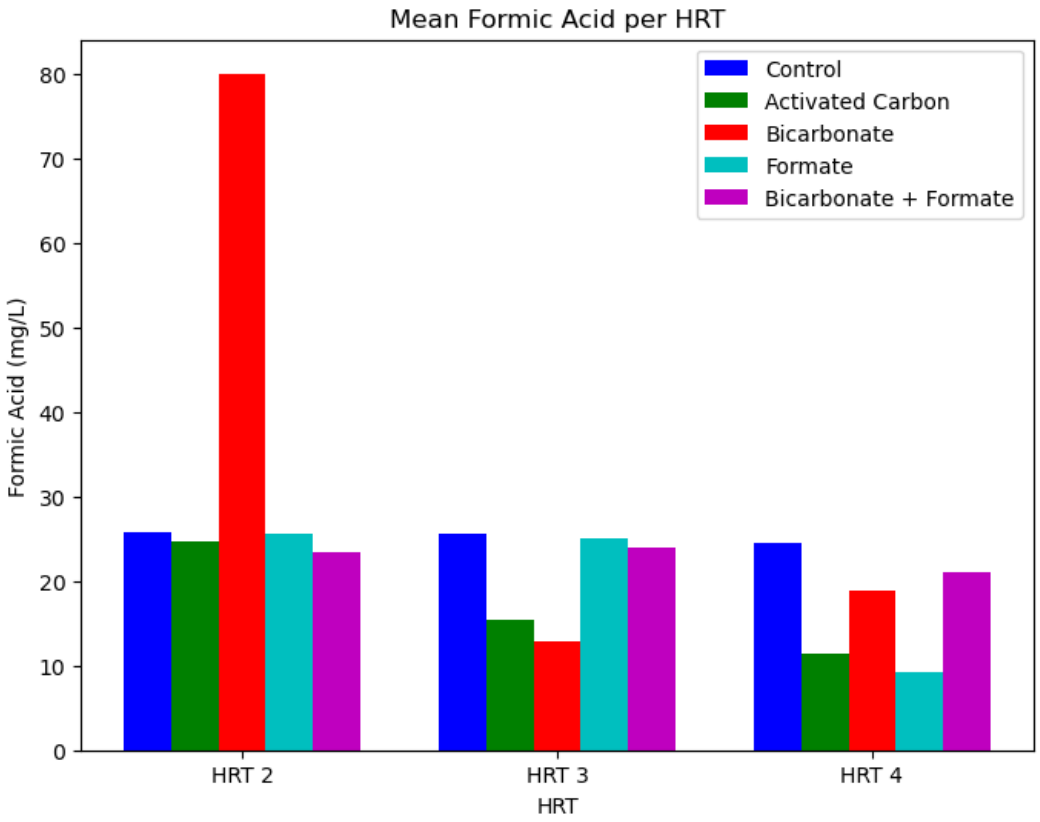

Figure 3.5.1. Mean Formic Acid per HRT

All samples, with one exception, lie in the 9-30 mg/L range for formic acid concentration. However, the formate and carbonate/formate reactors had feedstock with a 100 mg/L concentration of formate. This means that the formate reactors were able to metabolize all of the formic acid in the feedstock. In the case of the HRT 4, the formate only reactor has lower formic acid than any of the other reactors, indicating that the formic acid produced in the culture was also metabolized more efficiently. The activated carbon only reactors also demonstrated a reduced formic acid concentration, indicating that the presence of the conductive material aided in the metabolism of formic acid.

In the first HRT, the bicarbonate-only reactors indicated a much larger formic acid concentration than any other reactors. This is due to two measurements in that HRT that are much larger than the other measurements. However, these values fell to values in the normal range by the next HRT. Formic acid is produced and consumed quickly, so it is plausible that these reactors were able to change concentration relatively quickly compared to the other feeding conditions. While there may have been temporary formic acid build-up in those reactors, by the time reactor stability was reached, bicarbonate addition did not seem to have caused continuing formic acid accumulation.

3.6 Acetic Acid, Propionic Acid, Butyric Acid, Valeric Acid

3.6.1 Acetic Acid

Acetic acid is a main intermediary product in AD. A difference in acetic acid concentrations would suggest a difference in the rate of acetogenesis or acetate-consuming methanogenesis. The concentration of acetic acid decreased over the course

of the experiment as the reactors approached the stable phase. Figure 3.6.1 shows the mean concentration of acetic acid in each feeding condition in each HRT. For VFA data, values are the average for samples from that HRT, but from one replicate each.

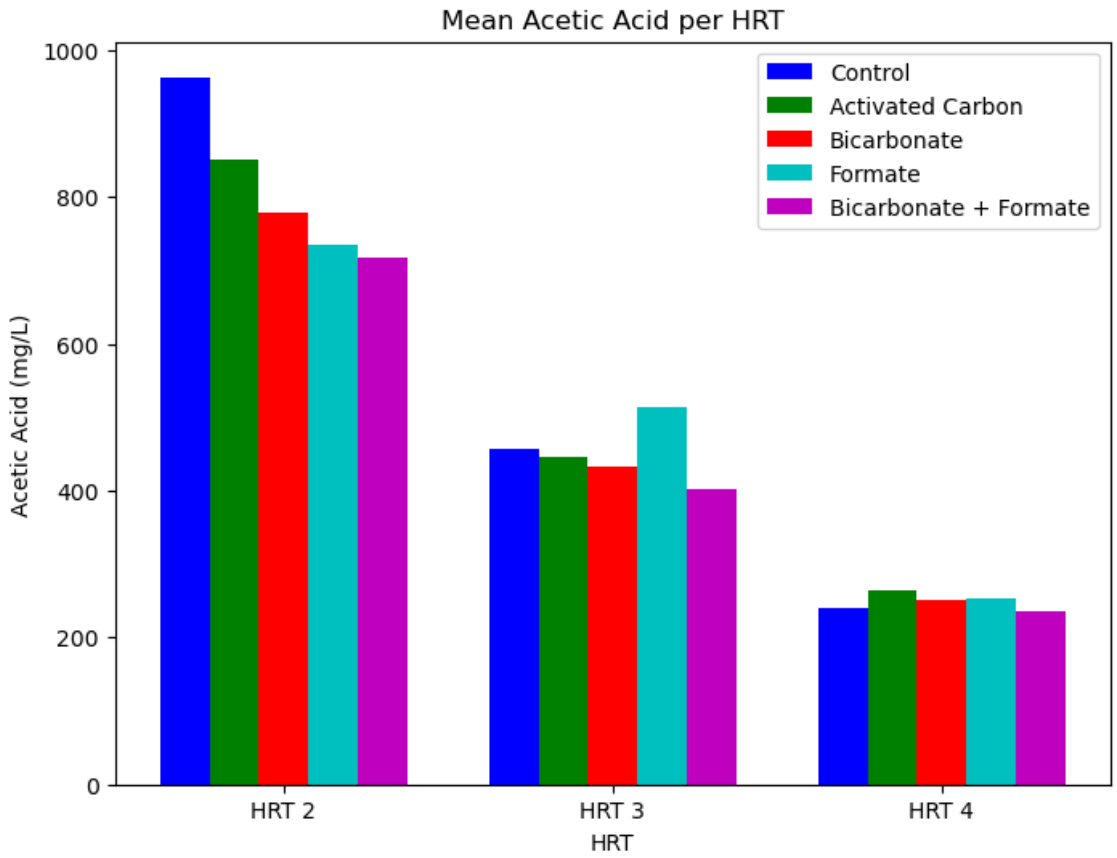

Figure 3.6.1. Mean Acetic Acid per HRT

In the fourth HRT, by which time the reactors had stabilized, the concentration of acetic acid was even across all feeding conditions. This suggests that the presence or absence of activated carbon, bicarbonate, or formate did not have a large impact on the rate of acetate metabolism or acetate production.

3.6.2 Propionic Acid

Figure 3.6.2 shows the mean concentration of propionic acid in each reactor condition during each HRT, obtained by averaging the propionic acid measurements for each condition in that HRT. For VFA data, values are the average for samples from that HRT, but from one replicate each.

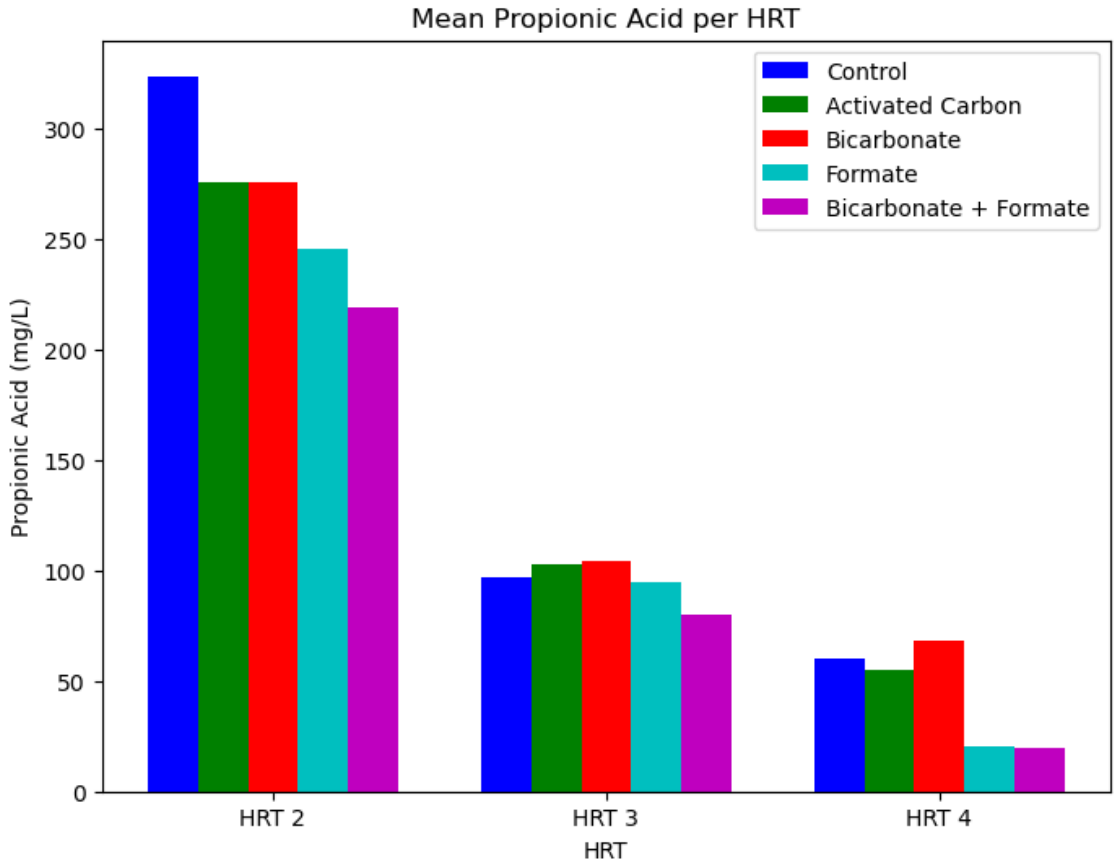

Figure 3.6.2. Mean Propionic Acid per HRT

As with acetic acid, the concentration of propionic acid fell over the course of the experiment. In the fourth HRT, there is a notable difference in the concentration of propionic acid between the reactors treated with formic and the reactors that lack formic acid; that is, reactors with formic acid show a decrease in the concentration of propionic acid. This suggests that formate addition plays a role in enhancing the metabolism of

propionic acid. This is consistent with the notion discussed in the literature that formic acid addition serves to enhance IET, as that process is essential to the efficient degradation of propionic acid.

3.6.3 Butyric and Isobutyric Acid

Figure 3.6.3 shows the mean concentration of butyric acid in each HRT, while Figure 3.6.4 shows the mean concentration of isobutyric acid. For VFA data, values are the average for samples from that HRT, but from one replicate each.

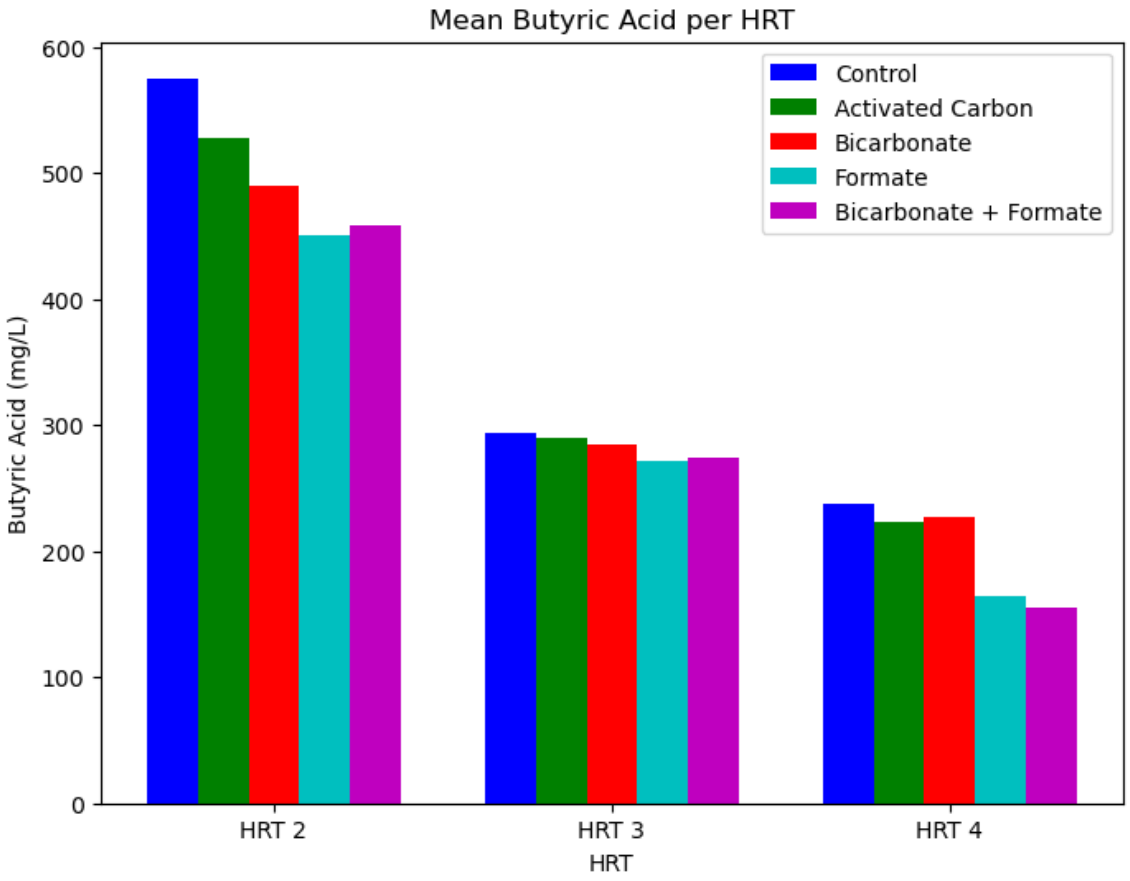

Figure 3.6.3. Mean Butyric Acid per HRT

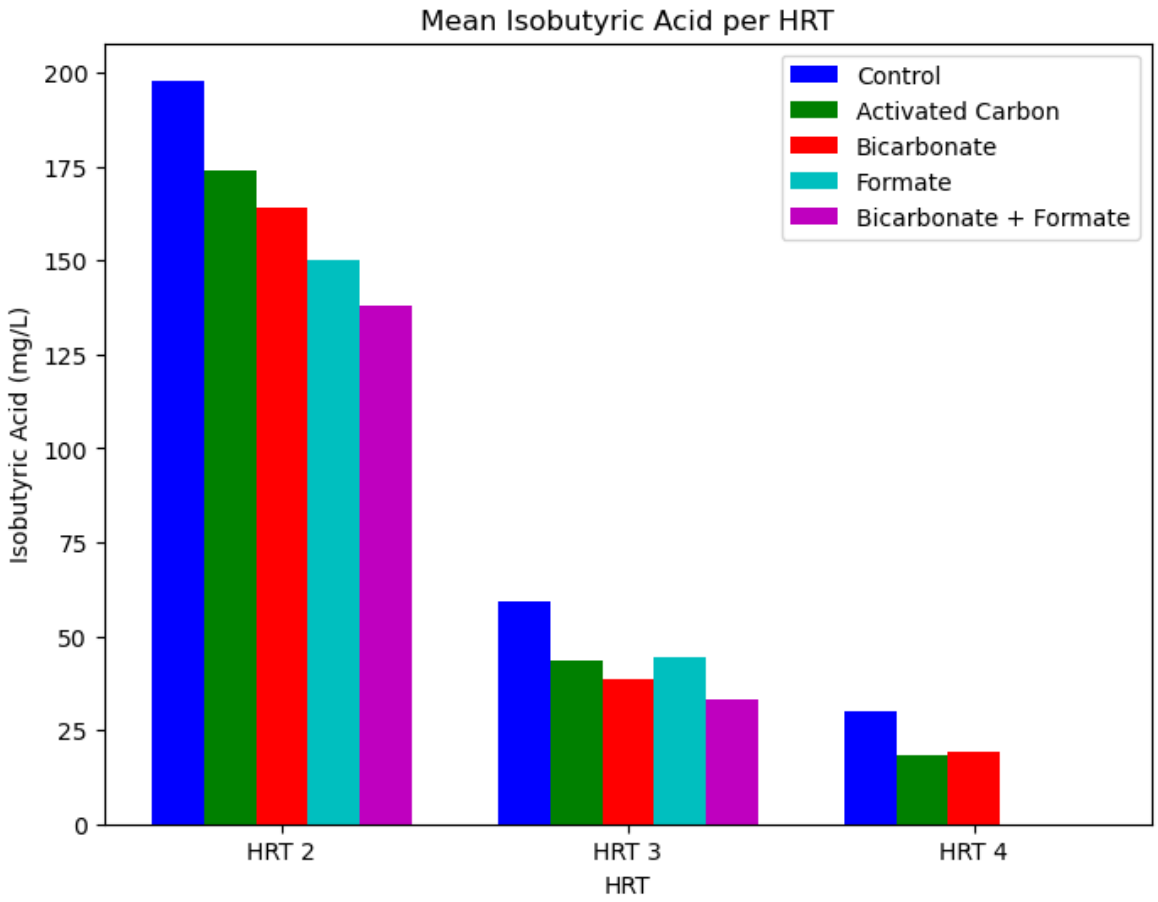

Figure 3.6.3. Mean Isobutyric Acid per HRT

Butyric acid and isobutyric acid follow similar trends, both to one another and other VFAs. There is a decrease in concentration as the experiment progresses. In the fourth HRT, there is again a decreased concentration of the acid in reactors that received formic acid. There is no bar shown for isobutyric acid for formate and bicarbonate/formate in the fourth HRT because the concentration was below the detection threshold, and is therefore listed as 0 mg/L. Like propionic acid, butyric acid is thermodynamically unfavorable to degrade and therefore benefits from the enhancement of IET.

3.6.4 Isovaleric Acid

Figure 3.6.5 shows the mean concentration of isovaleric acid in each HRT. For VFA data, values are the average for samples from that HRT, but from one replicate each.

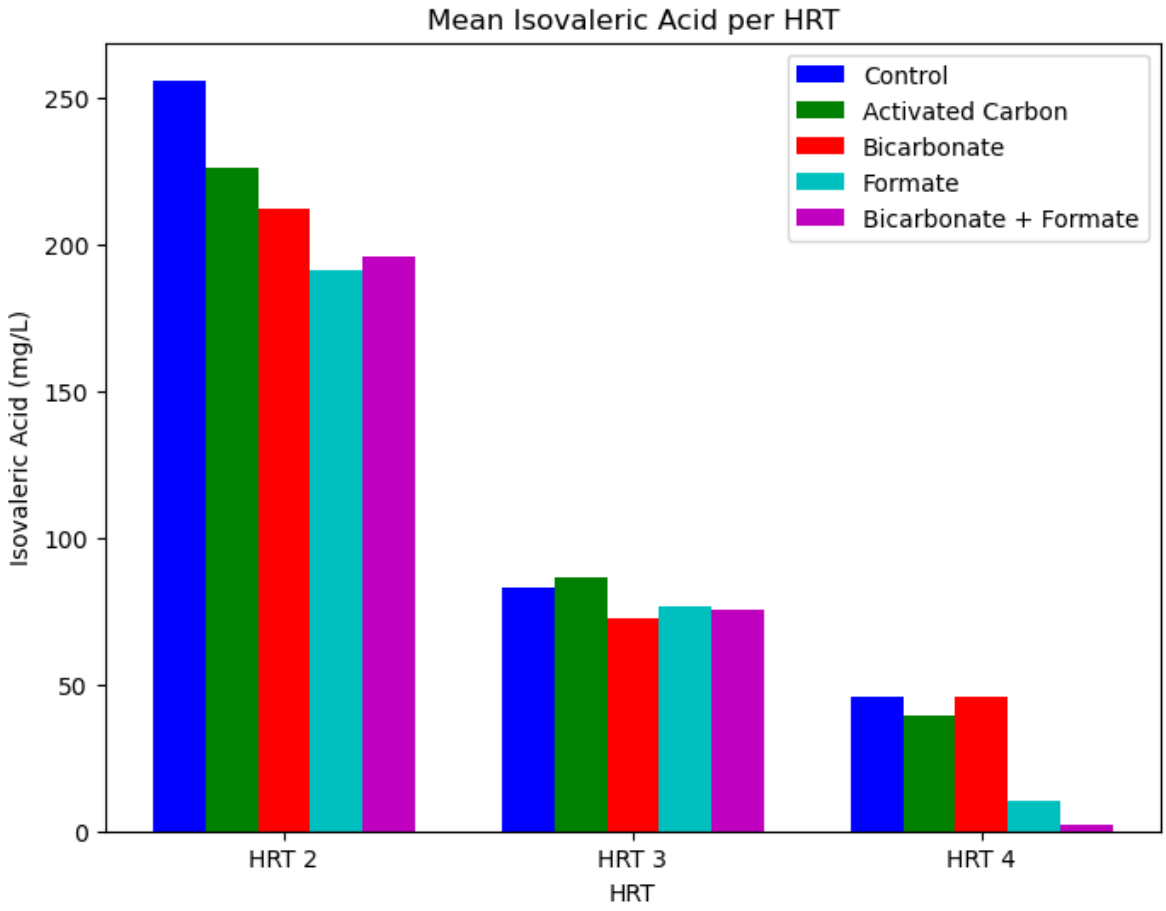

Figure 3.6.4. Mean Isovaleric Acid per HRT

The last VFA with a detectable quantity was isovaleric acid. The concentration follows a similar trend to propionic, butyric, and isobutyric acid, with the concentration decreasing as the experiment progresses. In the fourth HRT, the reactors treated with formic acid show decreased acid concentration compared to reactors without formic acid addition. Notably, the bicarbonate/formic acid combined treatment shows a greater reduction in isovaleric acid concentration as compared to formate alone.

3.7 Microbial DNA

In the microbial DNA analysis section, the reactors are labeled Ctrl-AD, Ctrl-AC, R1, R2, and R3. These correspond to Control (C), Activated Carbon (A), Bicarbonate (B), Formate (F), and Bicarbonate and Formate (CF) respectively. Table 3.7.1 provides these equivalents for ease of reference. The analysis of the microbial communities focuses on HRT 3 and 4, which are the stable periods of digestion.

Table 3.1. Reactor Name Equivalencies

Reactor	Experimental Designation	Microbial Analysis Designation
Control	C	Ctrl-AD
Activated Carbon	A	Ctrl-AC
Bicarbonate	B	R1
Formate	F	R2
Bicarbonate plus Formate	CF	R3

3.7.1 Alpha Diversity Shannon, Simpson, and Sobs Indices

A statistical analysis of the microbial community was performed to determine the Simpson, Shannon, and Sobs indices of alpha diversity. Alpha diversity measures the diversity of a single community or sample. The Simpson Index of Diversity quantifies species dominance. Higher values indicate greater diversity, as lower values indicated domination by a single species. The Shannon Index indicates community diversity and evenness. Higher values indicate a more diverse ecology with a more even distribution of species. The Sobs Index measures community abundance.

Figure 3.7.1 shows the distribution of Shannon, Simpson, and Sobs indices for culture bacteria.

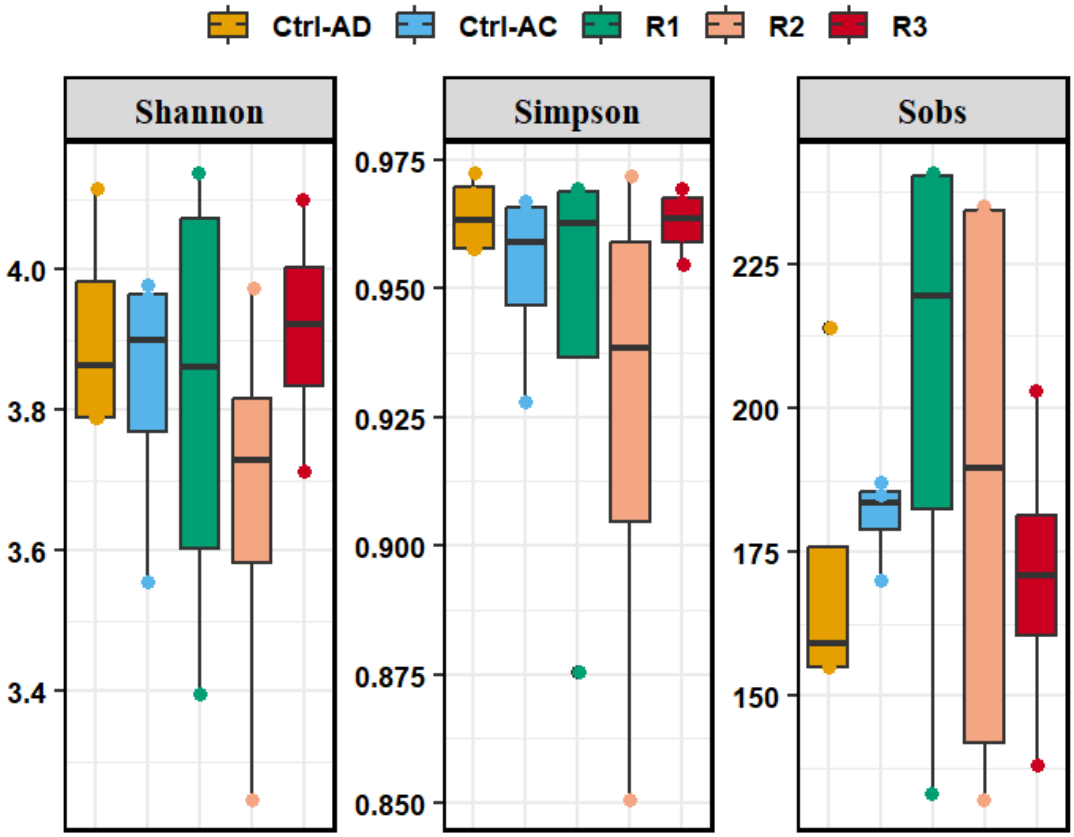

Figure 3.7.1. Shannon, Simpson, and Sobs Indices of Alpha Diversity for Bacteria

Between HRT 3 and HRT 4, both the Shannon and Simpson index increased, while the Sobs index decreased, particularly in formate-only and bicarbonate-only reactors. This indicates that while a more balanced microbial community emerged as stabilization approached, the selective pressures created by the formate or bicarbonate rich environments of the digesters may have reduced the richness of bacterial species present.

Figure 3.7.2 shows the distribution of Shannon, Simpson, and Sobs indices for culture archaea.

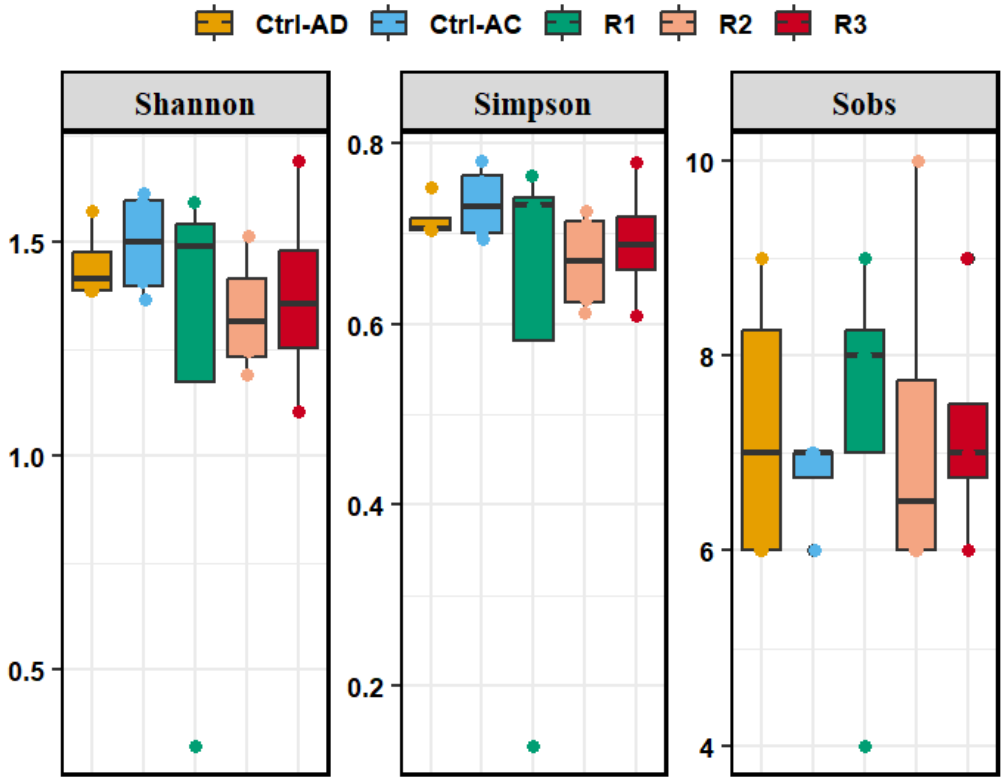

Figure 3.7.2. Shannon, Simpson, and Sobs Indices of Alpha Diversity for Archaea

The diversity and richness of the archaea community were much lower compared to the bacterial community, indicating more similar community members were present. Bicarbonate-only and formate-only reactors had lower Simpson indices, indicating that both formate and bicarbonate, when added separately, contributed to a more even archaea community.

3.7.2 Beta Diversity

Beta diversity measures differences in community between multiple groups. A non-metric multidimensional scale (NMDS) analysis was conducted to evaluate the beta diversity. The stress value of the model indicates reliability, with values below 0.1 indicating

a more reliable model. Figure 3.7.3 shows the beta diversity plot for the bacterial community.

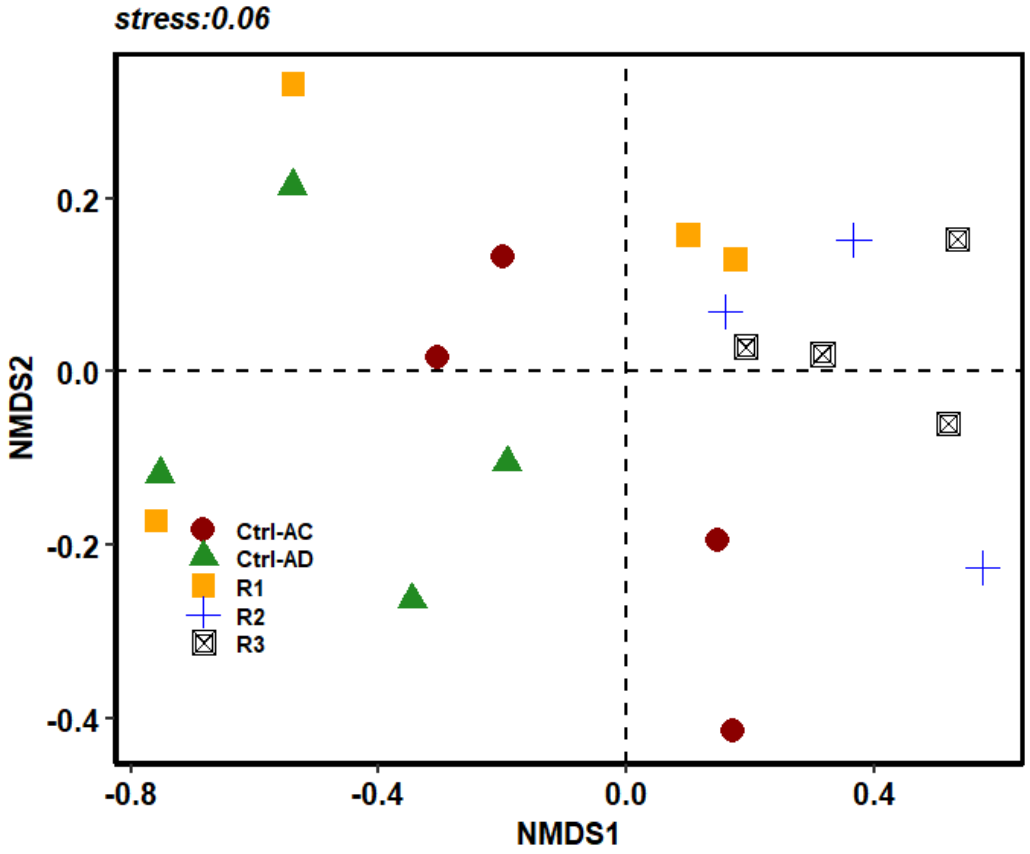

Figure 3.7.3. Beta Diversity of Bacteria

The stress value for the bacterial community model was 0.06. A significant difference ($P < 0.05$) was indicated between reactors, suggesting that the different feedstock conditions had a significant impact on the composition of the microbial community. The archaea model yielded similar results, with a stress value of 0.04. That analysis is displayed in Figure 3.7.4.

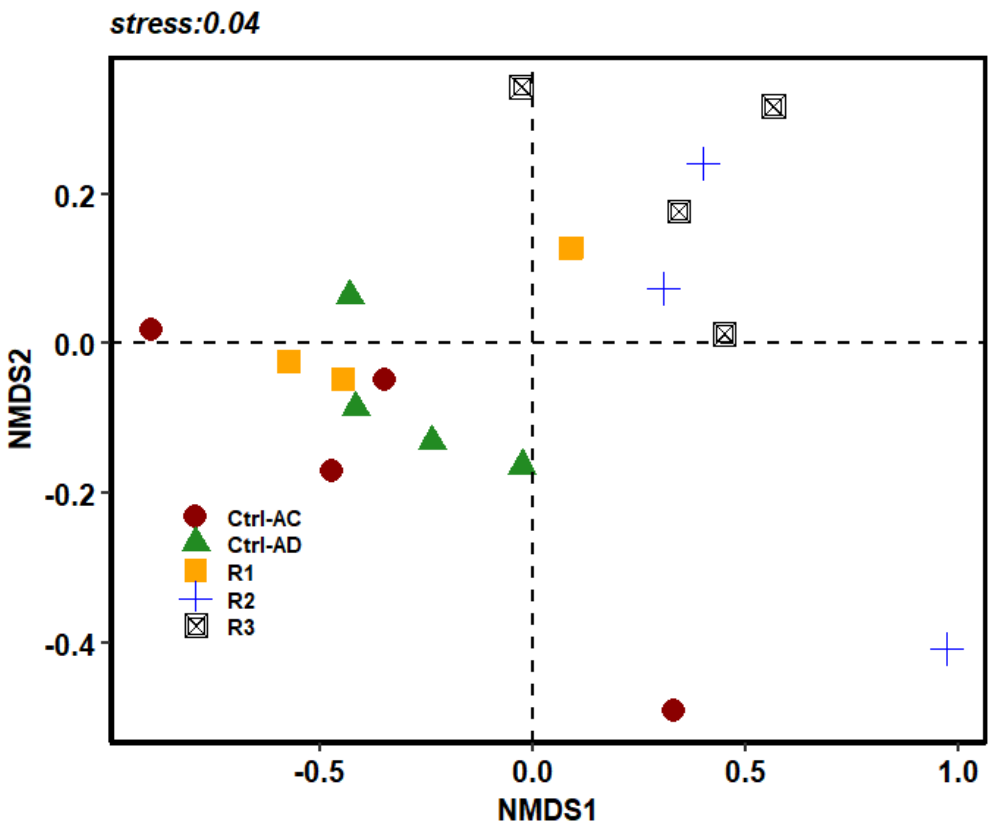

Figure 3.7.4. Beta Diversity of Archaea

3.7.3 Bacterial Community Analysis

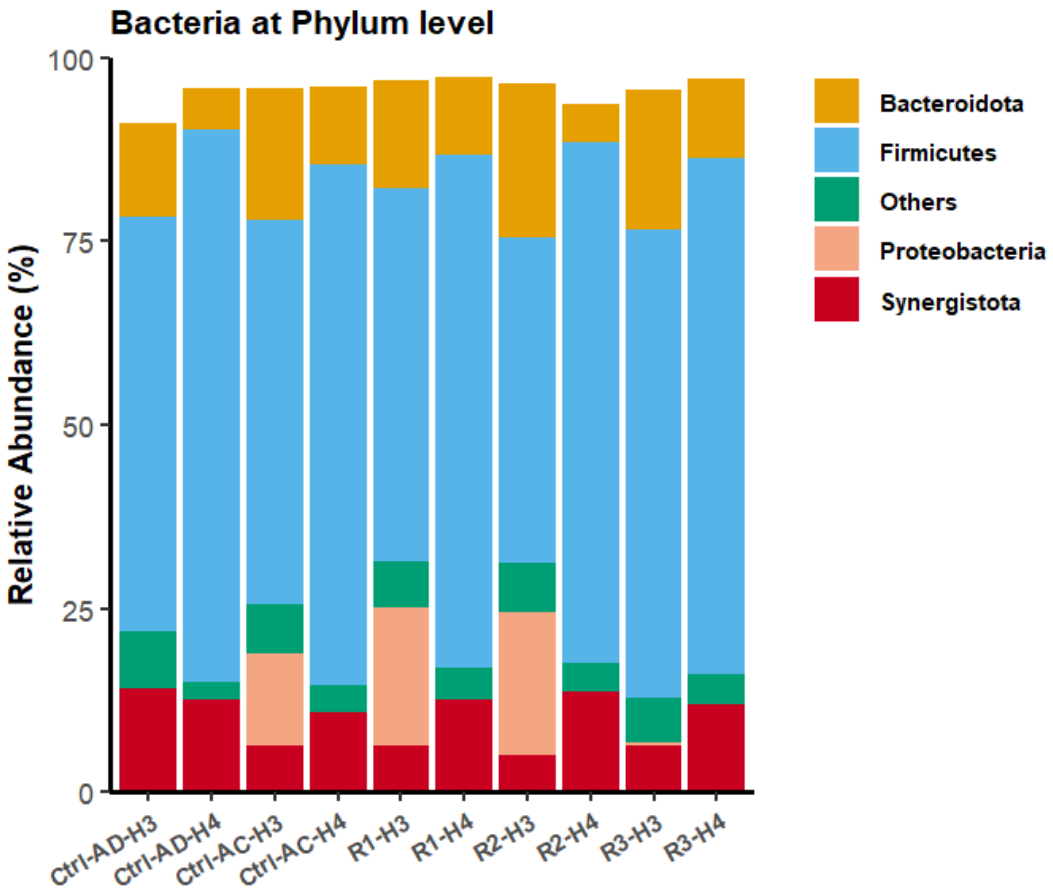

Figure 3.7.5. Phylum-Level Distribution of Bacteria

The primarily dominant bacterial phyla were *Fimicutes*, *Bacteroidota*, *Proteobacteria*, and *Synergistota*. In particular, the *Firmicutes* phylum dominated in all reactors, ranging from 57.56% abundance in bicarbonate reactors to 67.01% in bicarbonate and formate reactors. Its abundance in all reactors increased from HRT 3 to HRT 4. This increase was particularly large in the formate-treated reactors, increasing from 44.6% to 70.86%.

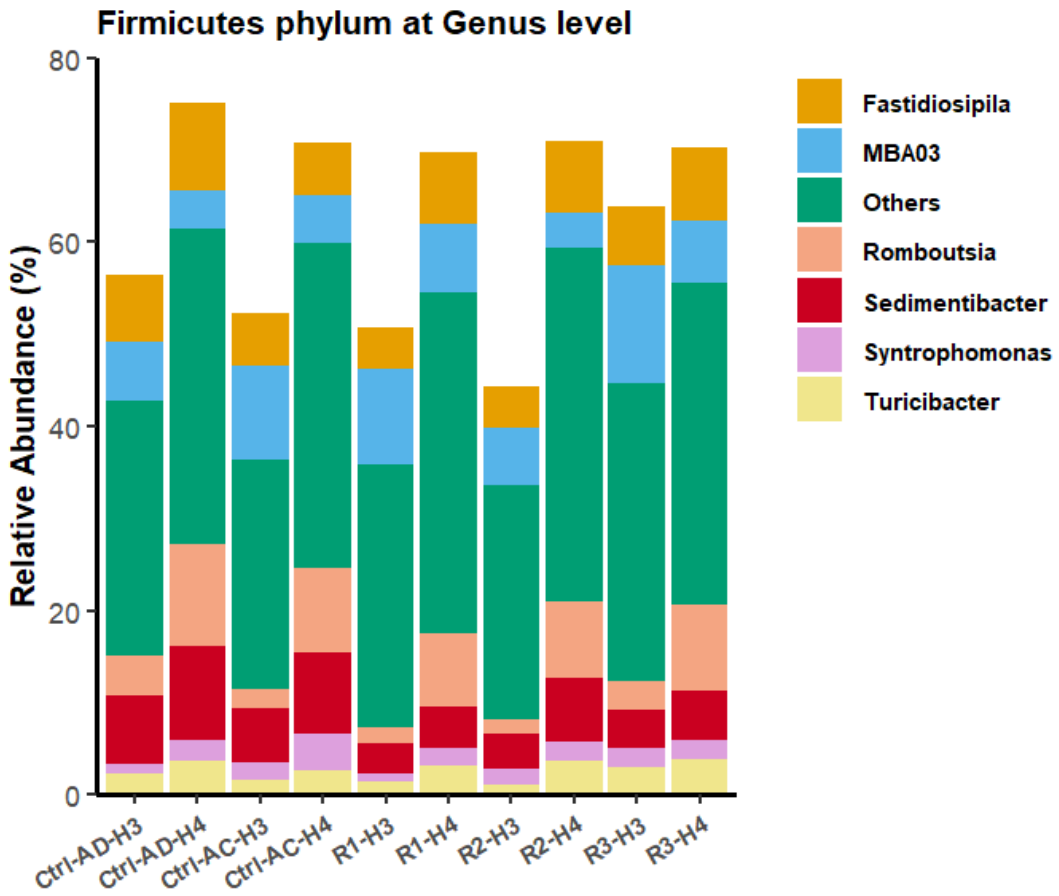

Figure 3.7.6. Genus-Level Distribution of *Firmicutes* Phylum

In contrast to the phylum's dominance, there was no single dominant genus within *Firmicutes*. Only the *Fastidiosipila*, *MBA03*, *Romboutsia*, and *Sedimentibacter* genera exhibited an average realitve abundance greater than 5% across all reactors. Another notable phylum-level change was the increase in abundance of *Synergistota*, which increased in all reactors from HRT 3 to HRT 4, but particularly increased in the formate reactors.

3.7.4 Archaeal Community Analysis

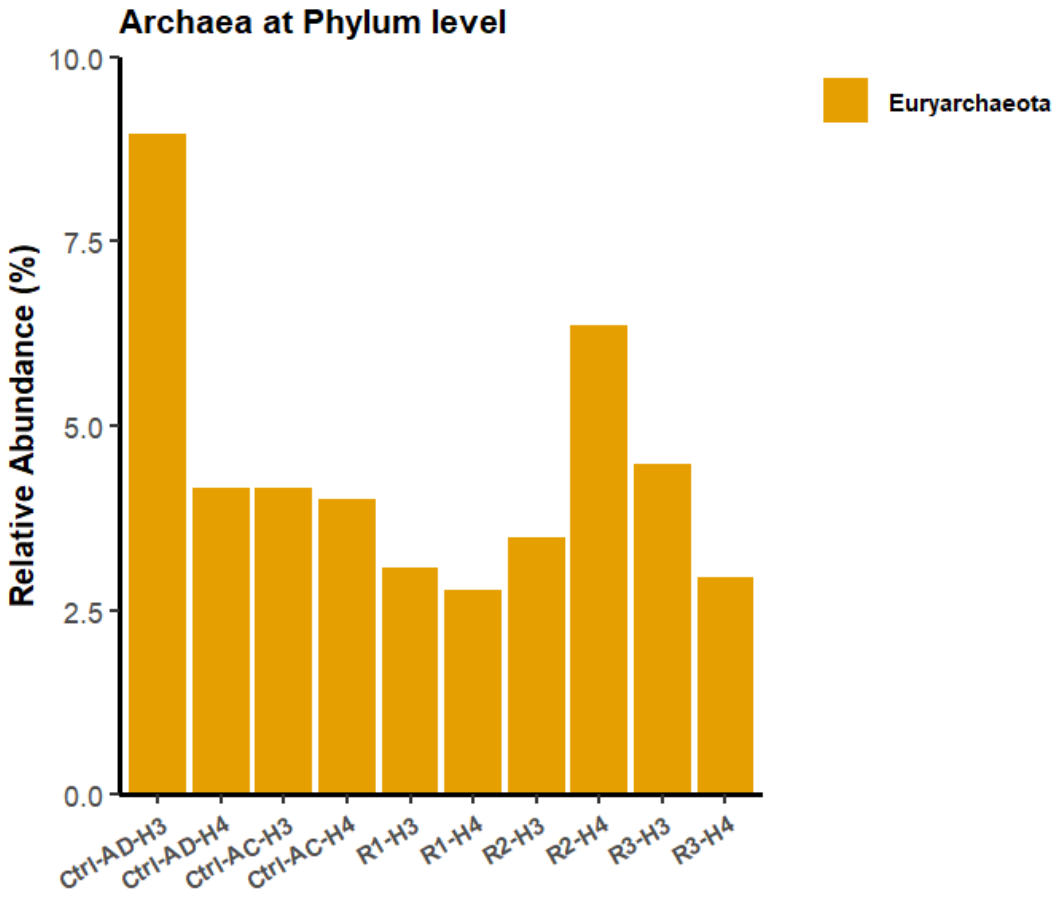

Figure 3.7.7. Phylum-Level Distribution of Archaea

The dominant and in fact only archaeal phylum observed in all reactors was *Euryarchaeota*. In the fourth HRT, where the CH_4 production differences were the most pronounced, there is a notable difference in the abundance of archaea in the formate-enhanced reactors as compared to the other reactors in that HRT. This suggests that formate enhancement of methanogens contributes to increased CH_4 yield.

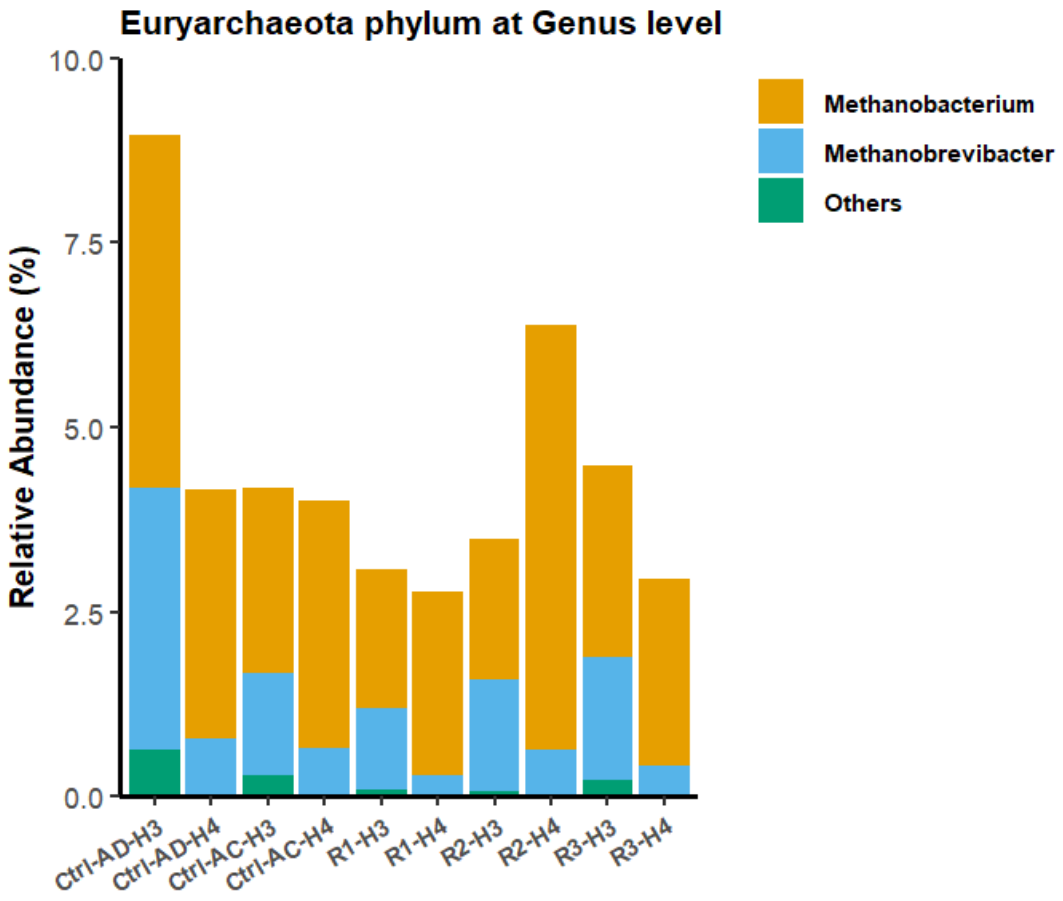

Figure 3.7.8. Genus-Level Distribution of *Euryarchaeota* Phylum

At the genus level, *Methanobacterium*, and *Methanobrevibacter* dominated, with a relative abundance above 95%. In HRT 4, 26.73% of archaea in formate reactors were *Methanobrevibacter*, as compared to 22.99% in the bicarbonate reactors or 25.80% in the formate and bicarbonate reactors. *Methanobrevibacter* contains species capable of formate utilization for methanogenesis. This finding indicates that the presence of formic acid in the reactor enhances the abundance of microbes adapted for the utilization of formate in methanogenesis.

CHAPTER 4: LIFE CYCLE ASSESSMENT

4.1 Introduction

Building on the findings presented in Chapter 3, a comprehensive LCA was conducted to evaluate the environmental performance of a formate-enhanced AD system. In this system, a secondary digestion is performed using the liquid digestate of a primary digester to utilize the residual carbon in the digestate and CO_2 from the primary digester. The LCA aims to assess the environmental impacts associated with implementing formate-enhanced AD to provide insights into its potential as a sustainable, value-added pathway to improve digester performance.

4.1.1 Studied System

Figure 4.1.2 depicts a diagram of the studied system. SCAD produces, on average, 17,081 metric tons/year of liquid digestate, which was used as the feedstock for this study.

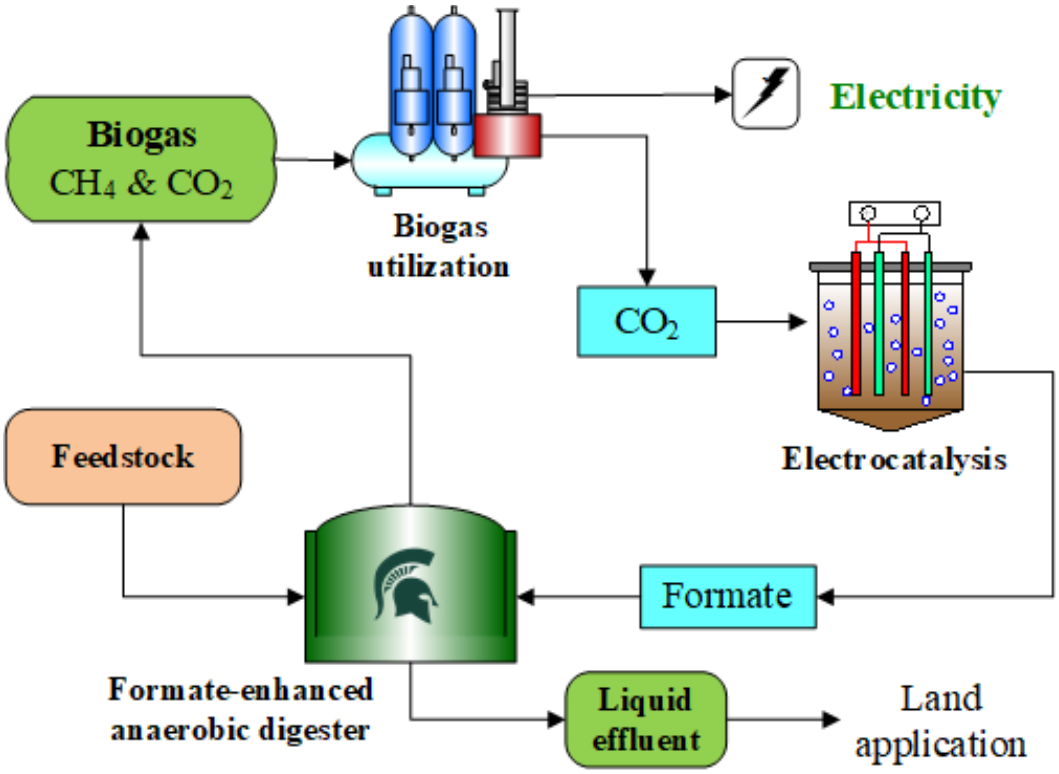

Figure 4.1.1. Diagram of the Studied Formate Digester System

First, the feedstock enters a formate-enhanced AD from SCAD. The digestion produces biogas, a mixture consisting mainly of CH_4 and CO_2. The biogas is directed to a biogas utilization unit that combusts CH_4 to generate electricity. The flue gas from biogas combustion is then fed into an electrocatalysis unit that converts CO_2 into formic acid using electricity generated biogas consumption. The formic acid is recycled back into the formate-enhanced digester to stimulate methanogenesis, enhancing the carbon conversion efficiency. Activated carbon is added during digester start-up based on the results from Chapter 3. The system produces a liquid effluent which is used in land application for nutrient recycling.

4.1.2 Goal and Scope

The goal of this LCA is to determine the environmental impacts of formate-enhanced anaerobic digester operation. To assess the potential environmental benefits and/or trade-offs of this enhanced configuration, two control scenarios were selected for comparison based on experimental setups described in Chapter 3. AD alone served as the baseline system, while AD with activated carbon served as an enhancement control for comparison to the formate-enhanced configuration. The bicarbonate and bicarbonate-formate reactors were not evaluated. While the digestion was not unsuccessful, the performance of the bicarbonate-treated reactors was not deemed to exceed the activated carbon reactors enough to be considered for full-scale estimation. Similarly, the performance of the bicarbonate-formate reactor was lower than formate alone, and therefore not considered for full-scale.

The scope of the LCA includes all major processes directly related to digestion, biogas production and utilization, formate production via electrocatalysis, and digestate management. Environmental burdens from upstream or unrelated processes, such as feedstock generation, infrastructure construction, and unrelated agricultural activities, are excluded from the system boundary. This LCA aims to provide a system-level understanding of how formate addition as a strategy for CO_2 valorization impacts the mass and energy balances, greenhouse gas emissions, and environmental sustainability as compared to conventional AD configurations.

4.2 Mass and Energy Balance

The mass balance analysis compares the formate-enhanced AD system to the control, a conventional AD system, and the enhanced control, a conventional AD system using activated carbon. All three systems use liquid digestate effluent from MSU's SCAD as feedstock, with a total amount of 17,081 tons/year. The characteristics of AD filtrate are 4.1% TS, 3.0% VS, 26,625 mg/kg TC, 53,380 mg/kg COD, 3,293 mg/kg TN, and 509 mg/kg TP, with those values based on SCAD operational data. The outputs of the mass balance were biogas production, energy usage and production, and effluent characteristics, which were quantified to evaluate digester performance and implications for environmental impacts. Figure 4.2.1 displays the mass balance for the formate-enhanced system.

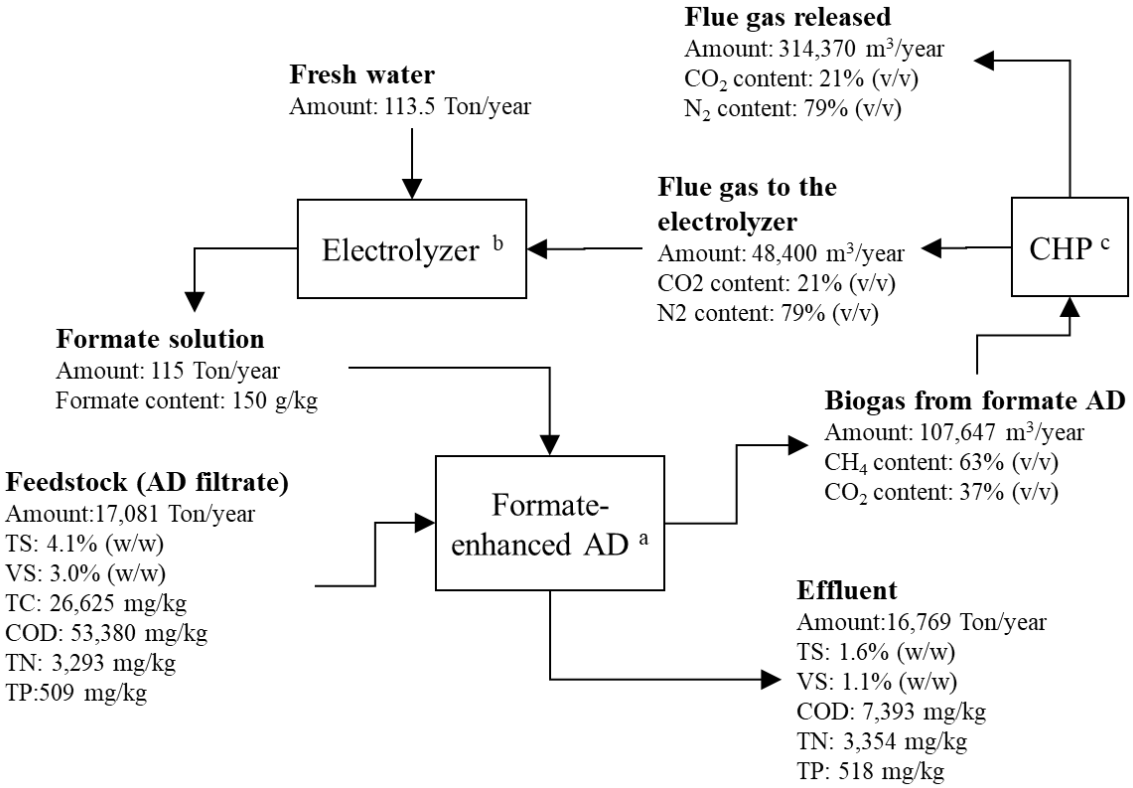

Figure 4.2.1. Mass Balance of the Formate-Enhanced AD System

a. The data used for the mass balance is from the 4th HRT. The formate-enhanced AD has a retention time of 20 days.
b. The electrolyzer runs 10 hours/day with a flow rate of 46 m^3/hr. The formic acid concentration generated from electrolyzer is 150 g/kg solution (*Complete 5 Cm2 Electrolyzer to Convert CO2 to Formic Acid | Dioxide Materials*, n.d.). The AD has a retention time of 20 days.
c. The lambda value for the CHP engine is 1.

In the formate-enhanced AD system model, 150 g additional formate per kg digestate was introduced into the digester via electrocatalytic conversion of captured CO_2 from flue gas. A total of 115 tons/year of formate solution was generated using 113.5 tons/year of water and 48,400 m^3/year of flue gas containing 21% CO_2. This system achieved the highest biogas production among all three systems, producing 107,647 m^3 biogas/year, consisting of 63% CH_4 and 37% CO_2. The effluent volume was 16,769 tons/year, with reduced TS (1.6%), VS (1.1%) and COD (7,393 mg/kg), indicating effective solids reduction. The flue gas output was 314,370 m^3/year, from which 48,400 m^3/year was diverted to the electrolyzer. Overall, the formate-enhanced AD model demonstrated improved carbon utilization and enhanced CH_4 yield.

Figure 4.2.2 shows the mass balance on a system with added activated carbon, but without formate addition. The activated carbon addition in this model slightly improved digestion performance relative to the control.

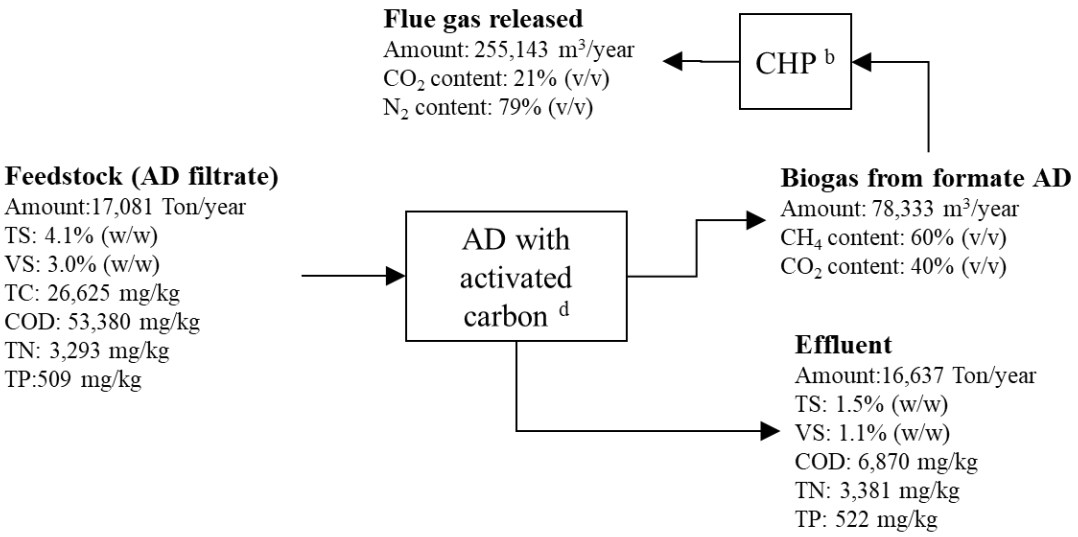

Figure 4.2.2. Mass Balance of the Activated Carbon AD System

b. The lambda value for the CHP engine is 1.
d. The data used for the mass balance is from the 4th HRT. The AD has a retention time of 20 days. Activated carbon was added at the beginning of the AD.

The biogas yield reached 78,333 m^3/year, with a CH_4 concentration of 60%, similar to the control configuration. Effluent output was 16,637 tons/year, with TS of 1.5% and VS of 1.1%. COD was reduced to 6,870 mg/kg. Flue gas release totaled 255,143 m^3/year, lower than in the formate-enhanced system, due to lower total biogas production. Although activated carbon facilitated improved CH_4 production, the enhancement was limited compared to the formate-enhanced digestion.

The final mass balance was performed on an AD with no additives using digester effluent as feedstock, which is shown in Figure 4.2.3.

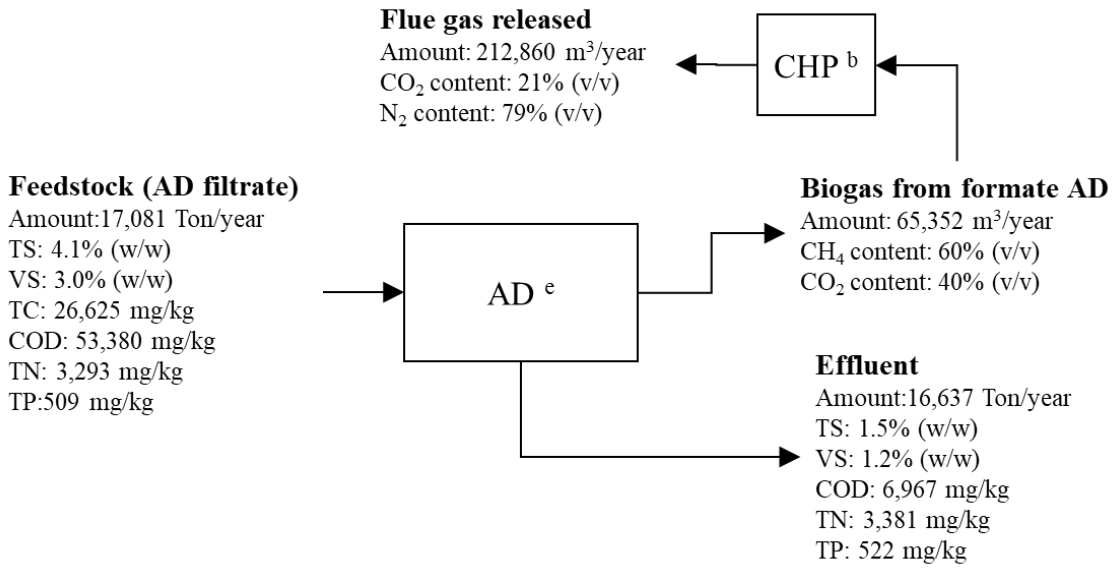

Figure 4.2.3. Mass Balance of the Conventional AD System

b. The lambda value for the CHP engine is 1.
e. The data used for the mass balance is from the 4th HRT. The AD has a retention time of 20 days. No additives were used.

The standard AD system produced the lowest biogas volume at 65,352 m^3/year, with a CH4 content of 60%. The effluent profile was comparable to that of the AD with activated carbon, with TS of 1.5%, VS of 1.2%, and COD of 6,967 mg/kg, indicating relatively less efficient organic degradation compared to formate-added digestion. Flue gas emissions were the lowest among the three systems at 212,860 m^3/year, attributed to lower CH_4 production and combustion activity.

The mass balance model data show that the formate-enhanced system outperformed the other configurations in terms of CH_4 yield and carbon recovery, demonstrating the value of recirculating CO_2 as a substrate via electrolyzation to formate. The integration of electrocatalysis enables a partial carbon loop closure, improving energy efficiency and potentially providing a beneficial effect on emissions. Activated carbon

showed moderate benefits but did not significantly impact effluent quality or CH_4 content compared to the control.

Following the mass balance results, an energy balance analysis was conducted to evaluate the energy performance of the formate-enhanced AD, AD with activated carbon, and conventional AD systems. Table 4.2.1 summarizes the energy inputs and outputs associated with each system, including electricity and heat requirements for system operation and energy recovery from combined heat and power (CHP) units.

Table 4.2.1. Summary of Energy Balances

Unit operation	Formate-enhanced AD	Control AD with activated carbon	Control AD
Electrolyzer energy input			
Electricity input (kWh-e/year)	-8,083	-	-
AD energy input			
Heat input (W_{heat}, kWh-e/year) [b]	-236,159	-236,159	-236,159
Electricity input ($W_{electricity}$, kWh-e/year) [c]	-135,504	-135,504	-135,504
CHP energy output from the formate AD			
Energy output as heat (Eheat, kWh-e/year) d	404,645	280,434	233,960
Energy output as electricity (Eelectricity, kWh-e/year) e	236,043	163,586	136,477
Net energy output			
Net heat output (kWh-e/year) f	168,487	44,275	-2,199
Net electricity output (kWh-e/year) g	92,456	28,082	972

a. Negative numbers indicate energy inputs; positive numbers indicate energy outputs.
b. Eq. 2.7.1 was used to calculate the heat input.
c. Eq. 2.7.2 was used to calculate the electricity input.
d. An annual biogas production of 1,323,757 m3 with 65% (v/v) of CH4 was used to calculate the energy content of the biogas. The LHV of CH4 is 35.8 MJ/m3 CH4. The thermal conversion efficiency of the CHP unit is 65%.
e. The electricity output is the metered number of the digestion operation.
f. Net heat output = Eheat - Wheat
g. Net electricity output = Eelectricity - Welectricity

In the formate-enhanced AD system, additional energy input is required for the electrolyzer to perform CO_2 to formic acid conversion. This unit consumed 8,083 kWh-e/year, which is the only difference in energy demand among the three systems. All three systems required the same operational energy inputs for digestion, which was 236,159 kWh-e/year of thermal energy to maintain thermophilic conditions and 135,504 kWh-e/year of electricity to power the operation, including pumps, mixers, and other auxiliary equipment. While the formate-enhanced system had slightly higher total energy input due to the electrolyzer, it generated significantly more recoverable energy than either of the control systems because of its higher biogas yield. Specifically, it produced 404,645 kWh-e/year of heat and 236,043 kWh-e/year of electricity from the CHP unit. Comparatively, the AD with activated carbon yielded 280,434 kWh-e/year of heat and 163,586 kWh-e/year of electricity, while the conventional AD produced the lowest outputs, at 233,960 kWh-e/year of heat and 136,477 kWh-e/year of electricity. These data reflect the proportional increase in CH_4 production seen in the mass balance data, as increased CH_4 production leads directly to increased energy production from combustion.

The most meaningful metric of the system's energy efficiency is the net energy output, which is calculated by subtracting energy inputs from energy outputs. The formate-enhanced AD achieved the highest net heat output at 168,487 kWh-e/year, and the highest net electricity output, at 92,456 kWh-e/year. The AD-activated carbon system showed moderate improvement at 44,275 kWh-e/year of net heat and 28,082 kWh-e/year of net electricity. The control AD system had a negative net heat output, at −2,199 kWh-e/year, meaning that the heat recovered from the CHP was insufficient to offset the heating energy

required for thermophilic operation. Its net electricity output was marginal, at only 972 kWh-e/year.

These results demonstrate that only the formate-enhanced AD system achieves a substantially positive energy balance, confirming its technical advantage in surplus energy generation and self-sufficiency. The superior CH_4 yield from formate supplementation not only enhances carbon conversion efficiency but also significantly boosts energy recovery. The improved energy balance of the formate-enhanced system suggests a viable pathway for increasing the sustainability of AD operations. Despite the additional electricity demand of the electrolyzer, the increase in energy output—especially electricity—makes the system less dependent on external energy inputs. This may allow excess energy to be exported or used to support adjacent agricultural or industrial operations. Furthermore, the activated carbon system, while offering moderate improvement over the baseline, did not achieve the same level of net energy gains and remained largely dependent on external heating sources.

4.3 Life Cycle Impact Assessment

To evaluate the environmental impacts of the three anaerobic digestion configurations studied, life cycle impact assessment (LCIA) was conducted focusing on two key categories: global warming potential (GWP) and water eutrophication potential (WEP). The systems assessed included formate-enhanced AD, AD with activated carbon, conventional AD, and a reference case involving direct land application of AD filtrate without secondary digestion.

The GWP results are presented in Figure 4.3.1, which breaks down the contributions from CH_4 and nitrous oxide (N_2O) emissions, as well as the GHG offset from energy recovery.

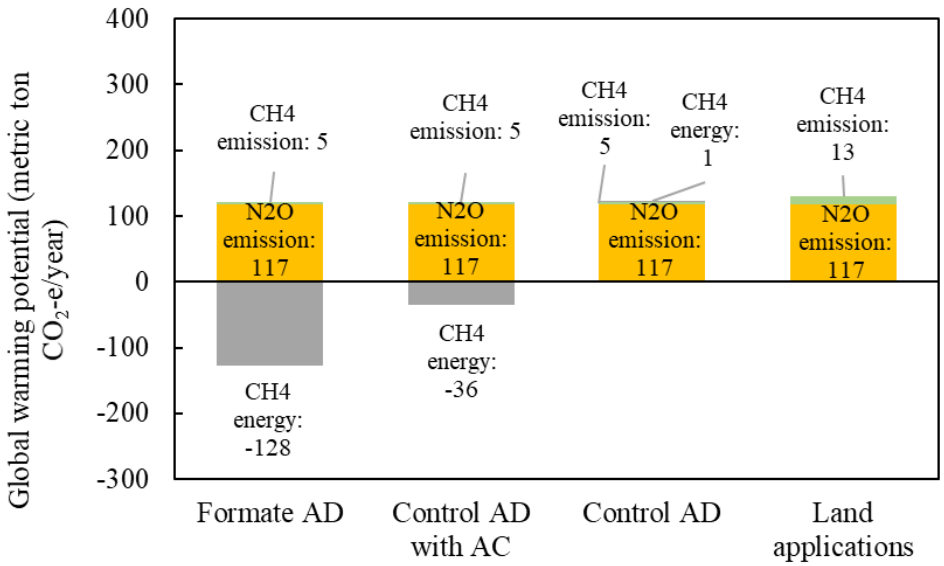

Figure 4.3.1. GWP Contribution Analysis

All three digestion systems resulted in comparable direct CH_4 emissions of approximately 5 metric tons CO_2-e per year, primarily from residual volatile solids in the digestate applied to land. In contrast, the land application of untreated digestate produced the highest CH_4 emissions, reaching 13 metric tons CO_2-e/year due to the absence of organic matter stabilization prior to land application. N_2O emissions were uniformly high across all scenarios involving land application—117 metric tons CO_2-e/year due to the consistent TN content in the effluent and the use of the same emission factor. These emissions are significant and often dominate the climate impact from digestate management. The most notable differences between systems arise from the CH_4 energy credit, which accounts for avoided emissions resulting from renewable electricity and heat

generation. The formate-enhanced AD system provided the largest GHG offset, with an energy credit of –128 metric tons CO_2-e/year due to its enhanced biogas yield and greater CH_4 recovery. The AD with activated carbon yielded a smaller credit of –36 metric tons CO_2-e/year, while the conventional AD system contributed minimally, with just -1 metric tons CO_2-e/year. As a result, the formate-enhanced system was the only one to achieve net-negative GWP, effectively compensating for all CH_4 and N_2O emissions through natural gas energy replacement. In contrast, the conventional AD system and direct land application scenario had net-positive GHG emissions, emphasizing the superior climate impacts of the formate-enhanced system.

Figure 4.3.2 presents the results for WEP, again comparing formate added-AD, activated carbon AD, untreated AD, and direct application of digestate without treatment.

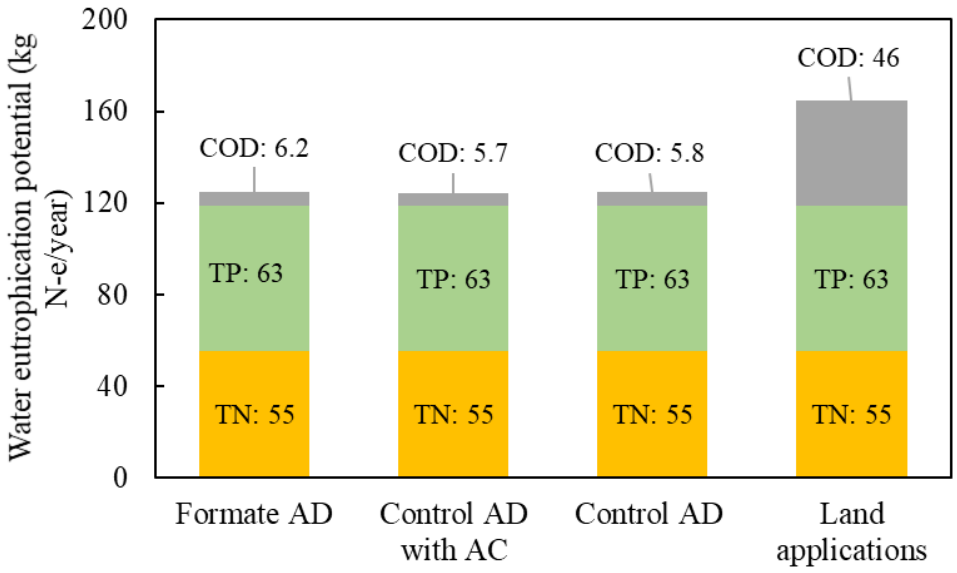

Figure 4.3.2. WEP Contribution Analysis

Across all digestion systems, the contributions from TN and TP remained consistent at 55 kg N-e/year and 63 kg N-e/year respectively, due to similar nutrient profiles in the final

effluent. The key difference was observed in the contribution of COD, which reflects the organic matter content of the effluent. The formate-enhanced AD system exhibited the lowest COD-related eutrophication potential at 6.2 kg N-e/year, followed by AD with activated carbon at 5.7 kg N-e/year, and conventional AD at 5.8 kg N-e/year. Land application of untreated digestate resulted in a significantly higher COD contribution of 46 kg N-e/year due to the lack of prior stabilization and degradation of organic matter.

These findings confirm that all anaerobic digestion systems offer environmental advantages over direct land application, particularly in reducing eutrophication risks. Among them, the formate-enhanced AD system demonstrates the best overall environmental performance, achieving both the lowest net GWP and WEP. This improvement is primarily attributed to the dual benefits of CO_2 utilization via formate recycling and enhanced CH_4 production. The activated carbon system, while offering some benefit over the control, does not deliver the same reduction of environmental impacts. In summary, the LCIA results support the conclusion that formate-enhanced AD not only improves energy recovery but also offers clear climate and water quality benefits, making it a promising strategy for sustainable waste-to-energy conversion.

4.4 Carbon Efficiency

To further compare the three operating conditions, the carbon efficiency of the reactors was evaluated. The carbon efficiency is expressed as the percentage of total carbon loaded that was converted into biogas. Figure 4.4.1 shows the carbon efficiency of the three situations based on the mass balance.

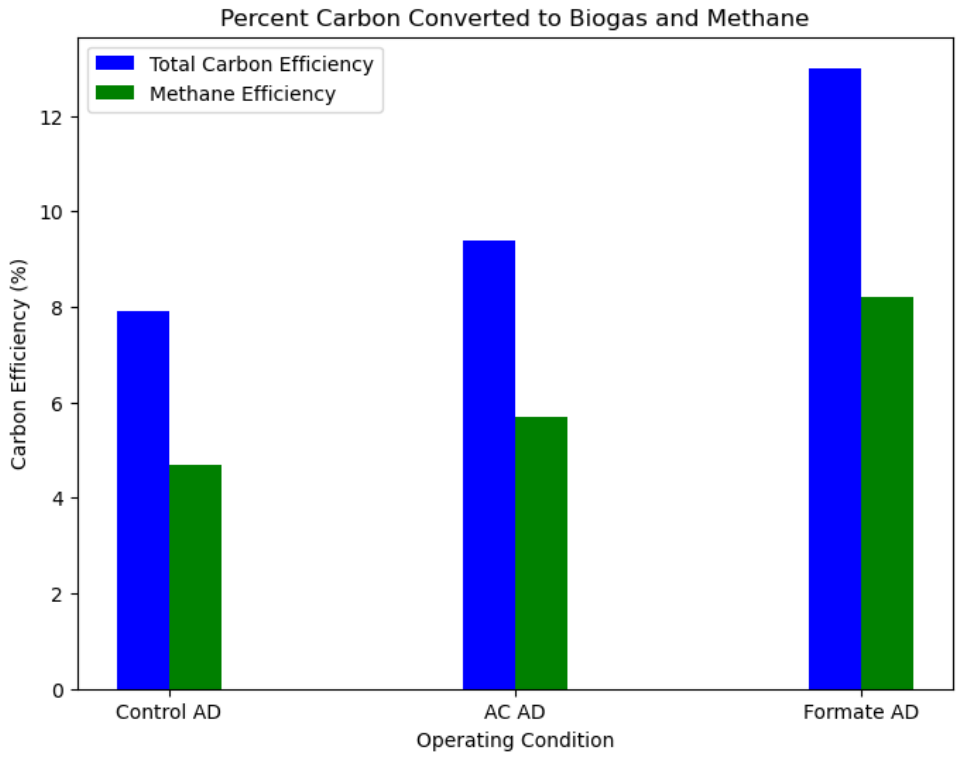

Figure 4.4.1. Carbon Efficiencies Based on Mass Balance

The figure shows both the efficiency for the entire biogas and the efficiency for methane only. The carbon efficiency is higher in the activated carbon AD compared to the control and further increased with formate enhancement. Comparing the control and the activated carbon treatment, the carbon efficiency is increased by 21% when comparing methane content. The formate-enhanced digester, when comparing methane content, shows a 76% carbon efficiency increase as compared to control. This result has significant implications for the viability of digester operations, as the effective number of cattle needed to ensure digester viability would be lowered by this increase in carbon efficiency.

CHAPTER 5: CONCLUSIONS

5.1 Findings

The study found that there was evidence to support a significant increase in biogas production when reactors were treated with formic acid. The mean biogas production between reactors with activated carbon and formic acid was approximately 21% greater than reactors treated only with activated carbon, and ~47% greater than reactors treated with neither activated carbon or formic acid. This was determined to be a statistically significant increase when equal variances and normality were assumed. The addition of sodium bicarbonate resulted in a 4% increase in biogas yield, which was not determined to be statistically significant under any assumptions. The combination of formate and bicarbonate resulted in an 8.8% increase in total gas production as compared to activated carbon only reactors and a 10% decrease in total gas production compared to the formate reactors. The addition of formic acid had a significant enhancement effect on gas production, while bicarbonate had a minimal or even detrimental effect on the ability of the anerobic microbes to metabolize the feedstock.

The concentration of formic acid measured largely did not vary in the different feeding conditions, and most samples had concentrations in the 1-3 mg/L range. However, two feeding conditions were receiving feedstock with a 100 mg/L formic acid concentration. This means that the reactors that received This means that the reactors that received formic acid were able to fully metabolize the formic acid from the feedstock. Further, reactors treated with formic acid showed lower concentrations of propionic,

butyric, isobutyric, and isovaleric acid, indicating a more efficient metabolism of VFAs when formate was present.

The microbial community analysis indicated a higher abundance of formate-scavenging organisms in formic acid reactors. This suggests that the microbial community could adapt under selective pressure to more effectively utilize formate, which is further reinforced by the lack of increased formic acid concentration in reactors treated with formic acid. Archaea abundance was shown to be enhanced by the addition of formic acid.

The LCA demonstrated that a system using formate-enhanced digestion would be capable of significantly reducing both GWP and WEP impacts of AD operation. Further, the energy efficiency of such a system was shown to be superior to a standard two-stage digester operation, as well as improving carbon efficiency and utilization. This suggests that formate-enhanced digestion as a viable strategy for improving anaerobic digester operation.

5.2 Recommendations for Future Work

Although the formate study presented in Chapter 2 of this thesis found that there was evidence to support the positive impact of formate addition on digester performance, it is unlikely that said findings would constitute sufficient evidence to motivate implementation of formate addition to full-scale digester operations. Further, existing data on formate addition remains sparse at the time of writing. Additional studies on the effects of formate addition to digesters, incorporating a variety of feedstocks, reactor conditions, additives, and formate concentrations, are a much-needed step to bring this technology to full-scale fruition. In specific, the author recommends that future studies perform

experiments with a greater number of biological replicates (n >= 3). A study that includes data from three or more reactors will have greater statistical power and be able to utilize tests for normality and variance more effectively. More detailed monitoring of effluent and influent COD and total solids data should be performed in future studies, as the suggested COD and TS reductions used in the mass balance are overly efficient, and evoke some skepticism. Additionally, studies on feedstocks other than liquid food waste/manure co-digestate would provide vital insight into the effects of formic acid on other cultures. Of particular utility would be a study on formic acid treatment for reactors fed with manure and/or food waste directly. Provided that future bench-scale studies support the viability of formate addition, pilot-scale studies should be performed to ensure that the technology remains viable as it approaches full-scale. This work was conducted in 500 mL bottles, and is in no way guaranteed to perform similarly at increased volumes.

The LCA portion would benefit from future work involving results from pilot-scale studies on codigestion operations treated with formic acid. Such results would be an important step in determining potential full-scale viability. Additionally, it may be possible that a more nutrient-rich environment could take on more formic acid addition, allowing further reductions GHG emissions and low- or negative-carbon energy production. A full review of the process's economic viability and optimization of that viability should also be conducted to determine the technology's fitness for commercial implementation. A comprehensive review of available electrocatalysis technology and other details of the formate-enhanced system could bring the technology into economic viability, providing

great environmental and financial benefits to the AD waste treatment and energy production system.

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APPENDIX A: DETAILED STATISTICAL RESULTS

Table 6.1. ANOVA Results Summary

Comparison	ANOVA Test	P-value	Significant?
Total gas production	Ordinary	0.0114	Yes
Gas quality	Ordinary	1.65e-5	Yes
Total gas production	Kruskal-Wallis	0.08148	No
Gas quality	Kruskal-Wallis	0.06829	No
pH, HRT 4	Ordinary	0.00134	Yes
pH, HRT 4	Kruskal-Wallis	0.07798	No
COD, HRT 4	Ordinary	6.1e-08	Yes
COD, HRT 4	Kruskal-Wallis	0.06829	No
Total Solids, HRT 4	Ordinary	2.98e-05	Yes
Total Solids, HRT 4	Kruskal-Wallis	0.09705	No
Volatile Solids, HRT 4	Ordinary	0.000312	Yes
Volatile Solids, HRT 4	Kruskal-Wallis	0.09292	No

Table 6.2. Tukey's HSD on Total Biogas Production Ordinary ANOVA Results Summary

Comparison	P-value	Significant?
Bicarbonate vs AC	0.96	No
C+F vs AC	0.65	No
Control vs AC	0.15	No
Formate vs AC	0.084	No
C+F vs Bicarbonate	0.94	No
Control vs Bicarbonate	0.077	No
Formate vs Bicarbonate	0.17	No
Control vs C+F	0.038	Yes
Formate vs C+F	0.36	No
Formate vs Control	0.007	Yes

Table 6.3. Tukey's HSD on Biogas Quality Ordinary ANOVA Results Summary

Comparison	P-value	Significant?
Bicarbonate vs AC	0.000022	Yes
C+F vs AC	0.00043	Yes
Control vs AC	0.00018	Yes
Formate vs AC	0.48	No
C+F vs Bicarbonate	0.00078	Yes
Control vs Bicarbonate	0.0026	Yes
Formate vs Bicarbonate	0.000029	Yes

Table 6.3 (cont'd)

Control vs C+F	0.27	No
Formate vs C+F	0.00093	Yes
Formate vs Control	0.00034	Yes

Table 6.4. Tukey's HSD on pH Ordinary ANOVA Results Summary

Comparison	P-value	Significant?
Bicarbonate vs AC	0.0044	Yes
C+F vs AC	0.038	Yes
Control vs AC	0.017	Yes
Formate vs AC	0.52	No
C+F vs Bicarbonate	0.16	No
Control vs Bicarbonate	0.42	No
Formate vs Bicarbonate	0.0017	Yes
Control vs C+F	0.87	No
Formate vs C+F	0.0098	Yes
Formate vs Control	0.0051	Yes

Table 6.5. Tukey's HSD on COD Ordinary ANOVA Results Summary

Comparison	P-value	Significant?
Bicarbonate vs AC	0.0000005	Yes
C+F vs AC	0.0000005	Yes

Table 6.5 (cont'd)

Control vs AC	0.16	No
Formate vs AC	0.00011	Yes
C+F vs Bicarbonate	0.107	No
Control vs Bicarbonate	0.0000005	Yes
Formate vs Bicarbonate	0.0000011	Yes
Control vs C+F	0.0000006	Yes
Formate vs C+F	0.0000018	Yes
Formate vs Control	0.00032	Yes

Table 6.6. Tukey's HSD on Total Solids Ordinary ANOVA Results Summary

Comparison	P-value	Significant?
Bicarbonate vs AC	0.000109	Yes
C+F vs AC	0.00012	Yes
Control vs AC	0.997	No
Formate vs AC	0.797	No
C+F vs Bicarbonate	0.995	No
Control vs Bicarbonate	0.00012	Yes
Formate vs Bicarbonate	0.00016	Yes
Control vs C+F	0.00014	Yes
Formate vs C+F	0.00018	Yes

Table 6.6 (cont'd)

Formate vs Control	0.92	No

Table 6.7. Tukey's HSD on Volatile Solids Ordinary ANOVA Results Summary

Comparison	P-value	Significant?
Bicarbonate vs AC	0.0012	Yes
C+F vs AC	0.0014	Yes
Control vs AC	0.49	No
Formate vs AC	0.94	No
C+F vs Bicarbonate	0.994	No
Control vs Bicarbonate	0.00296	Yes
Formate vs Bicarbonate	0.0008	Yes
Control vs C+F	0.0037	Yes
Formate vs C+F	0.00097	Yes
Formate vs Control	0.23	No

APPENDIX B: PYTHON AND R CODE

B.1 Grouped Bar Charts Generation (COD, TS, VS, GC)

```
#plot to generate COD data

import numpy as np

import matplotlib.pyplot as plt

def generate_grouped_bar_chart():

  # Define parameters

  num_groups = 4

  num_bars_per_group = 5

  bar_width = 0.15  # Width of individual bars

  # Manually set all data values to 1 without using np.ones or a loop

  data = [14860, 9213.4, 11236.7,

9750,10916.7],[9610,8415,10075,10770,10315],[6566.7,5243.4,6493.4,5280,6666.7],[696

6.666667,6870,8846.666667,7393.333333,8736.666667]

   # Define the positions of the groups on the x-axis

  group_positions = np.arange(num_groups)

  # Define colors and labels for the bars

  colors = ['b', 'g', 'r', 'c', 'm']

  labels = ['Control', 'Activated Carbon', 'Bicarbonate', 'Formate', 'Bicarbonate + Formate']

  # Create figure and axis

  fig, ax = plt.subplots(figsize=(8, 6))

  # Plot each bar in the group
```

```
    for i in range(num_bars_per_group):
        ax.bar(group_positions + i * bar_width, [row[i] for row in data], width=bar_width,
color=colors[i], label=labels[i])
    # Formatting
    ax.set_xlabel('HRT')
    ax.set_ylabel('COD (mg/L)')
    ax.set_title('Mean COD per HRT')
    ax.set_xticks(group_positions + (num_bars_per_group - 1) * bar_width / 2)
    ax.set_xticklabels(['HRT 1', 'HRT 2', 'HRT 3', 'HRT 4'])
    ax.legend()
    # Show plot
    plt.show()
# Run the function
generate_grouped_bar_chart()
```

Line Chart Generation (Cumulative Gas Production)

```
import numpy as np
import matplotlib.pyplot as plt
import pandas as pd
# Load data from CSV file
data = pd.read_csv('formate_gas_prod.csv',header=None)
# Generate x values
x = data.iloc[0, :]
```

```
# Define y values for each line
y1 = data.iloc[1, :]  # Adjust column indices as needed
y2 = data.iloc[2, :]
y3 = data.iloc[3, :]
y4 = data.iloc[4, :]
y5 = data.iloc[5, :]
y6 = data.iloc[6, :]
y7 = data.iloc[7, :]
y8 = data.iloc[8, :]
y9 = data.iloc[9, :]
y10 = data.iloc[10, :]
plt.figure(figsize=(15, 10), dpi=600)
# Plot each line separately
plt.plot(x, y1, label='Control 1')
plt.plot(x, y2, label='Control 2')
plt.plot(x, y3, label='AC 1')
plt.plot(x, y4, label='AC 2')
plt.plot(x, y5, label='Bicarbonate 1')
plt.plot(x, y6, label='Bicarbonate 2')
plt.plot(x, y7, label='Formate 1')
plt.plot(x, y8, label='Formate 2')
plt.plot(x, y9, label='C+F 1')
```

```
plt.plot(x, y10, label='C+F 2')

# Add title and labels

plt.title('Cumulative Gas Production')

plt.xlabel('Days of Operation')

plt.ylabel('Total Biogas (mL)')

plt.legend()

plt.grid(True)

# Show the plot

plt.show()
```

5.2.1 B.2 Ordinary ANOVA and Tukey's HSD Test R Code

```
pH <- read.csv(file = "ph_data.csv", header = TRUE, sep = ",")

pH

reactor <- c(rep('Control', 2),rep('AC', 2),rep('Bicarb', 2),rep('Formate', 2),rep('C+F', 2))

pH_4 <-c(pH$Control, pH$AC, pH$Bicarb, pH$Formate, pH$C.F)

df_pH <- data.frame(reactor, pH_4)

ph.aov <- aov(pH_4 ~ reactor, data = df_pH)

summary(ph.aov)

cod_4 <- c(cod$Control, cod$AC, cod$Bicarb, cod$Formate, cod$C.F)

df_cod <- data.frame(reactor, cod_4)

cod.aov <- aov(cod_4 ~ reactor, data = df_cod)

summary(cod.aov)

ts_4 <- c(ts$Control, ts$AC, ts$Bicarb, ts$Formate, ts$C.F)
```

```
df_ts <- data.frame(reactor, ts_4)

ts.aov <- aov(ts_4 ~ reactor, data = df_ts)

summary(ts.aov)

vs_4 <- c(vs$Control, vs$AC, vs$Bicarb, vs$Formate, vs$C.F)

df_vs <- data.frame(reactor, vs_4)

vs.aov <- aov(vs_4 ~ reactor, data = df_vs)

summary(vs.aov)

TukeyHSD(ph.aov)

TukeyHSD(cod.aov)

TukeyHSD(ts.aov)

TukeyHSD(vs.aov)
```

5.2.2 B.3 Kruskal-Wallis Test R Code

```
#arranging the data for each measurement

gasProd <- read.csv(file = "gas_prod_data.csv", header = TRUE, sep = ",")

print(gasProd)

gasConc <- read.csv(file = "gas_conc_data.csv", header = TRUE, sep = ",")

print(gasConc)

pH <- read.csv(file = "ph_data.csv", header = TRUE, sep = ",")

print(pH)

#

cod <- read.csv(file = "cod_data.csv", header = TRUE, sep = ",")

print(cod)
```

```
ts <- read.csv(file = "ts_data.csv", header = TRUE, sep = ",")

print(ts)

vs <- read.csv(file = "vs_data.csv", header = TRUE, sep = ",")

print(vs)

# run KW test for each - if p < a, reject the null

kwTest <- kruskal.test(gasProd)

kwTest

kwTest_conc <- kruskal.test(gasConc)

kwTest_conc

kwTest_pH <-kruskal.test(pH)

kwTest_pH

kwTest_cod <-kruskal.test(cod)

kwTest_cod

kwTest_ts <-kruskal.test(ts)

kwTest_ts

kwTest_vs <-kruskal.test(vs)

kwTest_vs
```

Table 6.8. Biogas Production Values

Reactor	Final Biogas Production (mL)
Control 1	9365
Control 2	9600
Activated Carbon 1	11105
Activated Cabon 2	11890
Bicarbonate 1	11800
Bicarbonate 2	12125
Formate 1	14470
Formate 2	13350
Bicarbonate/Formate 1	11635
Bicarbonate/Formate 2	13310

Table 6.9. Mean Biogas Quality Values

HRT	**Reactor**	**Mean Value**
1	Control	0.58
	Activated Carbon	0.59
	Bicarbonate	0.49
	Formate	0.61
	Bicarbonate + Formate	0.57
2	Control	0.52

Table 6.9 (cont'd)

	Activated Carbon	0.56
	Bicarbonate	0.56
	Formate	0.58
	Bicarbonate + Formate	0.54
3	Control	0.51
	Activated Carbon	0.53
	Bicarbonate	0.50
	Formate	0.54
	Bicarbonate + Formate	0.51
4	Control	0.60
	Activated Carbon	0.63
	Bicarbonate	0.59
	Formate	0.60
	Bicarbonate + Formate	0.60

Table 6.10. Methane Production per HRT (mL)

	Control	AC	Bicarbonate	Formate	Formate + Bicarbonate
HRT-1	1221	1654	1603	2173	1847
HRT-2	1369	1890	1806	1930	1708
HRT-3	1434	1739	1443	1939	1706
HRT-4	1149	1383	1507	1983	1662

Table 6.10 (cont'd)

Total CH_4	**5172**	**6666**	**6358**	**8025**	**6923**
Percent Increase vs Control	**28.88%**	**22.94%**	**55.17%**	**33.86%**	

Table 6.11. Complete Table of pH Results

	Control	AC	Bicarbonate	Formate	Formate + Bicarbonate
Day 6	8.06	8.15	7.87	7.95	7.97
Day 13	8.53	8.38	8.60	8.43	8.55
Day 20	8.66	8.50	8.69	8.46	8.58
Day 27	8.43	8.32	8.50	8.38	8.26
Day 34	8.26	8.14	8.37	8.06	8.24
Day 40	8.22	8.07	8.35	8.09	8.23
Day 48	8.07	7.97	8.20	7.95	8.10
Day 55	8.17	8.07	8.30	8.01	8.20
Day 64	8.18	8.05	8.28	7.97	8.18
Day 69	8.13	7.90	8.14	7.86	8.04

Table 6.11 (cont'd)

Day 76	8.19	8.01	8.27	7.96	8.18

Table 6.12. Mean pH Measurement Values per HRT

HRT	Reactor	Mean Value
1	Control	8.42
	Activated Carbon	8.34
	Bicarbonate	8.38
	Formate	8.28
	Bicarbonate + Formate	8.37
2	Control	8.35
	Activated Carbon	8.23
	Bicarbonate	8.44
	Formate	8.22
	Bicarbonate + Formate	8.25
3	Control	8.15
	Activated Carbon	8.04
	Bicarbonate	8.28
	Formate	8.02
	Bicarbonate + Formate	8.18
4	Control	8.17

Table 6.12 (cont'd)

	Activated Carbon	7.98
	Bicarbonate	8.23
	Formate	7.93
	Bicarbonate + Formate	8.13

Table 6.13. Complete Table of COD Results (mg/L)

	Control	AC	Bicarbonate	Formate	Formate + Bicarbonate
Day 6	14490	8040	10030	9420	9730
Day 13	12360	7000	8420	7500	8500
Day 20	17730	12600	15260	12330	14520
Day 27	9820	7430	8850	9740	7730
Day 34	9400	9400	11300	11800	12900
Day 40	6910	5120	6450	5230	6010
Day 48	7110	5650	6730	5800	7630
Day 55	5680	4960	6300	4810	6360
Day 64	7010	6540	7350	7120	8300
Day 69	7430	7280	9800	7740	9260
Day 76	6460	6790	9390	7320	8650

Table 6.14. Mean COD Measurement Values

HRT	**Reactor**	**Mean Value**

Table 6.14 (cont'd)

1	Control	14860
	Activated Carbon	9210
	Bicarbonate	11240
	Formate	9750
	Bicarbonate + Formate	10920
2	Control	9610
	Activated Carbon	8420
	Bicarbonate	10080
	Formate	10770
	Bicarbonate + Formate	10320
3	Control	6570
	Activated Carbon	5340
	Bicarbonate	6490
	Formate	5280
	Bicarbonate + Formate	6670
4	Control	6970
	Activated Carbon	6870
	Bicarbonate	8850
	Formate	7390
	Bicarbonate + Formate	8740

Table 6.15. Complete Table of Total Solids Results (mg/L)

	Control	AC	Bicarbonate	Formate	Formate + Bicarbonate
Day 6	19155	16045	18355	17890	19150
Day 13	15950	15170	18995	16540	17370
Day 20	14915	14020	16200	14415	16105
Day 27	14720	13970	17345	16925	18120
Day 34	14035	13310	17210	14730	17295
Day 40	12350	12640	15375	13305	16285
Day 48	14155	12440	16560	12330	17000
Day 55	14725	13630	18070	13795	18675
Day 64	19985	17465	28270	18460	27855
Day 69	11910	13355	17840	14180	17795
Day 76	14700	15395	19405	14960	19400

Table 6.16. Mean Total Solids Value Measurements per HRT

HRT	**Reactor**	**Mean Value**
1	Control	16.67
	Activated Carbon	15.08
	Bicarbonate	17.85
	Formate	16.28
	Bicarbonate + Formate	17.54
2	Control	14.38

Table 6.16 (cont'd)

	Activated Carbon	13.64
	Bicarbonate	17.28
	Formate	15.83
	Bicarbonate + Formate	17.71
3	Control	13.74
	Activated Carbon	12.90
	Bicarbonate	16.67
	Formate	23.24
	Bicarbonate + Formate	27.32
4	Control	15.53
	Activated Carbon	15.41
	Bicarbonate	21.84
	Formate	15.87
	Bicarbonate + Formate	21.68

Table 6.17. Complete Table of Volatile Solids Results

	Control	AC	Bicarbonate	Formate	Formate + Bicarbonate
Day 6	19155	16045	18355	17890	19150
Day 13	15950	15170	18995	16540	17370
Day 20	14915	14020	16200	14415	16105
Day 27	14720	13970	17345	16925	18120

Table 6.17 (cont'd)

Day 34	14035	13310	17210	14730	17295
Day 40	12350	12640	15375	13305	16285
Day 48	14155	12440	16560	12330	17000
Day 55	14725	13630	18070	13795	18675
Day 64	19985	17465	28270	18460	27855
Day 69	11910	13355	17840	14180	17795
Day 76	14700	15395	19405	14960	19400

Table 6.18. Mean VS Measurement Values per HRT

HRT	Reactor	Mean Value
1	Control	10.45
	Activated Carbon	9.25
	Bicarbonate	10.87
	Formate	10.33
	Bicarbonate + Formate	10.84
2	Control	8.23
	Activated Carbon	7.70
	Bicarbonate	9.19
	Formate	8.61
	Bicarbonate + Formate	8.53
3	Control	7.55

Table 6.18 (cont'd)

	Activated Carbon	6.12
	Bicarbonate	7.43
	Formate	6.67
	Bicarbonate + Formate	7.29
4	Control	11.63
	Activated Carbon	11.05
	Bicarbonate	14.27
	Formate	10.80
	Bicarbonate + Formate	14.14

Table 6.19. Average Formic Acid Concentrations

HRT	Reactor	Measurement (mg/L)
2	Control	2.58
	Activated Carbon	2.48
	Bicarbonate	8.01
	Formate	2.55
	Bicarbonate + Formate	2.34
3	Control	2.57
	Activated Carbon	1.55
	Bicarbonate	1.28
	Formate	2.50

Table 6.19 (cont'd)

	Bicarbonate + Formate	2.39
4	Control	2.46
	Activated Carbon	1.14
	Bicarbonate	1.88
	Formate	0.930
	Bicarbonate + Formate	2.11

Table 6.20. Average Acetic Acid Concentration

HRT	Reactor	Measurement (mg/L)
2	Control	963
	Activated Carbon	851
	Bicarbonate	778
	Formate	735
	Bicarbonate + Formate	718
3	Control	456
	Activated Carbon	445
	Bicarbonate	433
	Formate	416
	Bicarbonate + Formate	403
4	Control	240

Table 6.20 (cont'd)

	Activated Carbon	265
	Bicarbonate	252
	Formate	253
	Bicarbonate + Formate	236

Table 6.21. Mean Propionic Acid Concentrations

HRT	Reactor	Measurement (mg/L)
2	Control	324
	Activated Carbon	276
	Bicarbonate	276
	Formate	247
	Bicarbonate + Formate	219
3	Control	96.8
	Activated Carbon	103
	Bicarbonate	104
	Formate	94.8
	Bicarbonate + Formate	80.0
4	Control	60.2
	Activated Carbon	55.3
	Bicarbonate	68.2
	Formate	20.7

Table 6.21 (cont'd)

	Bicarbonate + Formate	20.0

Table 6.22. Mean Butyric Acid Concentrations

HRT	Reactor	Measurement (mg/L)
2	Control	576
	Activated Carbon	527
	Bicarbonate	490
	Formate	451
	Bicarbonate + Formate	458
3	Control	294
	Activated Carbon	290
	Bicarbonate	285
	Formate	271
	Bicarbonate + Formate	274
4	Control	237
	Activated Carbon	224
	Bicarbonate	227
	Formate	164
	Bicarbonate + Formate	155

Table 6.23. Mean Isobutyric Acid Concentrations

HRT	Reactor	Measurement (mg/L)
2	Control	198
	Activated Carbon	174
	Bicarbonate	164
	Formate	150
	Bicarbonate + Formate	138
3	Control	59.2
	Activated Carbon	43.4
	Bicarbonate	38.7
	Formate	44.6
	Bicarbonate + Formate	33.4
4	Control	29.9
	Activated Carbon	18.2
	Bicarbonate	19.4
	Formate	0
	Bicarbonate + Formate	0

Table 6.24. Mean Isovaleric Acid Concentration

HRT	Reactor	Measurement (mg/L)
2	Control	256
	Activated Carbon	226

Table 6.24 (cont'd)

	Bicarbonate	212
	Formate	191
	Bicarbonate + Formate	196
3	Control	83.0
	Activated Carbon	86.4
	Bicarbonate	72.8
	Formate	76.8
	Bicarbonate + Formate	75.5
4	Control	45.6
	Activated Carbon	39.4
	Bicarbonate	45.6
	Formate	10.2
	Bicarbonate + Formate	2.04

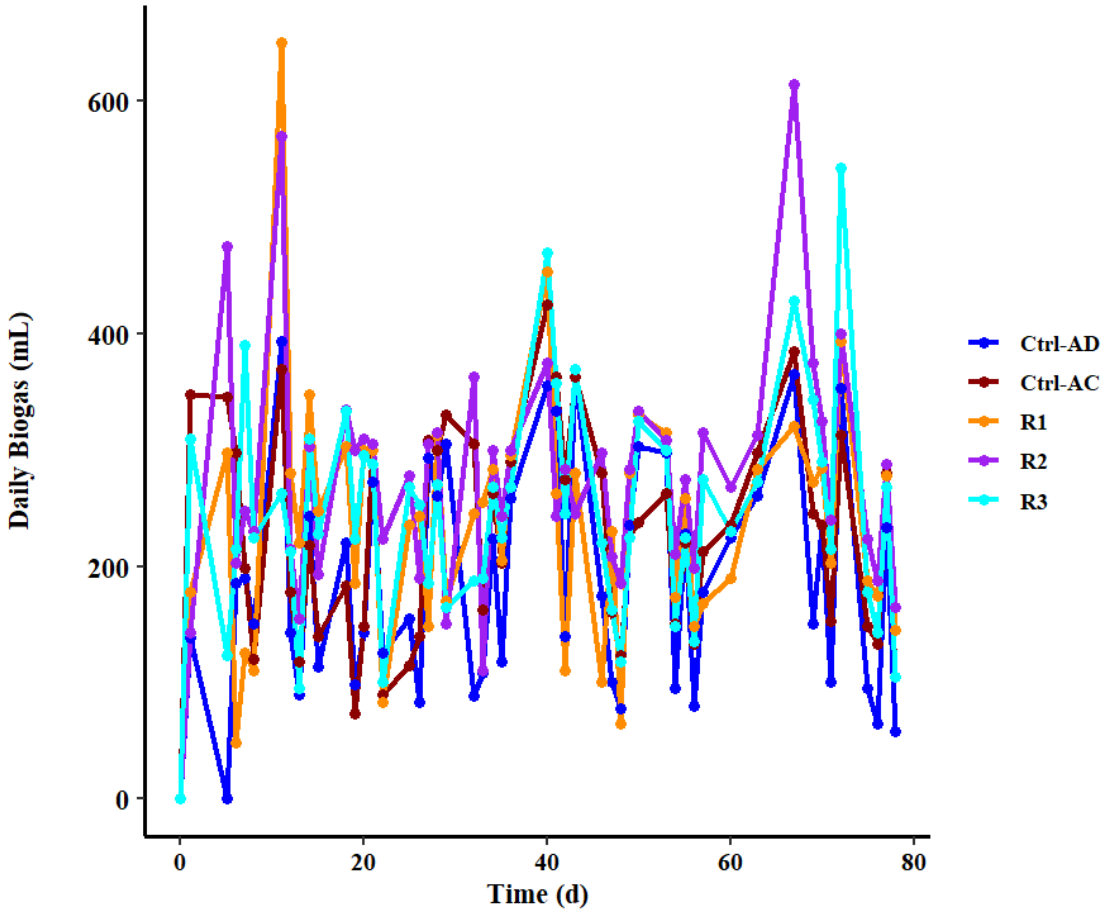

Figure 5.2.1. Average Daily Biogas Production Rate

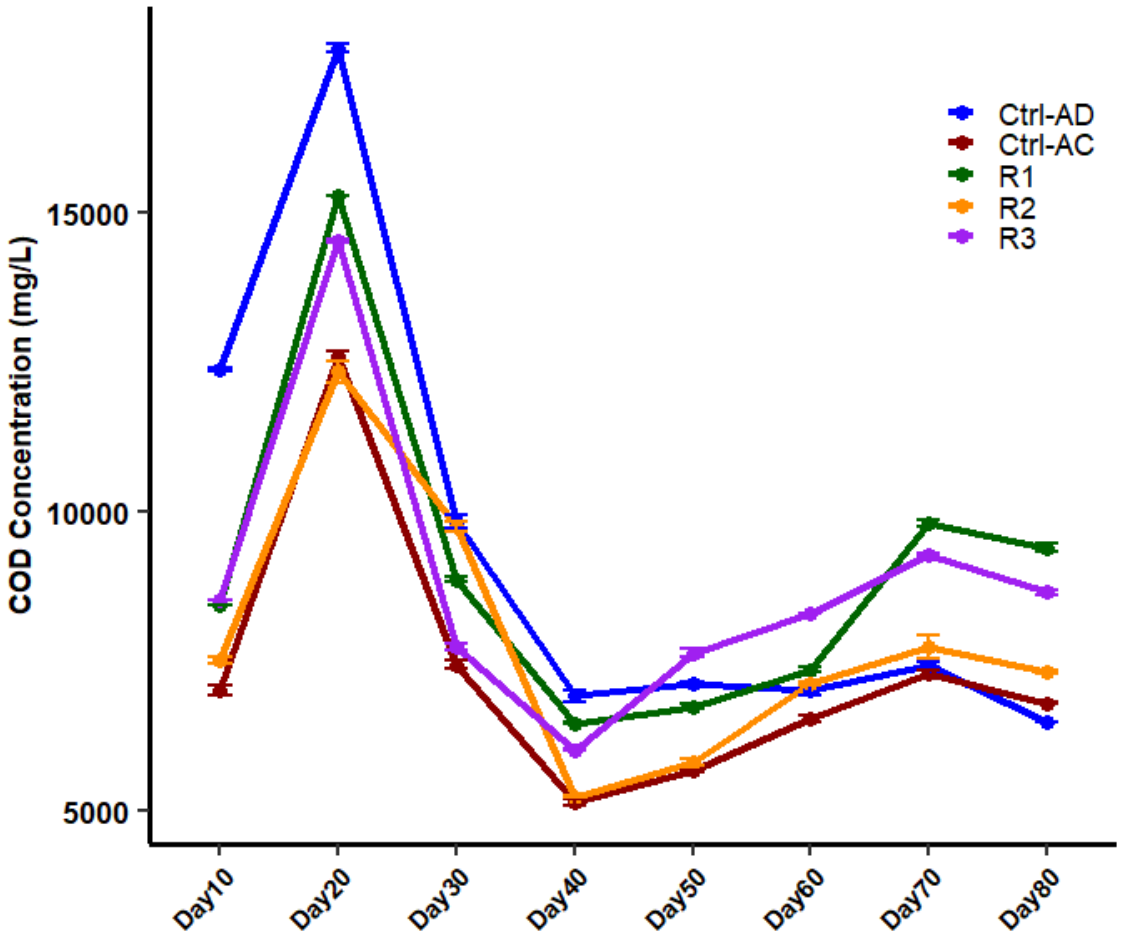

Figure 5.2.2. Average Daily COD Measurements

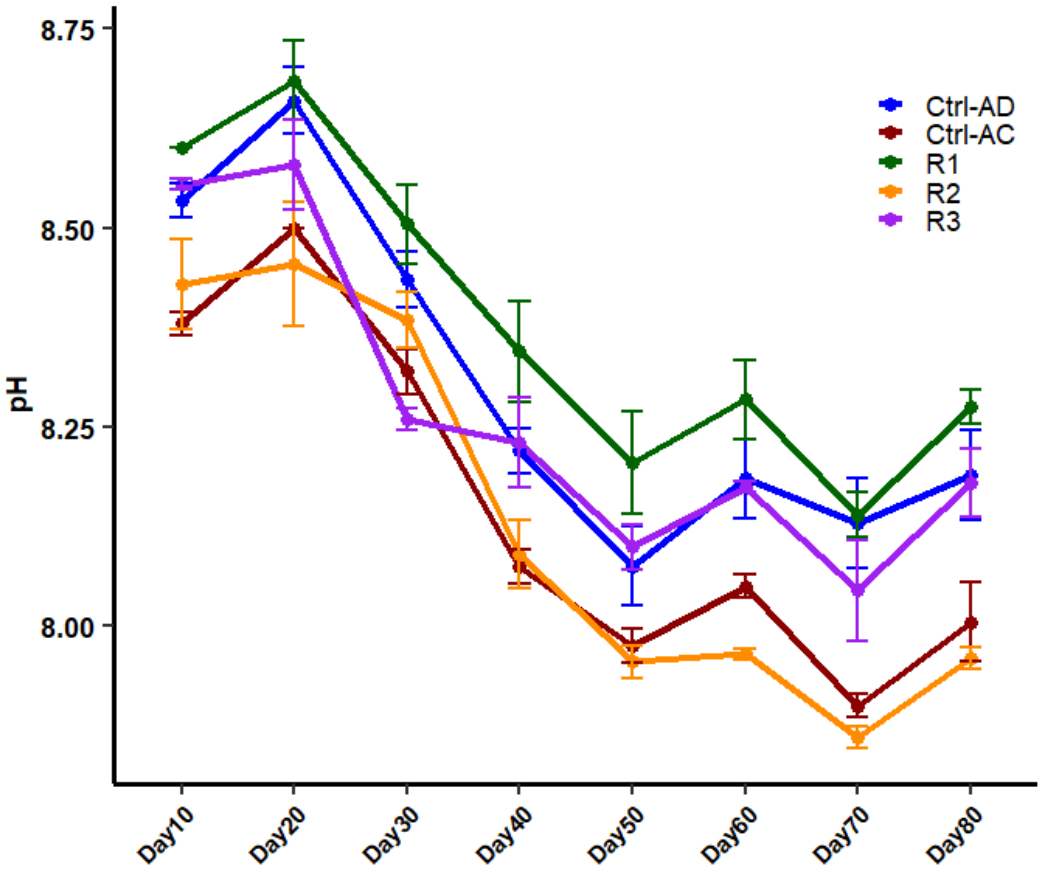

Figure 5.2.3. Average Daily pH Measurements

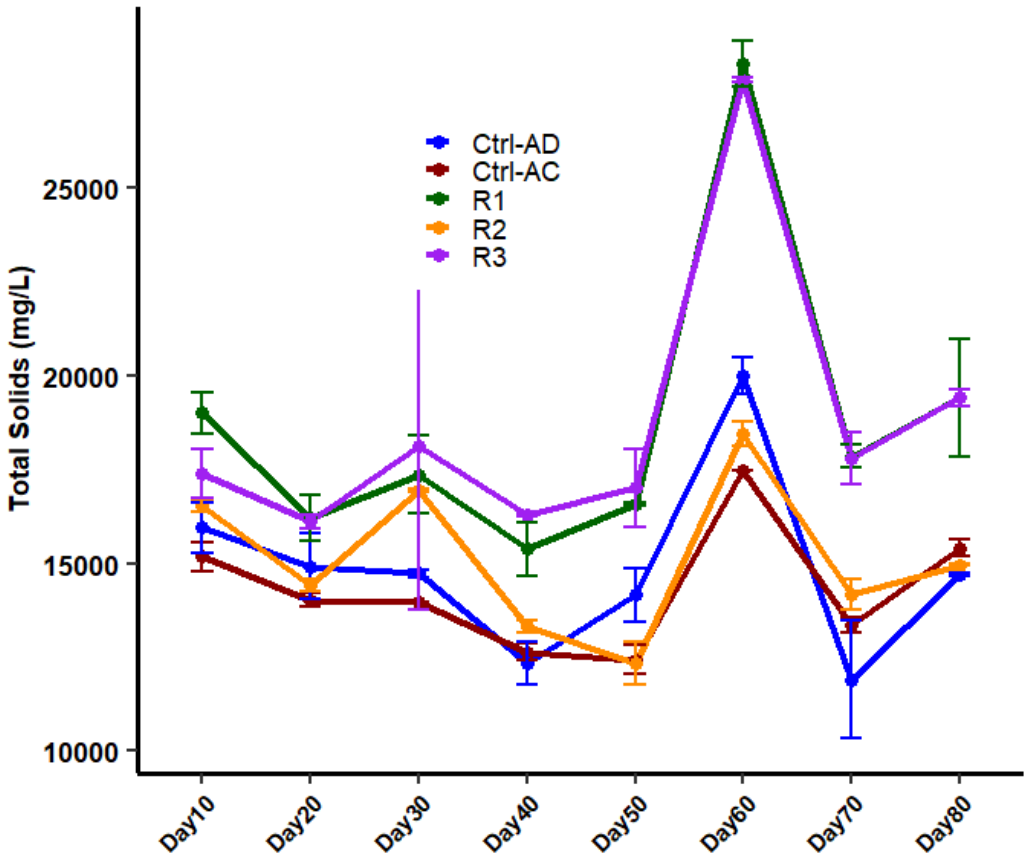

Figure 5.2.4. Average Daily Total Solids Measurements

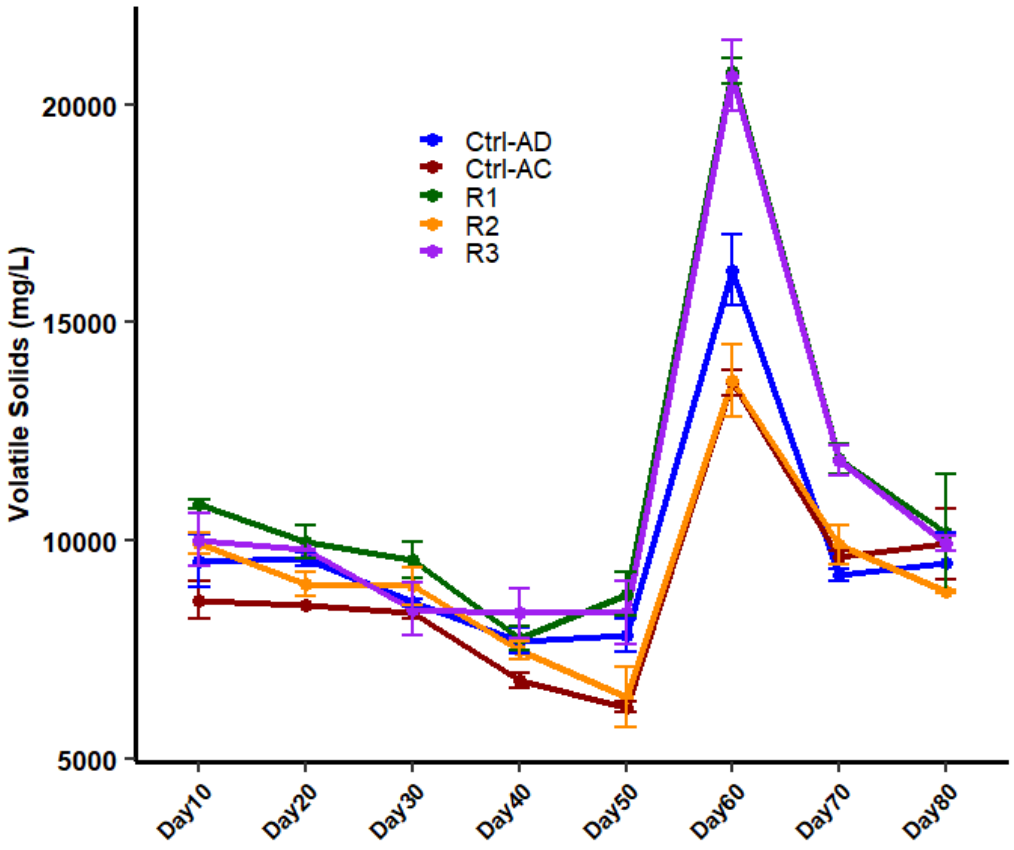

Figure 5.2.5. Average Daily Volatile Solids Measurements