Two Stage Anaerobic Digestion of Orange Peels

A Major Qualifying Project Report submitted to the Faculty of WORCESTER POLYTECHNIC INSTITUTE In partial fulfillment of the requirements for the Degree of Bachelor of Science in CHEMICAL ENGINEERING

22 March 2019

Submitted By:

Alexis Zoffreo

Project Advisor:

Professor Michael Timko

Table of Contents

1. Abstract

Rising energy costs, stronger environmental regulations, and increased public awareness of climate change has pushed industries to pursue renewable means of energy production. Anaerobic digestion is an effective method of obtaining energy in the form of biogas (mainly hydrogen and methane) from various waste products. Brazil is the largest exporter of orange juice in the world and exported 1.2 million tons of orange juice in 2012[1]. About half of the orange is discarded as waste during juice production including the seeds, peels, and pulp. Therefore, a significant economic and environmental benefit could be realized by repurposing these byproducts. The objective of this pilot experiment was to determine the feasibility of implementing two stage anaerobic digestion of orange peels for use in the fruit juice industry.

The reactors in this experiment were fed in a semi-batch configuration and were sampled every 3-4 days. Samples were analyzed for Total Solids, Volatile Solids, COD, Ammonia, and Alkalinity. The performance of the reactor was evaluated based on these parameters in addition to biogas composition and volume.

The findings from this experiment indicated a successful pilot trial for the methanogenic reactor which produced a high methane and gas volume yield. The VS and COD reduction percentages in both reactors were also promising. However, the acidogenic reactor produced extremely low amounts of hydrogen far less than those expected. High spikes of oxygen alternating with carbon dioxide spikes were observed during the start-up period of the experiment. Alkalinity levels in the acidogenic reactor indicated insufficient buffering capacity in the acidogenic reactor. Additionally, the ammonia levels in both reactors were very low which could have contributed to the low hydrogen production in the acidogenic reactor.

A possible solution to improve the performance of the system could be to mix the orange peel substrate with another substance. Codigestion could improve alkalinity and ammonia levels in both reactors in part by correcting the high C/N ratio of the orange peel substrate. Alternatively, the acidogenic reactor could be operated at a slightly higher pH to boost the alkalinity and attempt to better stabilize the operating conditions.

2. Introduction

Food Waste is an important issue facing many developed and developing countries. It it is estimated by the World Resources Institute (WRI) Brazil, that the country produces 41,000 tons

---

[1] Jeffrey T. Lewis, In Brazil, Farmers Ripping Out Orange Trees, *Wall Street Journal* (June 13, 2013)

of food waste per year.[2] There is a large orange juice industry in Sao Paulo and food waste including orange peels from processing facilities is currently being dumped into landfills. Maria Paula et al. seek to evaluate the possibility of using anaerobic digestion to produce methane and hydrogen from the byproducts of orange juice production that would otherwise go to waste. Nathia-Neves et al. previously studied and published findings on two-stage anaerobic co-digestion under mesophilic (moderate) conditions used to produce hydrogen and methane from restaurant food waste and vinasse (a byproduct of the ethanol industry). The goal of this experiment was to apply a similar method using a substrate of orange peels.

Anaerobic digestion includes multiple biological processes where microorganisms break down organic matter in the absence of oxygen. The main product of value produced from this process is biogas. Biogas consists of mainly methane and hydrogen with a small amount of CO_2. It has a high heat content and can be used as fuel for plant boilers in industry. Additionally, anaerobic digestion produces a nutrient rich digestate or "AD effluent" that is useful for soil enrichment. There are no waste products of anaerobic digestion as every end product has a beneficial use. There are three stages of anaerobic digestion including hydrolysis, acidogenesis, and methanogenesis. Hydrogen is generated during the hydrolytic and acidogenic stages while methane is generated during the methanogenesis stage.

In single stage anaerobic treatment, all three steps of the degradation reaction occur in the same reactor. In a two stage anaerobic digester, the hydrolysis step occurs in the first reactor and the acetogenesis and methanogenesis steps occur in the second reactor. This is achieved by altering the conditions of the reactors to make them more favorable to the specific types of bacteria that catalyze the desired reaction.

The objective of this project is to evaluate the feasibility of implementing two stage anaerobic digestion in the fruit industry by analyzing how much methane and hydrogen can be generated, and energy produced.

3. Background

3.1 Anaerobic Digestion

Anaerobic digestion is a natural process where organic matter is broken down by microorganisms without oxygen. Common materials used in anaerobic digestion include animal manure, food wastes, grease and oils, and sewage sludge. The biogas that is produced from anaerobic digestion can be used to power engines and produce heat and electricity. Low quality biogas can be used in internal combustion engines with a relatively low efficiency. However, biogas can be scrubbed of carbon dioxide and other trace contaminants to increase its value and to prepare for use in high efficiency engines. The digestate or anaerobic digestion effluent can be

---

[2] "A receipt to reduce food waste"https://wribrasil.org.br/en/blog/2017/09/receipt-reduce-food-loss-and-waste

repurposed as fertilizer, soil amendments, and livestock bedding. Anaerobic digestion tends to be extremely cost effective due to the relatively large amount of energy that is able to be produced and its low environmental impact.

3.1.1 Chemical Process

There are four steps in anaerobic digestion. These include hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The hydrolysis reaction can be written as Equation 1 using the approximate chemical formula $C_6H_{10}O_4$ for the mixture of organic waste. In hydrolysis, extracellular enzymes that are produced by hydrolytic microorganisms such as amylase, cellulase, lipase and protease break down organic polymers into elementary soluble monomers. The resulting organic compounds from the hydrolysis reaction include amino acids from proteins, long and short chain fatty acids from lipids, and monomeric sugars from carbohydrates. The hydrolysis step is regarded as the rate limiting step in anaerobic digestion of solid organic waste and can be written as Equation 1.[3]

In the acidogenic step, the compounds produced by the hydrolysis step are then converted by fermentative acidogenic bacteria to a mixture of volatile fatty acids (VFA's). These VFA's include acetic, propionic, and butyric acids and other minor products such as carbon dioxide and hydrogen sulfide.[4] The acidogenic step is the fastest step in anaerobic digestion and can be written as Equations 2 and 3.

In the acetogenic step, the VFA's produced in the acidogenic step are converted by the acetogenic bacteria to acetate, carbon dioxide, and hydrogen. Various bacteria contribute to acetogenesis including *Syntrophobacter wolinii*, a bacterium that decomposes propionate as well as *Syntrophomonos wolfei*, a bacterium that decomposes butyrate.[5] The acetogenesis reaction can be written as Equation 4.

Lastly, the methanogenic step produces methane gas using various intermediate products from previous steps of anaerobic digestion including carbon dioxide, hydrogen, and acetic acid. The bacteria involved in methanogenesis include *Methanobacterium, Methanobacillus, Methanococcus, and Methanosarcina.*[6] Overall, in the process of anaerobic digestion, these methanogenic bacteria are more sensitive to changes in operational parameters than hydrolytic

---

[3] Adekunle, K. and Okolie, J. (2015) A Review of Biochemical Process of Anaerobic Digestion. *Advances in Bioscience and Biotechnology*, 6, 205-212. doi: 10.4236/abb.2015.63020.

[4] Adekunle, K. and Okolie, J. (2015) A Review of Biochemical Process of Anaerobic Digestion. *Advances in Bioscience and Biotechnology*, 6, 205-212. doi: 10.4236/abb.2015.63020.

[5] Adekunle, K. and Okolie, J. (2015) A Review of Biochemical Process of Anaerobic Digestion. *Advances in Bioscience and Biotechnology*, 6, 205-212. doi: 10.4236/abb.2015.63020.

[6] Adekunle, K. and Okolie, J. (2015) A Review of Biochemical Process of Anaerobic Digestion. *Advances in Bioscience and Biotechnology*, 6, 205-212. doi: 10.4236/abb.2015.63020.

and acidogenic bacteria.[7] Common reactions that occur in methanogenesis are written as Equations 5, 6, 7, and 8.

Table 1: Stages and Reactions in Anaerobic Digestion

Stage	Reaction	Eqn. Number
Hydrolysis	$C_6H_{10}O_4 + 2\,H_2O \rightarrow C_6H_{12}O_6 + H_2$	1
Acidogenesis	$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2$	2
	$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O$	3
Acetogenesis	$CH_3CH_2COOH + 2H_2O \leftrightarrow CH_3COOH + CO_2 + 3H_2$	4
Methanogenesis	$2CH_3CH_2OH + CO_2 \leftrightarrow 2CH_3COOH + CH_4$	5
	$CH_3COOH + CO_2 \leftrightarrow CH_4 + 2CO_2$	6
	$CH_3OH + H_2 \leftrightarrow CH_4 + H_2O$	7
	$CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O$	8

3.2 Two Stage Anaerobic Digestion

The theory behind two stage anaerobic digestion is to optimize conditions for different steps of the anaerobic digestion process in order to enhance the yield and composition of biogas. In single stage anaerobic digestion, the operating conditions are generally optimized to generate the highest production of methane. Usually single stage reactors produce only negligible amounts of hydrogen but two stage configurations can be used to achieve the sequential production of hydrogen and methane. The two stage configuration also allows for greater control over the digestion process. In two stage anaerobic digestion, conditions for hydrolysis, acidogenesis, and acetogenesis are typically optimized in one reactor while conditions for methanogenesis are optimized in the second reactor. Advantages of two stage anaerobic digestion include better pH control, higher stability, a higher methane yield, and increased reduction of volatile solids. Disadvantages of two-stage anaerobic digestion can include higher capital and operational costs.

---

[7] Babaei, Azadeh & Shayegan, Jalal. (2011). Effect of Organic Loading Rates (OLR) on Production of Methane from Anaerobic Digestion of Vegetables Waste. 411-417. 10.3384/ecp11057411.

3.3 Effect of Operational Parameters on Anaerobic Digestion

Various operational parameters impact the performance of anaerobic digesters. These parameters include the reactor configuration, sample procedure, temperature, organic loading rate, substrate composition, pH and hydraulic retention time.

3.3.1 Reactor Configuration and Water Content

Two of the most common reactor types for anaerobic digestion include CSTR and batch configurations. In a continuous reactor, there is a constant input of feedstock to the reactor and reactions occur at approximately the same rate. In a batch reactor, there are distinct stages to the process. Advantages of a continuous reactor include that the reactor volume is more heavily utilized. An advantage to a batch process is that the removed material should be completely digested where in continuous processes, oftentimes partially digested material is removed from the reactor.

The water content of the reactor is also an important parameter in anaerobic digestion. Anaerobic digestion systems can be classified as either wet or dry with wet anaerobic digestion systems having a Total Solids (TS) content of less than 15% and dry systems having a TS of greater than 15%. Water is essential to anaerobic digestion since it acts as a buffering agent and it fosters microbial growth.[8] The addition of water is also important for industrial scale AD so that the mixture can be pumped. Challenges with dry anaerobic digestion include retarded mass transfer as a result of limited mixing.

3.3.2 Temperature

Temperature is considered one of the most important parameters affecting anaerobic digestion. It has a strong influence on the level of activity of various enzymes and coenzymes. Anaerobic digestion typically is conducted under three different temperature ranges, psychrophilic (10-20°C), mesophilic (20-45°C), and thermophilic (50-65°C). Mesophilic anaerobic digestion is currently the most popular since these conditions work with a wider range of substrates. A temperature of 35°C under mesophilic conditions is shown to produce higher methane gas yields and COD efficiencies.[9] Anaerobic digestion at a higher temperature has other advantages including faster digestion, reduced vessel size, decreased system cost, and a more organically stable effluent. The greatest disadvantage of thermophilic anaerobic digestion is that there is a

---

[8] Richa Kothari, A.K. Pandey, S. Kumar, V.V. Tyagi, S.K. Tyagi,"Different aspects of dry anaerobic digestion for bio-energy: An overview", Renewable and Sustainable Energy Reviews,Volume 39, 2014, Pages 174-195, ISSN 1364-0321, https://doi.org/10.1016/j.rser.2014.07.011. (http://www.sciencedirect.com/science/article/pii/S1364032114004638)

[9] Budiastuti H, Widyabudiningsih D, Kurnia D R D 2017 temperature effect towards methane gas production and performances of anaerobic fixed bed reactors Mat. Sci. and Eng. 162 pp 1-6

greater risk of failure. This is why thermophilic anaerobic digestion is often used in highly controlled, large scale industry operations.[10]

3.3.3 Carbon to Nitrogen Ratio (C/N)

The ratio of carbon to nitrogen in the substrate also has an effect on the outcome of anaerobic digestion. Optimal C/N ratios range between 20 and 30.[11] A too high C/N ratio can result in a lower methane yield since the methanogenic bacteria consume the existing nitrogen too quickly. A too low C/N ratio can cause the accumulation of Ammonia and a resulting increase in pH. As temperature increases, the optimal C/N ratio increases since higher temperatures increase the likelihood of ammonia inhibition. For agricultural waste, a C/N ratio ranging from 22-25 at mesophilic conditions is considered optimal.[12]

3.3.4 pH

A factor identified as being essential to the success of pilot runs is that that an optimal pH is maintained throughout the system. The pH of an anaerobic digestion system can be controlled by the addition of an acid or base or can stabilize by having sufficiently high carbonate and ammonium levels as these are natural pH buffers. There are separate optimal pH values for the various stages of anaerobic digestion. The methanogenic bacteria are especially sensitive to acidic pH which can inhibit their growth. It was determined by Huber et al. that the best pH for the methanogenic process lies around 7.0.[13] For the acidogenic and hydrolysis stages, optimal pH ranges are lower and range from 5.5 to 6.5 as established by Kim et al.[14]

3.3.5 Hydraulic Retention Time and Organic Loading Rate

The Hydraulic Retention Time (HRT) of the anaerobic digestion process is how long a substrate resides in the digester and is equal to the volume of the reactor divided by the flow rate leaving the reactor as seen below in Equation 9. HRT affects the microbial load and nutrient content of the effluent as well as the biogas yield. Optimal HRT's for anaerobic digestion depend on the rate of decomposition of the raw materials. For a given Organic Loading Rate (OLR) a longer HRT will result in a bigger digester and higher capital costs.[15]

---

[10] Budiastuti H, Widyabudiningsih D, Kurnia D R D 2017 temperature effect towards methane gas production and performances of anaerobic fixed bed reactors Mat. Sci. and Eng. 162 pp 1-6

[11] Wang, Xiaojiao et al. "Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition" *PloS one* vol. 9,5 e97265. 9 May. 2014, doi:10.1371/journal.pone.0097265

[12] Wang, Xiaojiao et al. "Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition" *PloS one* vol. 9,5 e97265. 9 May. 2014, doi:10.1371/journal.pone.0097265

[13] Huber H, Thomm M, Konig H, Thies G, Stetter KO. Methanococeus hermolithotrophicus, a novel thermophilic lithotrophic methanogen. Arch Microbiol 1982;132:47–50.

[14] Kim J, Park C, Kim TH, Lee M, Kim S, Kim SW, et al. Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. J Biosci Bioeng 2003;95:271–5.

[15] Manyi-Loh, Christy E et al. "Microbial anaerobic digestion (bio-digesters) as an approach to the decontamination of animal wastes in pollution control and the generation of renewable energy" *International journal of environmental research and public health* vol. 10,9 4390-417. 17 Sep. 2013, doi:10.3390/ijerph10094390

$$\tau = \frac{Reactor\ Liquid\ Volume}{Feed\ Rate} \qquad \text{Equation 9}$$

The Organic Loading Rate (OLR) also influences the amount and quality of biogas produced. OLR can be expressed as the amount of volatile solids fed to the system daily as seen below in Equation 10. The OLR should be optimal when the COD of the system remains constant over time.

$$OLR = \frac{Daily\ Flow\ Rate * Volatile\ Solids\ Concentration}{Reactor\ Liquid\ Volume} \qquad \text{Equation 10}$$

An organic loading rate that is too high can cause the biogas yield and the rate of volatile solids reduction to decrease. This is because it can lead to accumulation of Volatile Fatty Acids (VFAs) which can lead to acidification and reactor failure.[16]

3.4 Potentials and Challenges of Orange Peel Substrate

The fruit juice industry is very large in Brazil, especially its orange juice industry where it is the largest supplier in the world. [17] This suggests that if anaerobic digestion of orange peels could be applied large scale in industry, the amount of money saved, renewable energy produced, and biomass prevented from being landfilled could be extremely significant. However, in regard to methane generation potential of orange peels, that from fruit and vegetable waste tends to be lower than other types of food waste. Fats, oils, and greases have the highest methane potential of up to 1.1 $m^3CH_4/kgVS_{added}$. Fruit and vegetable waste has a much lower methane yield of 0.16-0.35 $m^3CH_4/kgVS_{added}$. This is because the methane potential for lipids is much higher than for that of carbohydrates and proteins. Additionally orange peels are highly acidic, which is significant since lower pH values have a negative impact on anaerobic digester performance since they expend digester alkalinity. [18]

A high carbon to nitrogen ratio (C/N) is important to anaerobic digestion, a C/N ratio of between 20 and 30 is considered superior for anaerobic digestion.[19] Citrus waste has a very high C/N ratio of 45. This poses a challenge since this is well outside the optimal range of C/N ratios. However, since orange peels have a high C/N ratio, the Total Ammonia Nitrogen (TAN) is expected to be low as is the likelihood of ammonia inhibition.

---

[16] Babaei, Azadeh & Shayegan, Jalal. (2011). Effect of Organic Loading Rates (OLR) on Production of Methane from Anaerobic Digestion of Vegetables Waste. 411-417. 10.3384/ecp11057411.

[17] Jeffrey T. Lewis, In Brazil, Farmers Ripping Out Orange Trees, *Wall Street Journal* (June 13, 2013)

[18] Fuqing Xu, Yangyang Li, Xumeng Ge, Liangcheng Yang, Yebo Li, Anaerobic digestion of food waste – Challenges and opportunities, Bioresource Technology, Volume 247, 2018, Pages 1047-1058, ISSN 0960-8524, https://doi.org/10.1016/j.biortech.2017.09.020. (http://www.sciencedirect.com/science/article/pii/S0960852417315687)

[19] Wang, Xiaojiao et al. "Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition" *PloS one* vol. 9,5 e97265. 9 May. 2014, doi:10.1371/journal.pone.0097265

3.5 Characterization Parameters

Total Solids (TS), Volatile Solids (VS), Chemical Oxygen Demand (COD), Alkalinity, and Ammonia are all parameters that are important for evaluating the efficiency of microbial activity in the degradation of organic matter.

3.5.1 Total and Volatile Solids

The Total Solids (TS) content is the total dry matter in a sample and is the sum of the volatile solids and the inert solids that pass through the digesters. The Volatile Solids (VS) content is a measure of the organic biodegradable content that contributes to biogas production. Additionally, the effectiveness of an anaerobic digester is partially measured by the extent of VS reduction. In a two stage anaerobic digestion experiment using a substrate of food waste and vinasse, a VS reduction of 64% was observed.[20]

3.5.2 COD

The Chemical Oxygen Demand (COD) is a measure of how much oxygen is required to oxidize all of the organic material in a sample into water and carbon dioxide. It is a way to measure the amount of organic material in a sample. There is a direct correlation between the COD and the Total Volatile Solids (TVS) content of a sample. The effectiveness of a digester is partially measured by the extent of COD degradation.

3.5.3 Alkalinity

A high alkalinity concentration results in greater digester stability due to a higher ability to resist pH changes. This is because carbonate is a natural buffer. Typical alkalinity values for mesophilic digesters vary from 2500 to 5000 mg/L as $CaCO_3$.[21] A decrease in alkalinity is often followed by a drastic change in pH. Decreases in alkalinity are most often caused by an accumulation of VFA's due to an inhibited methanogenic step that has failed to convert these to methane.

---

[20] Grazielle Náthia-Neves; Thiago de Alencar Neves; Mauro Berni; Giuliano Dragone; Solange I. Mussatto; Tânia Forster-Carneiro. "Start-up phase of a two-stage anaerobic co-digestion process: hydrogen and methane production from food waste and vinasse from ethanol industry". *Biofuel Research Journal*, 5, 2, 2018, 813-820. doi: 10.18331/BRJ2018.5.2.5

[21] Water Environment Federation. *Design of Municipal Wastewater Treatment Plants*. WEF Press, 2010.

3.5.4 Ammonia

Ammonia is produced as nitrogenous organic matter is degraded. It exists in the form of NH_3 and the ammonium ion (NH_4^+). Typical measurements for ammonia in mesophilic digesters range from 800 to 2000 mg/L.[22] Though ammonia is a nutrient essential for bacterial growth, it may act as an inhibitor to anaerobic digestion systems at high concentrations. Ammonia greatly inhibits methanogenesis but only has a mildly negative effect on hydrolysis and acidogenesis.[23]

4. Methodology

4.1 Materials and Methods for Orange Peels Experiment

In the pilot anaerobic digestion experiment that was performed using the substrate of orange peels, there were two reactors. Hydrolysis, acidogenesis, and acetogenesis occurred in the first reactor and methanogenesis occurred in the second reactor. Samples were taken from each reactor periodically and were evaluated for target parameters.

4.1.1 Reactor Configuration and Conditions

In the experiments to be performed on orange peels, two 4.3 L reactors were used; an acidogenic reactor and a methanogenic reactor. The substrate was orange peels saturated with water obtained from the juice manufacturer CitrusJuice and the inoculum was from AmBev, a Brazilian brewing company's, anaerobic digesters. These reactors were operated under a semi-batch configuration and a temperature of 35°C (mesophilic conditions). This temperature was kept constant by a thermostatic bath. The reactors were each stirred for five minutes after being fed. The acidogenic reactor was kept at a pH of 5-6 and the methanogenic reactor was held at a pH of 7-8. The addition of NaOH and HCl were used to control the pH.

---

[22] Water Environment Federation. *Design of Municipal Wastewater Treatment Plants*. WEF Press, 2010.

[23] Effects of Ammonia on Anaerobic Digestion of Food Waste: Process Performance and Microbial Community, Hong Chen, Wen Wang, Lina Xue, Chang Chen, Guangqing Liu, and Ruihong Zhang, Energy & Fuels 2016 *30* (7), 5749-5757 DOI: 10.1021/acs.energyfuels.6b00715

Figure 1: Two Stage Reactor Configuration

4.1.2 Reactor Composition

Of the 4.3 liters, 40% (1.72 liters) were left empty to leave room in the reactor for the formation of biogas while 60% (2.58 liters) of the reactor was a mixture of wetted orange peel substrate, inoculum, and water. Orange peels composed 35% of the mixture for a total volume of 903 mL, while water composed 39% of the mixture for a total volume of 1,006 mL. The inoculum composed 26% of the mixture for a total volume of 671 mL. An orange peel composition of 35% was chosen since it was the highest substrate percentage that would still be easy to mix in the reactor. A greater percentage of substrate is preferable since this will cause more biogas to be produced however, the substrate composition should not be so great that adequate mixing cannot occur.

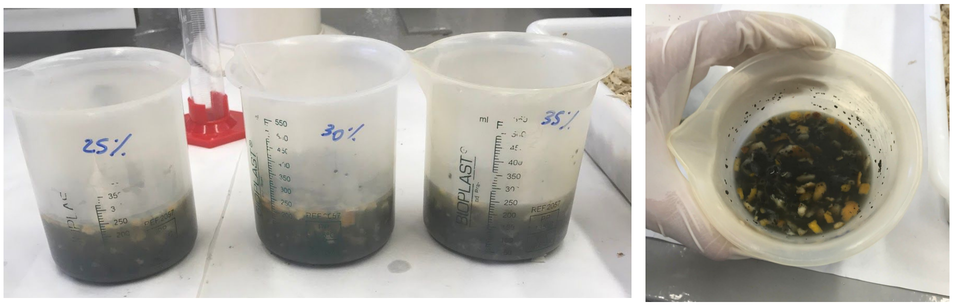


Figure 2: Left: Mixture Compositions with Various Percentages of Substrate

Right: Sample Mixture of Composition used in Reactors (35% solids)

4.1.3 Sampling Methodology

Analyses of target parameters were conducted on samples from both reactors every three or four days. However, the reactor was fed every working day. On each working day except for sampling days, a total volume of 100 mL with 35 mL of orange peels saturated with water and 65 mL of additional water was added to the acidogenic reactor. This resulted in an OLR of 1.49 gVS/(L·d) to the acidogenic reactor.

A volume of 100 mL from the mixture in the acidogenic reactor was then transferred to the methanogenic reactor after a volume of 100 mL from the methanogenic reactor was removed and discarded. This feed rate resulted in a HRT of 25.8. The resulting OLR to the methanogenic reactor was considerably less than that of the acidogenic reactor.

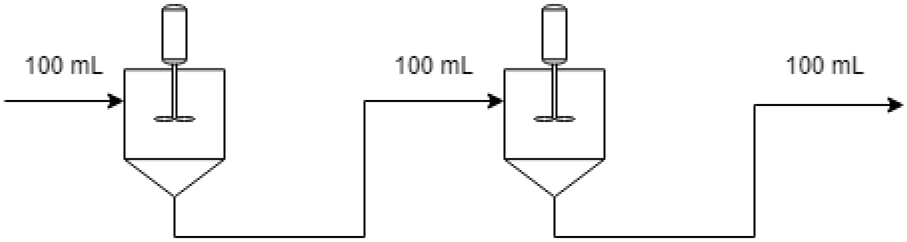


Figure 3: Feed Schematic for Non-Sampling Workdays

On the sample day, samples of 35 mL were taken from the mixtures in both the acidogenic and methanogenic reactors. To ensure that the HRT remained the same on this day, a total volume of 150 mL consisting of 52.5 mL of orange peels saturated with water and 97.5 mL of additional water was added to the acidogenic reactor. A volume of 150 mL was then taken from the mixture in the acidogenic reactor. A volume of 35 mL was used as a sample from the acidogenic reactor. A volume of 115 mL was then removed from the methanogenic reactor of which 35 mL was used as a sample. Lastly, the remaining 115 mL from the acidogenic reactor sample was transferred into the methanogenic reactor.

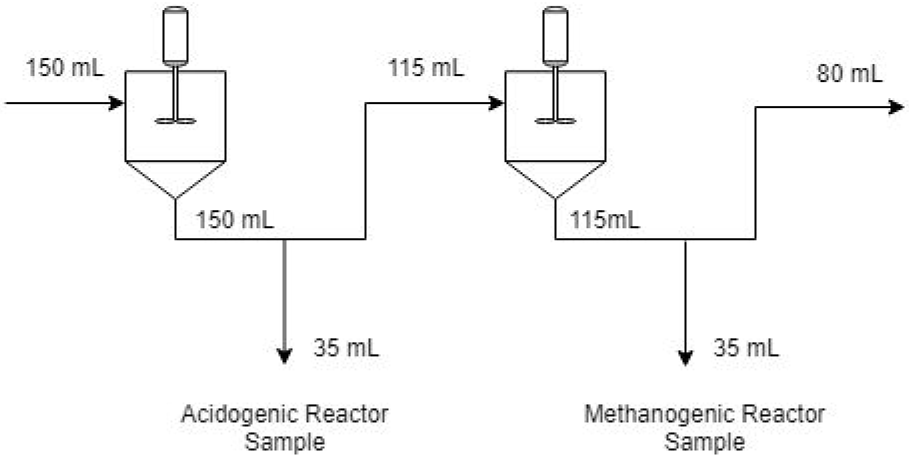


Figure 4: Sampling Day Feed Schematic

4.2 Characterization of Mixture

The mixture samples taken from the reactors were analyzed for parameters such as Total Solids (TS), Total Volatile Solids (TVS), Chemical Oxygen Demand (COD), Alkalinity, and Ammonia. All samples were analyzed in triplicate. These parameters are important for evaluating the efficiency of microbial activity in the degradation of organic matter and the effectiveness of the pilot experiment.

4.2.1 Total Solids (TS)

To determine the Total Solids (TS) composition of the mixture, a crucible was weighed on a scale and a mass of approximately 2 g of solution was placed into the crucible. The sample was then placed in the oven at 105°C and left for twelve hours. The weight of the sample was then weighed again and the TS was calculated using the formula below where P_1 is the weight of the dried sample and crucible, P_0 is the weight of the crucible, and P_{sample} is the weight of the sample.

$$TS\ [g/kg] = \frac{P_1 - P_0}{P_{sample}} * 1000 \qquad \text{Equation 11}$$

4.2.2 Volatile Solids (VS)

To determine the Volatile Solids (VS) composition of the mixture, the crucible after being dried in the oven for measurement of TS, was left in the Muffle Furnace at 550°C for two hours to ignite. The Volatile Solids was calculated using the formula below where P_2 is the weight of the sample after ignition, P_1 is the weight of the sample after drying, and P_{sample} is the weight of the initial sample .

$$VS\ [g/kg] = \frac{P_2 - P_1}{P_{sample}} * 1000 \qquad \text{Equation 12}$$

4.2.3 Chemical Oxygen Demand (COD)

To prepare the sample for analysis, 5 g of the reactor sample was added to a 250 mL Erlenmeyer flask. The flask was then filled to the 50 mL mark with deionized water. This mixture was then placed in a shaker at 25°C and 200 rpm for one hour. After the diluted sample was thoroughly mixed, the mixture was poured through funnel containing a layer of cotton into another Erlenmeyer flask to remove solid particles. A Buchner funnel was used then used with a vacuum to create a vacuum filtration apparatus to further filter solid particles out of the sample. At the end of the process, the liquid sample was separated from the total solids.

To analyze for the COD, HACH tubes were wiped and 2.5 mL of the diluted sample was put into the tubes. 1.5 mL of digestive solution and 3.5 mL of catalytic solution was also added. These HACH tubes were then placed in the HACH COD reactor for 150 °C for two hours. The tubes were then left to cool in the dark. The absorbance in the spectrophotometer (DR/4000, Hatch) was then read. A blank was also made to reset the equipment.

Figure 5: Samples in Hatch COD Reactors

The spectrophotometer gives values of the absorbance which are then converted to grams per liter of COD using the slope equation as determined by the calibration curve. This value was then corrected to account for the dilution of the sample by multiplying the COD result by ten.

The calibration curve was created by determining the absorbance values of multiple mixtures using the same procedure above. These mixtures were prepared from a solution with a known COD concentration that were diluted with various volumes of deionized water.

4.2.4 Alkalinity

To determine the Alkalinity of the sample, 5 g of the sample was placed into a Erlenmeyer flask. The flask was then filled to the 50 mL mark with deionized water. This mixture was then placed in a shaker at 25°C and 200 rpm for one hour.

After this sample was adequately mixed, 10 mL of diluted sample was placed in a beaker. The magnetic bar was placed in the beaker and the beaker was placed on the magnetic stirrer. The pH electrode was then immersed in the sample and the solution was titrated with 0.02 M H_2SO_4 until the sample reached a pH of 8.3. The volume spent as then used to calculate the alkalinity as in the equation below.

$$CaCO_3\,[mg/L]= [M_{H2SO4}*V_{1H2SO4}*50000]/V_{sample} \qquad \text{Equation 13}$$

4.2.5 Ammonia

To determine the Ammonia content of the sample, 5 g from each reactor's sample was poured into a 250 mL Erlenmeyer flask along with 50 mL of deionized water. This mixture was then placed in the shaker at 25°C and 200 rpm for one hour. After the diluted sample was thoroughly mixed, the mixture was poured through funnel containing a layer of cotton into another Erlenmeyer flask to remove solid particles. A Buchner funnel was used then used with a vacuum to create a vacuum filtration apparatus to further filter solid particles out of the sample. At the end of the process, the liquid sample was separated from the total solids.

Next, 5 mL of the resulting solution was added to a beaker along with 5 mL of a Borate buffer. The pH was measured and adjusted to 9.5 using a 0.5 M NaOH solution. This solution was then transferred to a Kjedahl digestion buffer tube.

Lastly, 10 mL of absorbing solution of boric acid were added to a 250 mL Erlenmeyer flask and the tube was then connected with the sample to the Kjedahl Nitrogen Distiller. The boiler heating was connected and it was made sure that the distillate was collected. The resulting solution in the Erlenmeyer flask was then titrated with a 0.018 M sulfuric acid solution until the indicator turned pink. The total amount of ammonia as N was calculated as below in Equation 14 where A is the volume of H_2SO_4 added to the solution, B is the volume of H_2SO_4 added to the blank, M H_2SO_4 is the molarity of the sulfuric acid and V_{sample} is the volume of the sample.

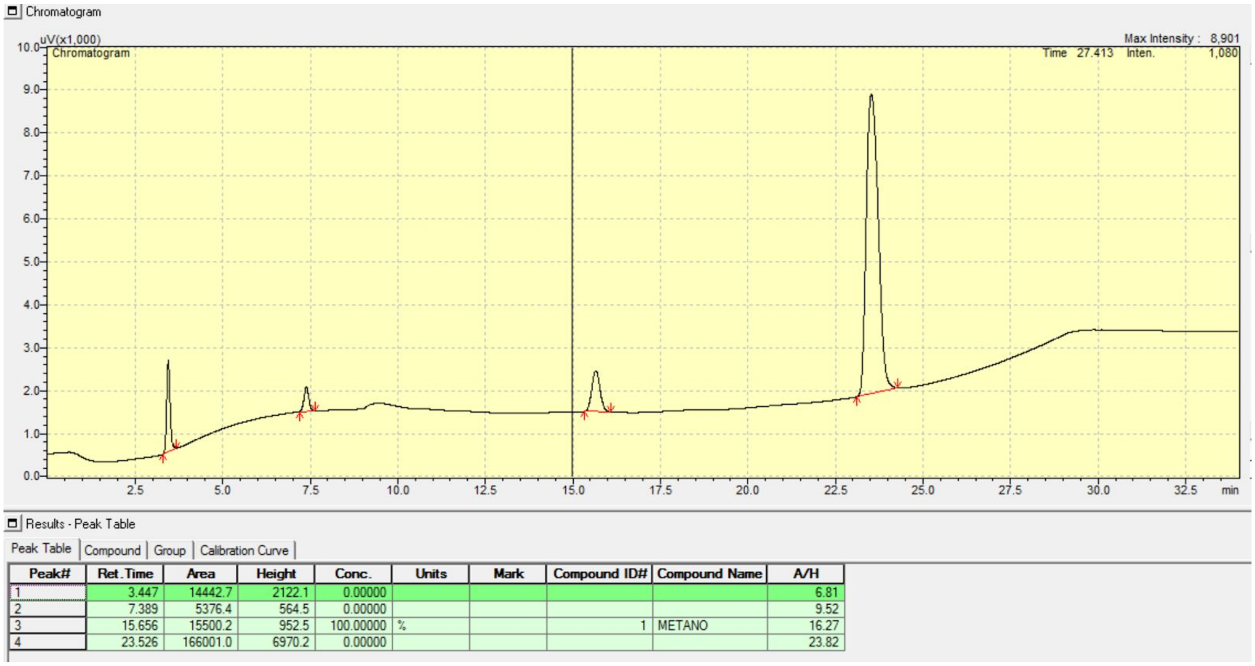
$$N - NH3 = \frac{(A-B)*14*M_{H2SO4}}{V_{sample}} \qquad \text{Equation 14}$$

4.3 Characterization of Biogas

4.3.1 Gas Composition

The composition of the biogas was determined daily by a gas chromatograph (GC-2014, Shimadzu) containing a thermal conductivity detector (TCD) using nitrogen as a carrier gas. An inert nitrogen carrier gas at 5 bar was used as the mobile phase. The following chromatographic conditions were used: temperatures of the injection port and detector were both set to 200 °C; the initial temperature of GC column was 50 °C (held for 3 min), and then increased by 5 °C.min-1 to 180 °C (held for 5 min); the sample volume injected was 0.5 mL; and N_2 was used as a carrier gas (35 mL.min-1, 5 bar). The results were analyzed for hydrogen, carbon dioxide, methane, and oxygen gas as predicted products of anaerobic digestion.

The identity and ratio of components were determined by the retention times and peak areas. Hydrogen, oxygen, methane, and carbon dioxide had approximate retention times at 3,7,16, and 23 respectively. The area of the peak of a particular component divided by the total area of all component peaks as measured by the gas chromatograph was determined to be the volume percent of the gas.



Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#	Compound Name	A/H
1	3.447	14442.7	2122.1	0.00000					6.81
2	7.389	5376.4	564.5	0.00000					9.52
3	15.656	15500.2	952.5	100.00000	%		1	METANO	16.27
4	23.526	166001.0	6970.2	0.00000					23.82

Figure 6: Sample Gas Chromatography Results

4.3.2 Gas Volume

Each day an amount of 600 mL of gas was removed from the reactor to relieve pressure. Additionally, the amount of gas in the bag was removed and measured. The total amount of gas produced was measured as the amount of gas removed from the bag.

5. Results

5.1 Total Solids and Volatile Solids

In Figure 7, a steady decreasing trend is observed for total and volatile solids in both the acidogenic and methanogenic reactors. This indicates that the reactor had processed the starting load of orange peels and that the OLR might have been on the low end for ideal results. For the acidogenic reactor the VS reduction percentage was 54.2%. This was about average for for two stage anaerobic digestion. For the methanogenic reactor the VS reduction percentage was 54.7%, also in a robust range for anaerobic digestion.

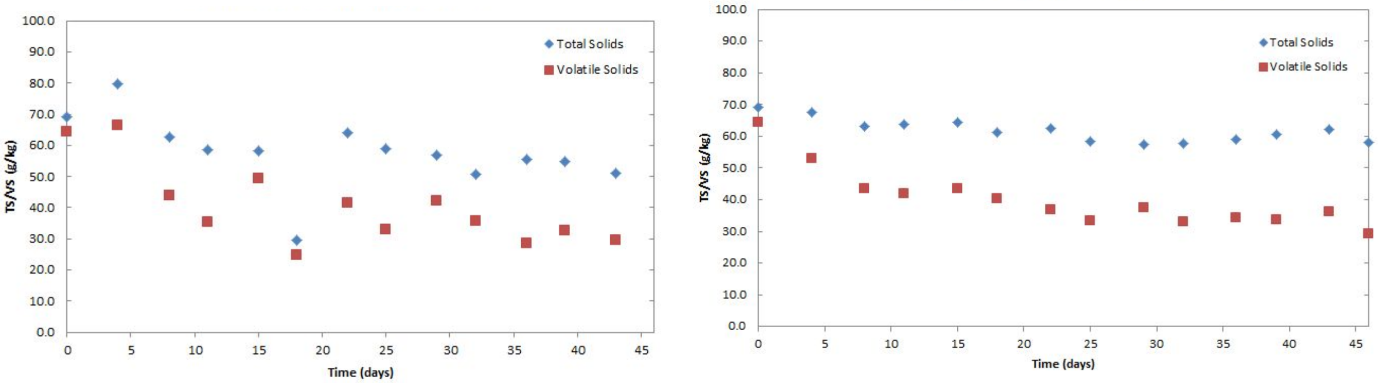


Figure 7: Left: Acidogenic Reactor Total Solids and Volatile Solids Content
Right: Methanogenic Reactor Total Solids and Volatile Solids Content

5.2 COD

As seen below in Figure 8, the COD in both reactors decreases significantly throughout the experiment. The COD is a significant parameter as it indicates the extent of degradation of organic matter. The COD of the acidogenic reactor decreased from 8382.5 mg/L to 1626.3 mg/L for a total reduction of 80.6%. The COD of the methanogenic reactor decreased from 8382.5 mg/L to 5807.5 mg/L for a total reduction of 30.7%. Differences in the reduction percentages can be attributed to the fact that the acidogenic reactor is fed food waste while the methanogenic reactor is fed material that has already been partially digested.

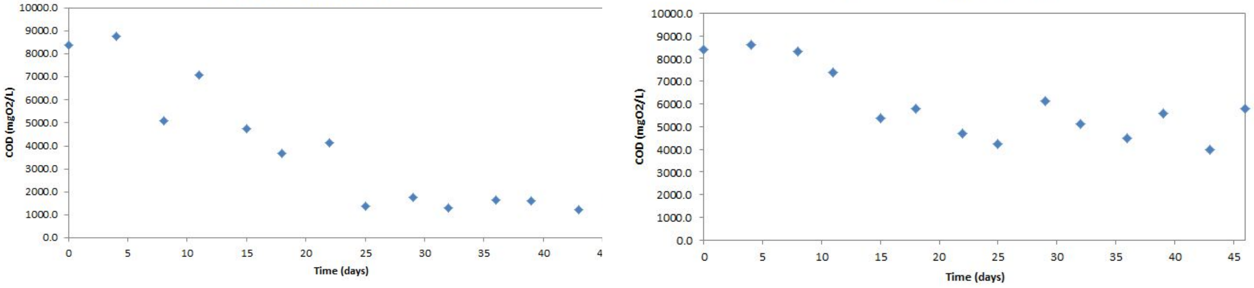


Figure 8: Left: Acidogenic Reactor Chemical Oxygen Demand
Right: Methanogenic Reactor Chemical Oxygen Demand

5.3 Alkalinity

As seen in Figure 9 below, the alkalinity of the acidogenic reactor was extremely low, ranging from 135.0 to 724.5 mgCaCO$_3$/L. This suggests that there is not sufficient buffering capacity of the reactor. The alkalinity in the acidogenic reactor was expected to be lower than that in the methanogenic reactor since it was operated at a lower pH. However, this low buffering capacity to protect against pH changes could explain some of the unexpected results in gas composition in the acidogenic reactor.

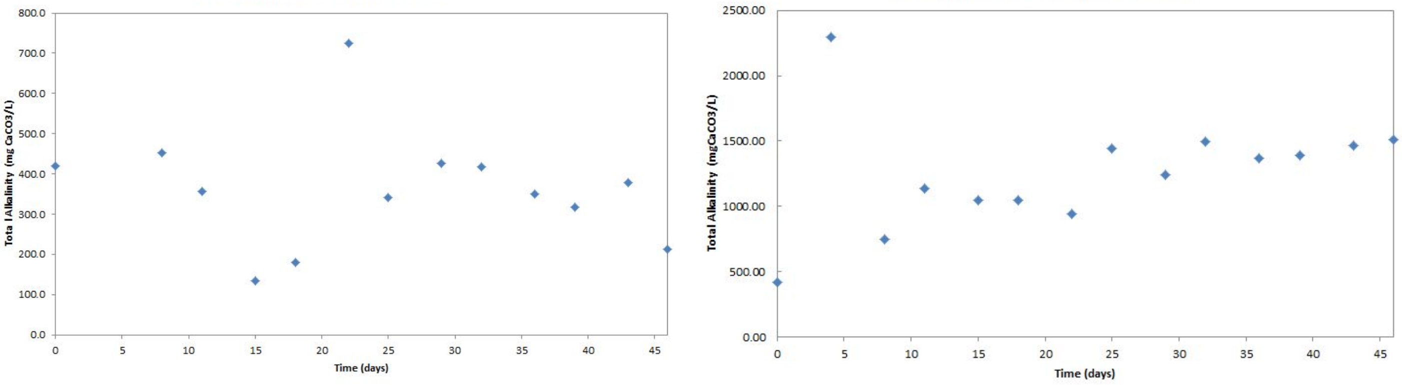


Figure 9: Left: Acidogenic Reactor Alkalinity
Right: Methanogenic Reactor Alkalinity

The alkalinity of the methanogenic reactor can be also be seen in Figure 9. It was consistently within the normal range of 800-2000 mgCaCO$_3$/L and was not as low as the alkalinity of the acidogenic reactor. These results suggest a sufficient buffering capacity to prevent abrupt pH changes, providing a high stability for the microorganisms.

5.4 Ammonia

The concentration of ammonia in both reactors were very similar and were relatively low. Higher ammonia concentrations were reported by other authors in two stage reactor experiments. Nathia-Neves et al. reported much higher levels of ammonia in the range of 1000-3000 mg N-NH$_3$/L. Too low or too high ammonia concentrations can lead to a reduction in methane production. Optimal concentrations of ammonia tend to be between 2100 and 3100 mg N-NH3/L. Since the ammonia concentration in both reactors is extremely low, the methane production could have resulted in a lower yield than as predicted.

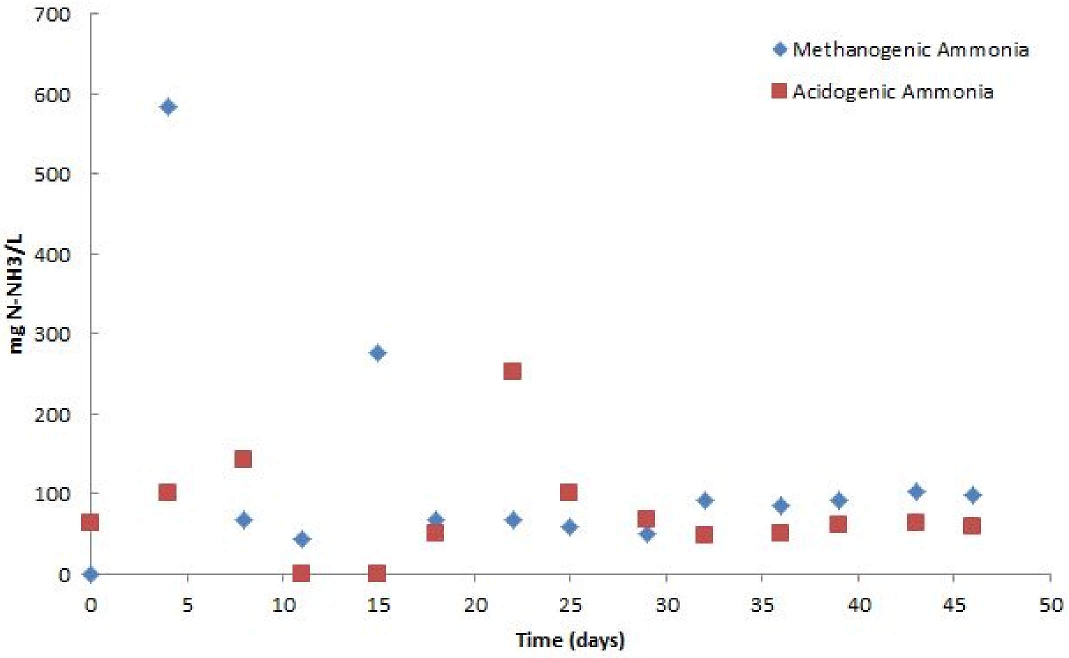


Figure 10: Ammonia expressed in mg N-NH3/L in Acidogenic and Methanogenic Reactors

5.5 Gas Composition

The gas composition in the acidogenic reactor along the 46 days that the experiment was run is shown in Figure 11 below. The results below only show significant hydrogen production during the first five days and during the last two days.

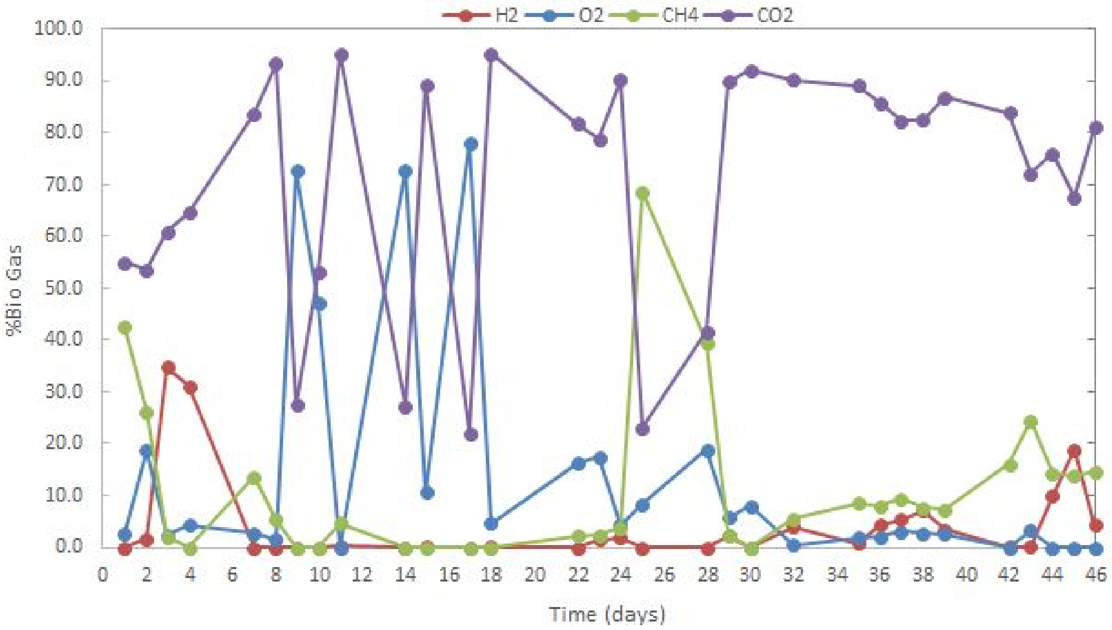


Figure 11: Gas Composition of Acidogenic Reactor

During days eight to seventeen, large oxygen spikes alternating with large spikes in carbon dioxide were seen. Since such high spikes of oxygen were not expected, a sample containing air was used as a sample in the Gas Chromatograph to confirm that the compound assumed to be oxygen was not a different compound with a similar retention time.

After it was confirmed that peaks were in fact oxygen, it was theorized that there was either an air leak or that a microorganism in the bacterial colony was producing oxygen. However, since nitrogen was being used as the carrier gas, it was impossible to determine if the amount of oxygen seen in the acidogenic reactor was higher that what is possible from air. It is also significant that the feeding procedures for both the acidogenic and methanogenic reactors were the same and that the methanogenic reactor only had minimal amounts of oxygen not greater than ten percent.

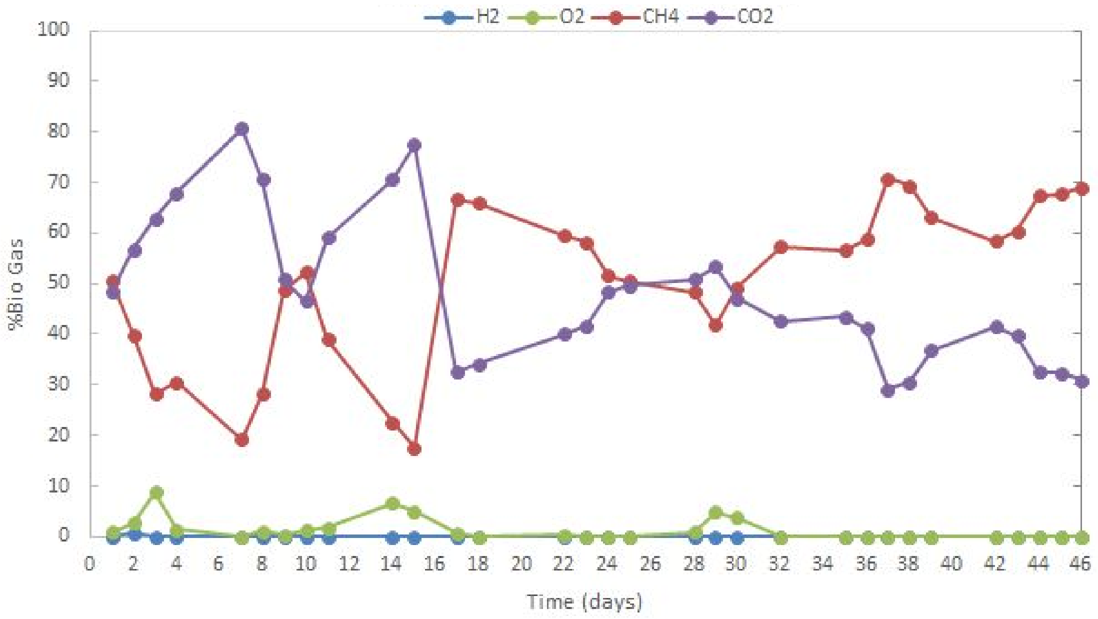


Figure 12: Gas Composition of Methanogenic Reactor

The methanogenic reactor produced results that were much more consistent of what was expected as shown in Figure 12. From day 22 onwards, the average composition of methane in the gas was approximately 59%. This was almost within the expected range of 60% to 70%. Low spikes of oxygen of up to ten percent were observed. These oxygen levels were drastically lower than those observed in the acidogenic reactor.

5.6 Gas Volume

The biogas yield from the acidogenic reactor was much greater than that of the methanogenic reactor and is shown below in Figure 13. The total gas yield at the end of the experiment was 9260 mL for the acidogenic reactor and 4193 mL for the methanogenic reactor.

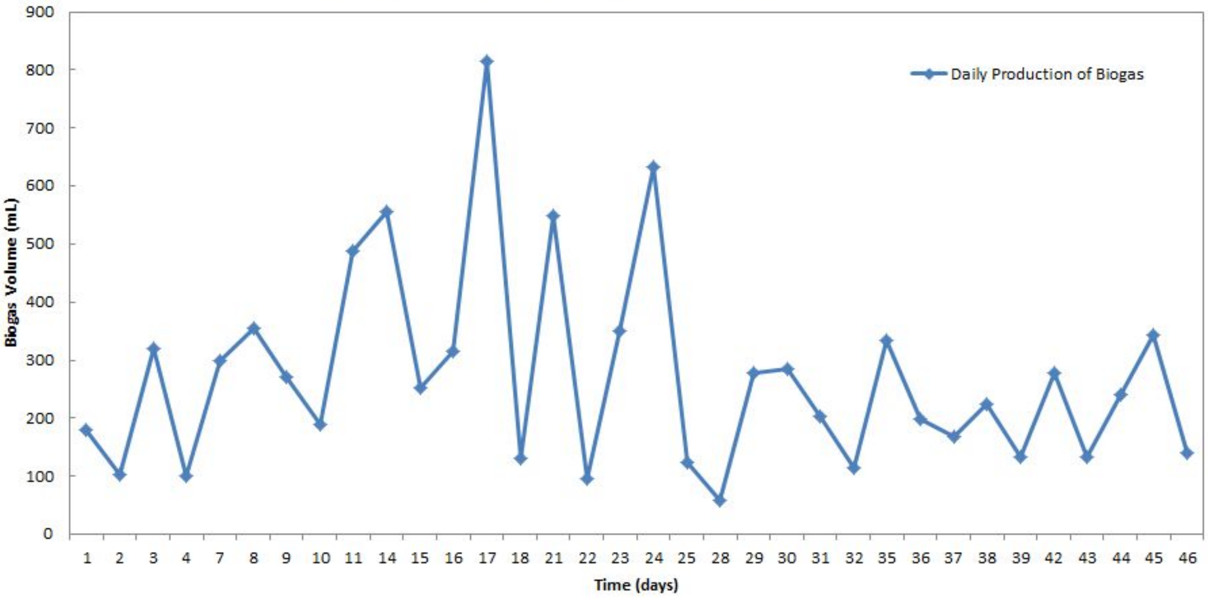


Figure 13: Daily Gas Volume Acidogenic Reactor

This results in a methane production of 2473.87 mL in the methanogenic reactor assuming an average methane percentage of 0.59.

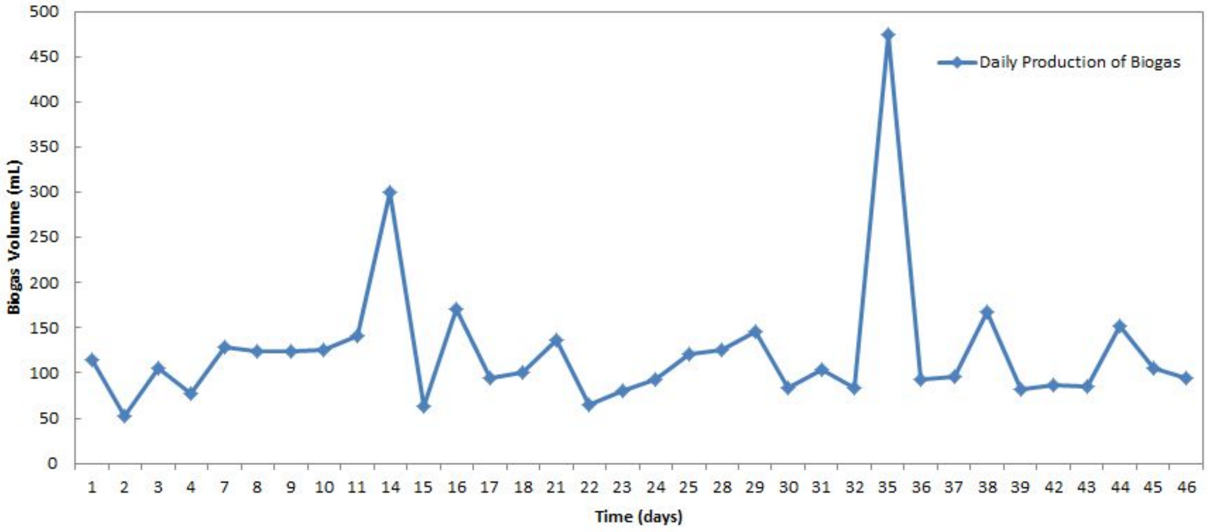


Figure 14: Daily Gas Volume Methanogenic Reactor

5.7 Energy Production

5.7.1 Acidogenic Reactor Energy Production

The total amount of biogas produced over the duration of the experiment in the acidogenic reactor was 9260 mL. The average hydrogen concentration of this biogas was 4.3%. Though hydrogen has a higher energy value than methane, because of the low yield the energy generated in this experiment was negligible. Other experiments using two stage anaerobic digestion have seen hydrogen yields above fifty percent and have had significant energy production potentials.

5.7.2 Methanogenic Reactor Energy Production

Equations 15 and 16 below were used to calculate the electricity production potential of the methanogenic reactor. The total amount of biogas produced over the duration of the experiment was 4193 mL. The average methane concentration of this biogas was 0.59.

$$PB = \frac{amount\ biogas\ produced\ (m3/year)}{percentage\ of\ methane\ in\ biogas}\,[m^3/year] \qquad \text{Equation 15}$$

$$PE = 5500\ kcal/m^3\ *\ PB\ =\ [kcal/year] \qquad \text{Equation 16}$$

Based on the equations above, the methanogenic reactor was found to produce 1.868 MJ/year of energy.

6. Conclusions

The pilot phase of the two stage anaerobic digestion is promising due to parameters such as VS and COD reduction. The methanogenic reactor on its own shows favorable results due to a high methane and gas volume yield. The acidogenic reactor shows much less promising results in terms of hydrogen production but appears to have been compromised by strange microbial activity characterized by large oxygen spikes alternating with carbon dioxide spikes during the start up phase.

Alkalinity in the acidogenic reactor as well as the ammonia concentration in both reactors should ideally be higher. A possible solution for this could be that another substance could be added to the substrate for codigestion. A possible substrate that could be mixed with orange peels could be animal manure or municipal waste. This would lower the high C/N ratio of the orange peel substrate. The acidogenic reactor may also be operated at a higher pH to boost the alkalinity to provide a better buffer against pH changes.

7. Appendix

Appendix A: TS and VS Calculations

ACIDOGENIC REACTOR

Day	Sample	P sample (g)	P0(g)	P1(g)	P2 (g)	TS(g/kg)	TS Avg(g/kg))	FS (g/kg)	FS Avg (g/kg)	VS (g/kg)	VS Avg (g/kg)
0	A	2.0636	40.170	40.301	40.179	63.772	69.0	4.6	4.5	59.2	64.5
	B	2.0320	38.360	38.507	38.368	72.589		3.8		68.7	
	C	2.0262	40.864	41.007	40.874	70.674		5.0		65.6	
4	A	1.1422	40.433	40.534	40.461	88.688	79.9	24.8	13.3	63.9	66.5
	B	1.3538	38.760	38.830	38.766	52.002		4.1		47.9	
	C	1.0244	40.862	40.963	40.873	98.887		11.1		87.8	
8	A	2.0127	39.6314	39.7506	39.6576	59.224	62.7	13.0	18.8	46.2	43.9
	B	2.0479	39.2511	39.3831	39.2978	64.456		22.8		41.7	
	C	2.0283	37.4907	37.6212	37.5324	64.340		20.6		43.8	
11	A	2.0361	34.527	34.649	34.580	59.820	58.6	26.3	23.1	33.5	35.5
	B	2.0804	39.259	39.378	39.301	57.345		19.9		37.4	
15	A	2.0467	34.522	34.644	34.540	59.901	58.4	8.9	9.0	51.0	49.3
	B	2.0471	37.495	37.612	37.514	57.007		9.1		47.9	
	C	2.0771	39.620	39.741	39.639	58.254		9.1		49.2	
18	A	2.0941	30.561	30.629	30.572	32.186	29.7	5.4	5.0	26.8	24.7
	B	2.1504	38.7631	38.8281	38.7734	30.22693452		4.8		25.4	
	C	2.1757	40.1735	40.2315	40.1839	26.65808705		4.8		21.9	
22	A	2.0914	35.5614	35.6943	35.6178	63.54595008	64.2	27.0	22.8	36.6	41.4
	B	2.008	34.5228	34.6506	34.5634	63.64541833		20.2		43.4	
	C	2.0682	37.5003	37.6353	37.544	65.27415144		21.1		44.1	
25	A	2.0467	40.0249	40.1454	40.07	58.87526262	59.0	22.0	26.0	36.8	33.1
	B	2.0113	31.9669	32.0903	32.0246	61.35335355		28.7		32.7	
	C	2.0643	51.8069	51.9244	51.8632	56.92002131		27.3		29.6	
29	A	2.0035	34.5231	34.6399	34.5559	58.29797854	56.9	16.4	14.6	41.9	42.3
	B	2.0178	29.4537	29.5656	29.4796	55.4564377		12.8		42.6	
32	A	2.0119	37.5511	37.6436	37.5792	45.97644018	50.8	14.0	15.0	32.0	35.8
	B	2.0112	40.8411	40.9434	40.8745	50.86515513		16.6		34.3	
	C	2.0666	38.0449	38.1595	38.0745	55.45340172		14.3		41.1	
36	A	2.0524	34.5222	34.6465	34.5799	60.56324303	55.6	28.1	27.0	32.4	28.6
	B	2.0385	37.503	37.6154	37.5617	55.13858229		28.8		26.3	
	C	2.0861	35.5562	35.6629	35.6064	51.14807536		24.1		27.1	
39	A	2.027	31.957	32.0568	31.9954	49.23532314	54.8	18.9	22.2	30.3	32.6
	B	2.0168	37.0303	37.1399	37.0743	54.34351448		21.8		32.5	
	C	2.1354	23.2953	23.4252	23.3503	60.8316943		25.8		35.1	
43	A	2.0013	42.8355	42.9396	42.8831	52.01618948	51.2	23.8	21.7	28.2	29.5
	B	2.1069	51.7993	51.9053	51.8405	50.31088329		19.6		30.8	
46	A	2.0507	40.4618	40.6248	40.5095	79.48505388	79.4	23.3	23.0	56.2	56.4
	B	2.1557	29.4413	29.6186	29.4881	82.24706592		21.7		60.5	
	C	2.0299	40.8655	41.0207	40.9145	76.45696832		24.1		52.3	

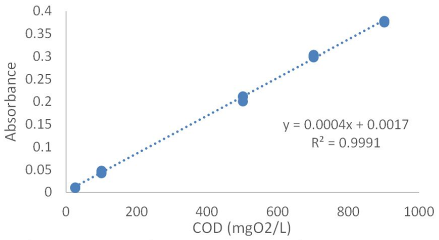
METHANOGENIC REACTOR

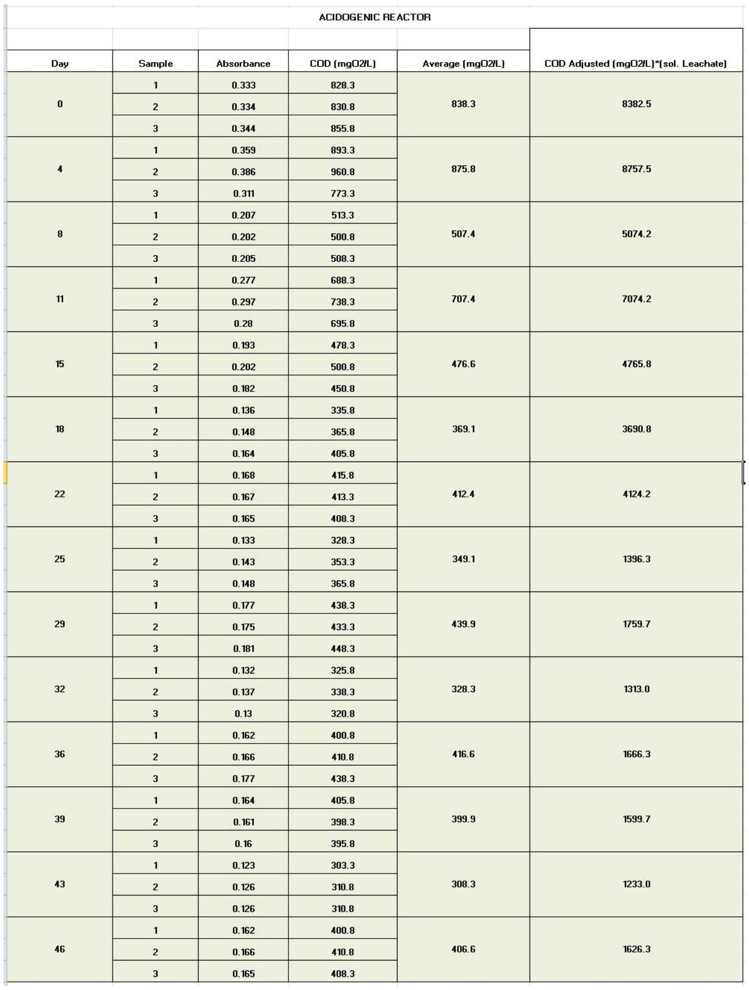
Day	Sample	P sample (g)	P0 (g)	P1 (g)	P2(g)	TS (g/kg)	TS Avg (g/kg)	FS (g/kg)	FS Avg(g/kg)	VS (g/kg)	VS Avg (g/kg)
0	A	2.0636	40.1698	40.3014	40.1792	63.8	69.0	4.6	4.5	59.2	64.5
	B	2.0320	38.3597	38.5072	38.3675	72.6		3.8		68.7	
	C	2.0262	40.8637	41.0069	40.8739	70.7		5.0		65.6	
4	A	1.0038	40.1682	40.2363	40.1847	67.8	67.6	16.4	14.7	51.4	52.9
	B	1.1746	37.5034	37.5777	37.5110	63.3		6.5		56.8	
	C	1.1262	45.3963	45.4771	45.4202	71.7		21.2		50.5	
8	A	2.0676	40.1690	40.2984	40.2104	62.6	63.1	20.0	19.8	42.6	43.3
	B	2.0736	38.7581	38.8920	38.8005	64.6		20.4		44.1	
	C	2.0536	38.3154	38.4430	38.3540	62.1		18.8		43.3	
11	A	2.0429	38.3160	38.4449	38.3580	63.1	63.8	20.6	21.8	42.5	41.9
	B	2.0992	37.4961	37.6314	37.5458	64.5		23.7		40.8	
	C	2.0510	39.6226	39.7533	39.6662	63.7		21.3		42.5	
15	A	2.0523	39.2526	39.3831	39.2941	63.6	64.5	20.2	20.9	43.4	43.6
	B	2.0190	35.5567	35.6876	35.6002	64.8		21.5		43.3	
	C	2.0118	38.3148	38.4459	38.3571	65.2		21.0		44.1	
18	A	2.1884	38.0890	38.2241	38.1342	61.7	61.4	20.7	21.2	41.1	40.2
	B	2.1659	40.3810	40.5128	40.4286	60.9		22.0		38.9	
	C	2.1964	35.0455	35.1805	35.0914	61.5		20.9		40.6	
22	A	2.0055	39.2610	39.3855	39.3108	62.1	62.4	24.8	25.7	37.2	36.7
	B	2.0504	38.3203	38.4502	38.3778	63.4		28.0		35.3	
	C	2.0548	39.6248	39.7517	39.6744	61.8		24.1		37.6	
25	A	2.0904	40.8688	40.9863	40.9226	56.2	58.3	25.7	25.0	30.5	33.3
	B	2.0847	23.2949	23.4196	23.3433	59.8		23.2		36.6	
	C	2.0323	37.0501	37.1699	37.1030	58.9		26.0		32.9	
29	A	2.0884	35.5594	35.6824	35.6017	58.9	57.4	20.3	20.0	38.6	37.4
	B	2.0686	37.5096	37.6222	37.5463	54.4		17.7		36.7	
	C	2.0530	39.6244	39.7454	39.6698	58.9		22.1		36.8	
32	A	2.0737	38.7094	38.8279	38.7590	57.1	57.7	23.9	24.7	33.2	33.0
	B	2.1525	37.0296	37.1548	37.0844	58.2		25.5		32.7	
36	A	2.0310	39.2594	39.3810	39.3118	59.9	59.0	25.8	24.7	34.1	34.2
	B	2.1432	39.6263	39.7541	39.6816	59.6		25.8		33.8	
	C	2.1166	29.4479	29.5693	29.4957	57.4		22.6		34.8	
39	A	2.0026	40.0069	40.1286	40.0582	60.8	60.6	25.6	26.9	35.2	33.7
	B	2.1130	30.5599	30.6868	30.6196	60.1		28.3		31.8	
	C	2.0383	30.8189	30.9433	30.8737	61.0		26.9		34.1	
43	A	2.0805	60.7527	60.8816	60.8089	62.0	62.1	27.0	26.0	34.9	36.1
	B	2.1537	54.1987	54.3314	54.2529	61.6		25.2		36.4	
	C	2.0707	59.9731	60.1028	60.0266	62.6		25.8		36.8	
46	A	2.0507	34.4803	34.6014	34.5417	59.1	58.1	29.9	28.9	29.1	29.2
	B	2.0214	35.0472	35.1619	35.1051	56.7		28.6		28.1	
	C	2.4422	37.2582	37.4012	37.3270	58.6		28.2		30.4	

Appendix B: COD Calculations

CALLIBRATION CURVE

COD(mgO2/L)	Absorbance
25	0.012
25	0.011
25	0.01
100	0.045
100	0.043
100	0.048
500	0.212
500	0.213
500	0.201
700	0.304
700	0.299
700	0.301
900	0.378
900	0.379
900	0.376





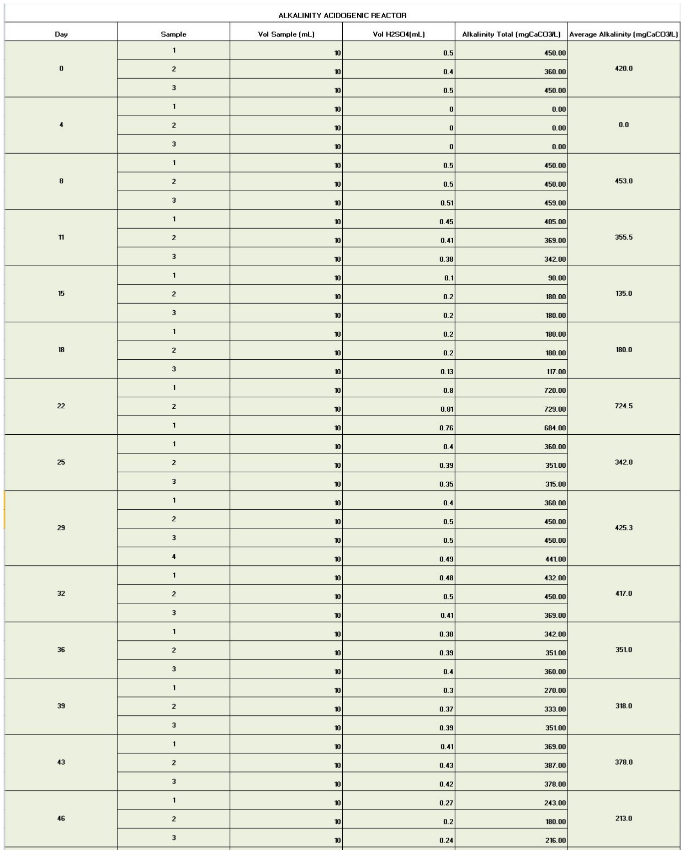
ACIDOGENIC REACTOR

Day	Sample	Absorbance	COD (mgO2/L)	Average (mgO2/L)	COD Adjusted (mgO2/L)*(sol. Leachate)
0	1	0.333	828.3	838.3	8382.5
	2	0.334	830.8		
	3	0.344	855.8		
4	1	0.359	893.3	875.8	8757.5
	2	0.386	960.8		
	3	0.311	773.3		
8	1	0.207	513.3	507.4	5074.2
	2	0.202	500.8		
	3	0.205	508.3		
11	1	0.277	688.3	707.4	7074.2
	2	0.297	738.3		
	3	0.28	695.8		
15	1	0.193	478.3	476.6	4765.8
	2	0.202	500.8		
	3	0.182	450.8		
18	1	0.136	335.8	369.1	3690.8
	2	0.148	365.8		
	3	0.164	405.8		
22	1	0.168	415.8	412.4	4124.2
	2	0.167	413.3		
	3	0.165	408.3		
25	1	0.133	328.3	349.1	1396.3
	2	0.143	353.3		
	3	0.148	365.8		
29	1	0.177	438.3	439.9	1759.7
	2	0.175	433.3		
	3	0.181	448.3		
32	1	0.132	325.8	328.3	1313.0
	2	0.137	338.3		
	3	0.13	320.8		
36	1	0.162	400.8	416.6	1666.3
	2	0.166	410.8		
	3	0.177	438.3		
39	1	0.164	405.8	399.9	1599.7
	2	0.161	398.3		
	3	0.16	395.8		
43	1	0.123	303.3	308.3	1233.0
	2	0.126	310.8		
	3	0.126	310.8		
46	1	0.162	400.8	406.6	1626.3
	2	0.166	410.8		
	3	0.165	408.3		

METHANOGENIC REACTOR

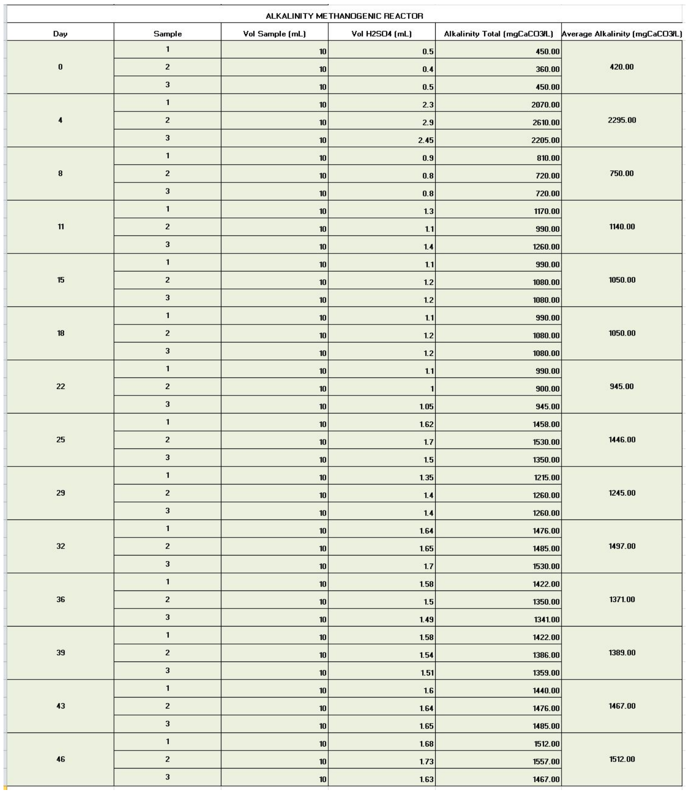
Day	Sample	Absorbance	COD (mgO2/L)	Average (mgO2/L)	COD Adjusted (mgO2/L)(sol. Leachate)
0	1	0.333	828.3	838.3	8382.5
	2	0.334	830.8		
	3	0.344	855.8		
4	1	0.383	953.3	862.4	8624.2
	2	0.316	785.8		
	3	0.341	848.3		
8	1	0.341	848.3	829.9	8299.2
	2	0.338	840.8		
	3	0.322	800.8		
11	1	0.302	750.8	740.8	7407.5
	2	0.303	753.3		
	3	0.289	718.3		
15	1	0.223	553.3	539.1	5390.8
	2	0.217	538.3		
	3	0.212	525.8		
18	1	0.232	575.8	577.4	5774.2
	2	0.232	575.8		
	3	0.234	580.8		
22	1	0.185	458.3	469.9	4699.2
	2	0.184	455.8		
	3	0.2	495.8		
25	1	0.17	420.8	422.4	4224.2
	2	0.17	420.8		
	3	0.172	425.8		
29	1	0.25	620.8	612.4	6124.2
	2	0.25	620.8		
	3	0.24	595.8		
32	1	0.207	513.3	511.6	5115.8
	2	0.208	515.8		
	3	0.204	505.8		
36	1	0.183	453.3	450.8	4507.5
	2	0.186	460.8		
	3	0.177	438.3		
39	1	0.222	550.8	559.1	5590.8
	2	0.224	555.8		
	3	0.23	570.8		
43	1	0.156	385.8	397.4	3974.2
	2	0.164	405.8		
	3	0.162	400.8		
46	1	0.232	575.8	580.8	5807.5
	2	0.231	573.3		
	3	0.239	593.3		

Appendix B: Alkalinity Calculations



ALKALINITY ACIDOGENIC REACTOR

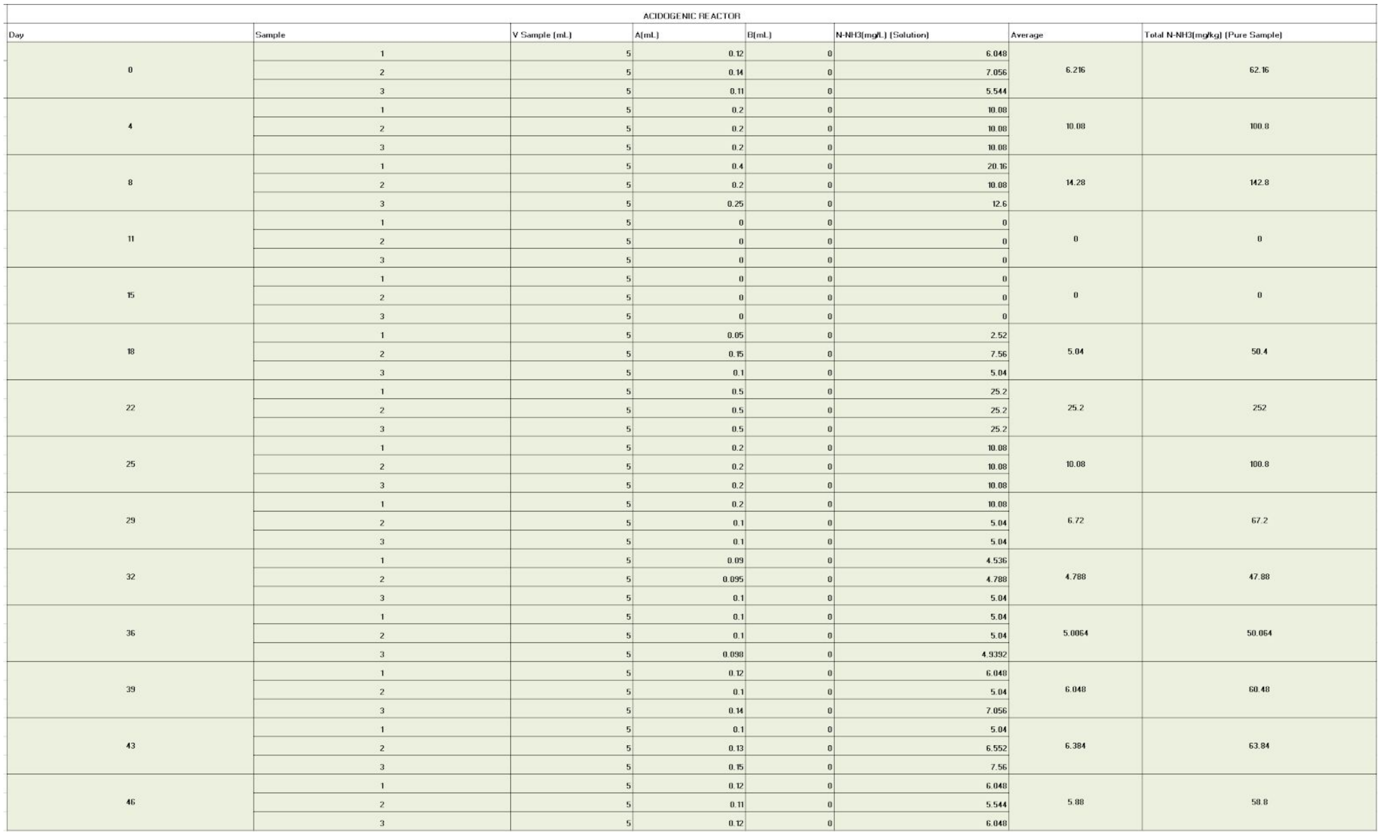
Day	Sample	Vol Sample (mL)	Vol H2SO4(mL)	Alkalinity Total (mgCaCO3/L)	Average Alkalinity (mgCaCO3/L)
0	1	10	0.5	450.00	420.0
	2	10	0.4	360.00	
	3	10	0.5	450.00	
4	1	10	0	0.00	0.0
	2	10	0	0.00	
	3	10	0	0.00	
8	1	10	0.5	450.00	453.0
	2	10	0.5	450.00	
	3	10	0.51	459.00	
11	1	10	0.45	405.00	355.5
	2	10	0.41	369.00	
	3	10	0.38	342.00	
15	1	10	0.1	90.00	135.0
	2	10	0.2	180.00	
	3	10	0.2	180.00	
18	1	10	0.2	180.00	180.0
	2	10	0.2	180.00	
	3	10	0.13	117.00	
22	1	10	0.8	720.00	724.5
	2	10	0.81	729.00	
	1	10	0.76	684.00	
25	1	10	0.4	360.00	342.0
	2	10	0.39	351.00	
	3	10	0.35	315.00	
29	1	10	0.4	360.00	425.3
	2	10	0.5	450.00	
	3	10	0.5	450.00	
	4	10	0.49	441.00	
32	1	10	0.48	432.00	417.0
	2	10	0.5	450.00	
	3	10	0.41	369.00	
36	1	10	0.38	342.00	351.0
	2	10	0.39	351.00	
	3	10	0.4	360.00	
39	1	10	0.3	270.00	318.0
	2	10	0.37	333.00	
	3	10	0.39	351.00	
43	1	10	0.41	369.00	378.0
	2	10	0.43	387.00	
	3	10	0.42	378.00	
46	1	10	0.27	243.00	213.0
	2	10	0.2	180.00	
	3	10	0.24	216.00	



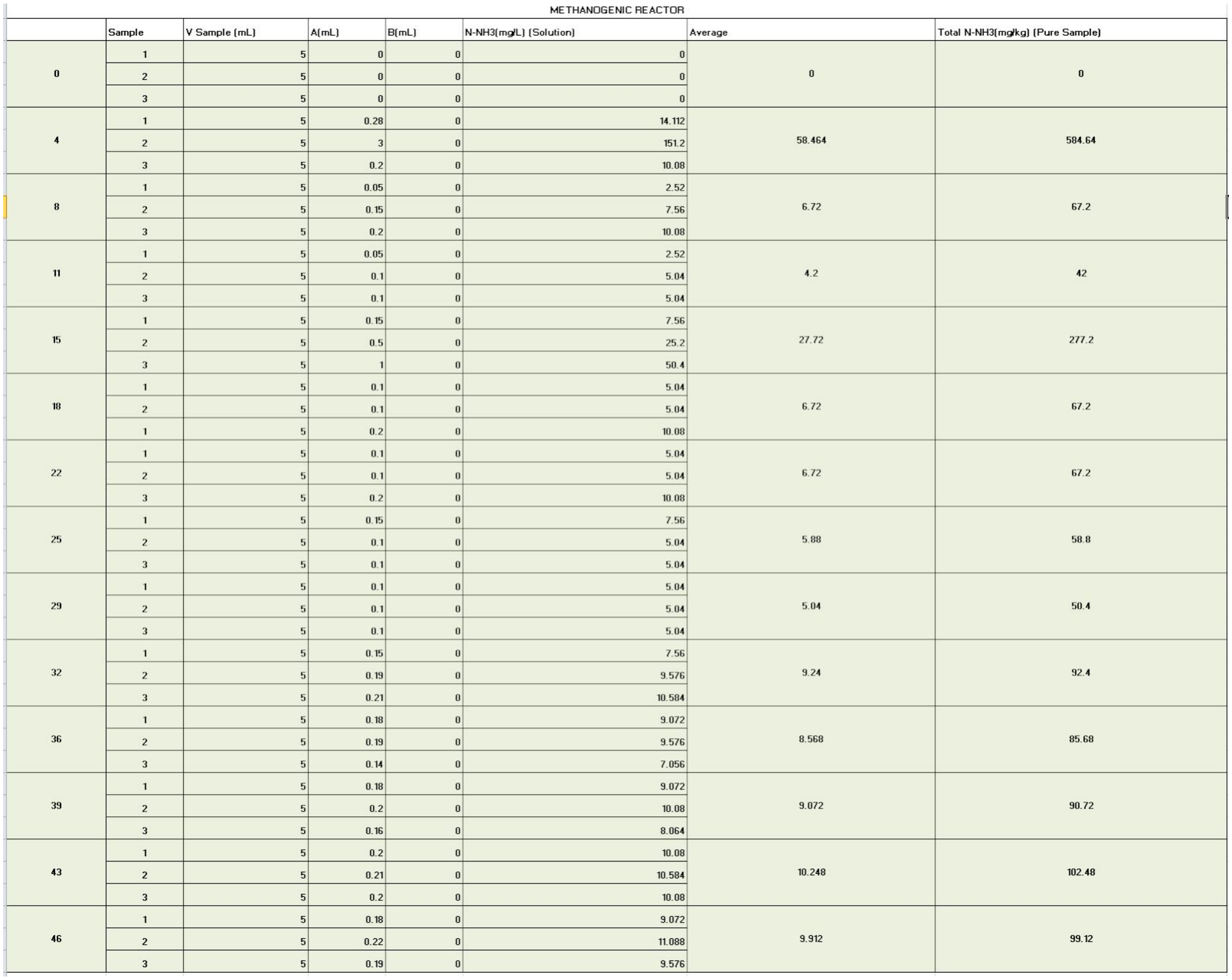
ALKALINITY METHANOGENIC REACTOR

Day	Sample	Vol Sample (mL)	Vol H2SO4 (mL)	Alkalinity Total (mgCaCO3/L)	Average Alkalinity (mgCaCO3/L)
0	1	10	0.5	450.00	420.00
	2	10	0.4	360.00	
	3	10	0.5	450.00	
4	1	10	2.3	2070.00	2295.00
	2	10	2.9	2610.00	
	3	10	2.45	2205.00	
8	1	10	0.9	810.00	750.00
	2	10	0.8	720.00	
	3	10	0.8	720.00	
11	1	10	1.3	1170.00	1140.00
	2	10	1.1	990.00	
	3	10	1.4	1260.00	
15	1	10	1.1	990.00	1050.00
	2	10	1.2	1080.00	
	3	10	1.2	1080.00	
18	1	10	1.1	990.00	1050.00
	2	10	1.2	1080.00	
	3	10	1.2	1080.00	
22	1	10	1.1	990.00	945.00
	2	10	1	900.00	
	3	10	1.05	945.00	
25	1	10	1.62	1458.00	1446.00
	2	10	1.7	1530.00	
	3	10	1.5	1350.00	
29	1	10	1.35	1215.00	1245.00
	2	10	1.4	1260.00	
	3	10	1.4	1260.00	
32	1	10	1.64	1476.00	1497.00
	2	10	1.65	1485.00	
	3	10	1.7	1530.00	
36	1	10	1.58	1422.00	1371.00
	2	10	1.5	1350.00	
	3	10	1.49	1341.00	
39	1	10	1.58	1422.00	1389.00
	2	10	1.54	1386.00	
	3	10	1.51	1359.00	
43	1	10	1.6	1440.00	1467.00
	2	10	1.64	1476.00	
	3	10	1.65	1485.00	
46	1	10	1.68	1512.00	1512.00
	2	10	1.73	1557.00	
	3	10	1.63	1467.00	

Appendix C: Ammonia Calculations



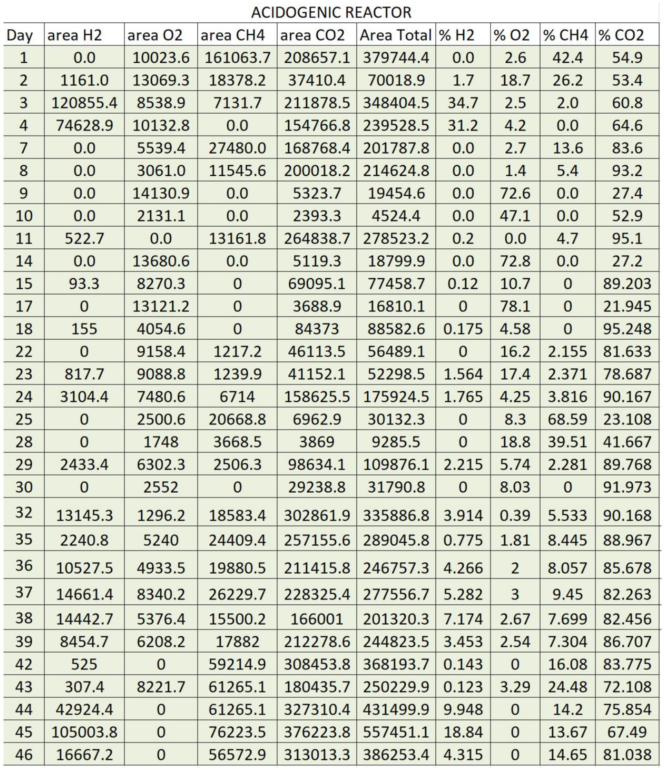
ACIDOGENIC REACTOR							
Day	Sample	V Sample (mL)	A(mL)	B(mL)	N-NH3(mg/L) (Solution)	Average	Total N-NH3(mg/kg) (Pure Sample)
0	1	5	0.12	0	6.048	6.216	62.16
	2	5	0.14	0	7.056		
	3	5	0.11	0	5.544		
4	1	5	0.2	0	10.08	10.08	100.8
	2	5	0.2	0	10.08		
	3	5	0.2	0	10.08		
8	1	5	0.4	0	20.16	14.28	142.8
	2	5	0.2	0	10.08		
	3	5	0.25	0	12.6		
11	1	5	0	0	0	0	0
	2	5	0	0	0		
	3	5	0	0	0		
15	1	5	0	0	0	0	0
	2	5	0	0	0		
	3	5	0	0	0		
18	1	5	0.05	0	2.52	5.04	50.4
	2	5	0.15	0	7.56		
	3	5	0.1	0	5.04		
22	1	5	0.5	0	25.2	25.2	252
	2	5	0.5	0	25.2		
	3	5	0.5	0	25.2		
25	1	5	0.2	0	10.08	10.08	100.8
	2	5	0.2	0	10.08		
	3	5	0.2	0	10.08		
29	1	5	0.2	0	10.08	6.72	67.2
	2	5	0.1	0	5.04		
	3	5	0.1	0	5.04		
32	1	5	0.09	0	4.536	4.788	47.88
	2	5	0.095	0	4.788		
	3	5	0.1	0	5.04		
36	1	5	0.1	0	5.04	5.0064	50.064
	2	5	0.1	0	5.04		
	3	5	0.098	0	4.9392		
39	1	5	0.12	0	6.048	6.048	60.48
	2	5	0.1	0	5.04		
	3	5	0.14	0	7.056		
43	1	5	0.1	0	5.04	6.384	63.84
	2	5	0.13	0	6.552		
	3	5	0.15	0	7.56		
46	1	5	0.12	0	6.048	5.88	58.8
	2	5	0.11	0	5.544		
	3	5	0.12	0	6.048		



METHANOGENIC REACTOR

	Sample	V Sample (mL)	A(mL)	B(mL)	N-NH3(mg/L) (Solution)	Average	Total N-NH3(mg/kg) (Pure Sample)
0	1	5	0	0	0	0	0
	2	5	0	0	0		
	3	5	0	0	0		
4	1	5	0.28	0	14.112	58.464	584.64
	2	5	3	0	151.2		
	3	5	0.2	0	10.08		
8	1	5	0.05	0	2.52	6.72	67.2
	2	5	0.15	0	7.56		
	3	5	0.2	0	10.08		
11	1	5	0.05	0	2.52	4.2	42
	2	5	0.1	0	5.04		
	3	5	0.1	0	5.04		
15	1	5	0.15	0	7.56	27.72	277.2
	2	5	0.5	0	25.2		
	3	5	1	0	50.4		
18	1	5	0.1	0	5.04	6.72	67.2
	2	5	0.1	0	5.04		
	1	5	0.2	0	10.08		
22	1	5	0.1	0	5.04	6.72	67.2
	2	5	0.1	0	5.04		
	3	5	0.2	0	10.08		
25	1	5	0.15	0	7.56	5.88	58.8
	2	5	0.1	0	5.04		
	3	5	0.1	0	5.04		
29	1	5	0.1	0	5.04	5.04	50.4
	2	5	0.1	0	5.04		
	3	5	0.1	0	5.04		
32	1	5	0.15	0	7.56	9.24	92.4
	2	5	0.19	0	9.576		
	3	5	0.21	0	10.584		
36	1	5	0.18	0	9.072	8.568	85.68
	2	5	0.19	0	9.576		
	3	5	0.14	0	7.056		
39	1	5	0.18	0	9.072	9.072	90.72
	2	5	0.2	0	10.08		
	3	5	0.16	0	8.064		
43	1	5	0.2	0	10.08	10.248	102.48
	2	5	0.21	0	10.584		
	3	5	0.2	0	10.08		
46	1	5	0.18	0	9.072	9.912	99.12
	2	5	0.22	0	11.088		
	3	5	0.19	0	9.576		

Appendix C: Gas Composition Calculations



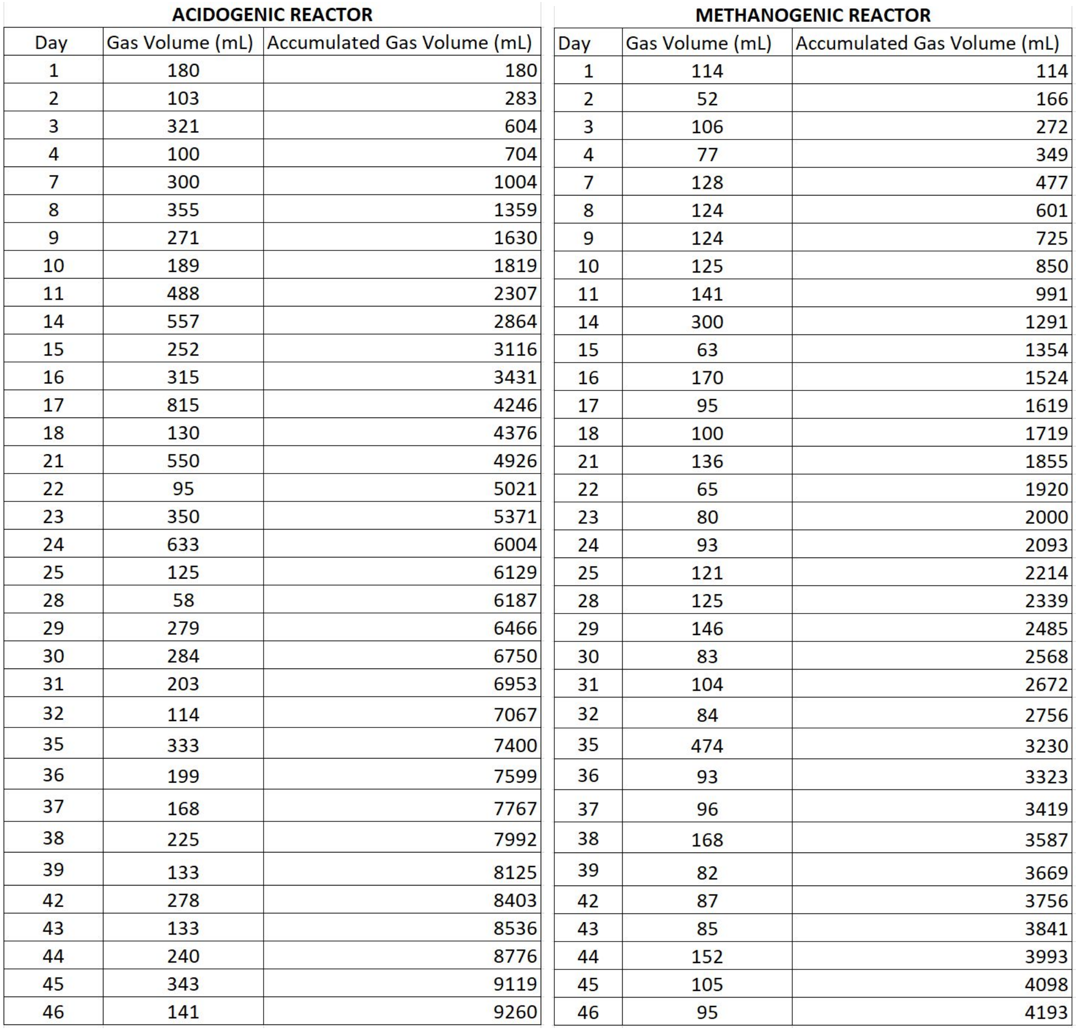
ACIDOGENIC REACTOR

Day	area H2	area O2	area CH4	area CO2	Area Total	% H2	% O2	% CH4	% CO2
1	0.0	10023.6	161063.7	208657.1	379744.4	0.0	2.6	42.4	54.9
2	1161.0	13069.3	18378.2	37410.4	70018.9	1.7	18.7	26.2	53.4
3	120855.4	8538.9	7131.7	211878.5	348404.5	34.7	2.5	2.0	60.8
4	74628.9	10132.8	0.0	154766.8	239528.5	31.2	4.2	0.0	64.6
7	0.0	5539.4	27480.0	168768.4	201787.8	0.0	2.7	13.6	83.6
8	0.0	3061.0	11545.6	200018.2	214624.8	0.0	1.4	5.4	93.2
9	0.0	14130.9	0.0	5323.7	19454.6	0.0	72.6	0.0	27.4
10	0.0	2131.1	0.0	2393.3	4524.4	0.0	47.1	0.0	52.9
11	522.7	0.0	13161.8	264838.7	278523.2	0.2	0.0	4.7	95.1
14	0.0	13680.6	0.0	5119.3	18799.9	0.0	72.8	0.0	27.2
15	93.3	8270.3	0	69095.1	77458.7	0.12	10.7	0	89.203
17	0	13121.2	0	3688.9	16810.1	0	78.1	0	21.945
18	155	4054.6	0	84373	88582.6	0.175	4.58	0	95.248
22	0	9158.4	1217.2	46113.5	56489.1	0	16.2	2.155	81.633
23	817.7	9088.8	1239.9	41152.1	52298.5	1.564	17.4	2.371	78.687
24	3104.4	7480.6	6714	158625.5	175924.5	1.765	4.25	3.816	90.167
25	0	2500.6	20668.8	6962.9	30132.3	0	8.3	68.59	23.108
28	0	1748	3668.5	3869	9285.5	0	18.8	39.51	41.667
29	2433.4	6302.3	2506.3	98634.1	109876.1	2.215	5.74	2.281	89.768
30	0	2552	0	29238.8	31790.8	0	8.03	0	91.973
32	13145.3	1296.2	18583.4	302861.9	335886.8	3.914	0.39	5.533	90.168
35	2240.8	5240	24409.4	257155.6	289045.8	0.775	1.81	8.445	88.967
36	10527.5	4933.5	19880.5	211415.8	246757.3	4.266	2	8.057	85.678
37	14661.4	8340.2	26229.7	228325.4	277556.7	5.282	3	9.45	82.263
38	14442.7	5376.4	15500.2	166001	201320.3	7.174	2.67	7.699	82.456
39	8454.7	6208.2	17882	212278.6	244823.5	3.453	2.54	7.304	86.707
42	525	0	59214.9	308453.8	368193.7	0.143	0	16.08	83.775
43	307.4	8221.7	61265.1	180435.7	250229.9	0.123	3.29	24.48	72.108
44	42924.4	0	61265.1	327310.4	431499.9	9.948	0	14.2	75.854
45	105003.8	0	76223.5	376223.8	557451.1	18.84	0	13.67	67.49
46	16667.2	0	56572.9	313013.3	386253.4	4.315	0	14.65	81.038

METHANOGENIC REACTOR

Day	area H2	area O2	area CH4	area CO2	Area Total	% H2	% O2	% CH4	% CO2
1	0	4937	287148	273882	565966.4	0	0.872	50.736	48.392
2	2312.1	9242.6	138986	198227	348767.8	0.6629	2.65	39.85	56.836
3	0	13658	44241.1	98389.7	156288.6	0	8.739	28.307	62.954
4	0	3351.2	67441.3	150183	220975	0	1.517	30.52	67.964
7	0	0	1338.7	5610.9	6949.6	0	0	19.263	80.737
8	0	1558.5	40057.9	100192	141808	0	1.099	28.248	70.653
9	0	2009.3	214688	224750	441447.2	0	0.455	48.633	50.912
10	0	3999.3	173499	154735	332233.3	0	1.204	52.222	46.574
11	0	5329.5	109883	167054	282265.8	0	1.888	38.929	59.183
14	0	8437.2	28698	89467.5	126602.7	0	6.664	22.668	70.668
15	0	7598.3	26756.7	118237	152591.9	0	4.979	17.535	77.486
17	0	2012.5	183785	89573.7	275371.5	0	0.731	66.741	32.528
18	0	0	12068.4	6246.8	18315.2	0	0	65.893	34.107
22	0	1340.6	262919	177272	441531.6	0	0.304	59.547	40.149
23	0	0	291341	208168	499509.3	0	0	58.325	41.675
24	0	0	271716	254626	526341.6	0	0	51.623	48.377
25	0	0	237436	233087	470523.2	0	0	50.462	49.538
28	0	1051.8	53534.9	56326.1	110912.8	0	0.948	48.268	50.784
29	0	6143.3	53456.2	68088.7	127688.2	0	4.811	41.865	53.324
30	0	6642	86409.8	82594.2	175646	0	3.781	49.195	47.023
32	0	0	260177	193425	453602	0	0	57.358	42.642
35	0	0	297354	228218	525571.4	0	0	56.577	43.423
36	0	0	290007	202652	492659.6	0	0	58.866	41.134
37	0	0	403970	166382	570351.6	0	0	70.828	29.172
38	0	0	396278	174389	570666.3	0	0	69.441	30.559
39	0	0	346421	202260	548681.4	0	0	63.137	36.863
42	0	0	370688	264065	634752.9	0	0	58.399	41.601
43	0	0	337155	221615	558770.4	0	0	60.339	39.661
44	0	0	376460	181917	558377.1	0	0	67.42	32.58
45	0	0	356283	169785	526068.3	0	0	67.726	32.274
46	0	0	397912	178594	576505.2	0	0	69.021	30.979

Appendix C: Gas Volume Calculations



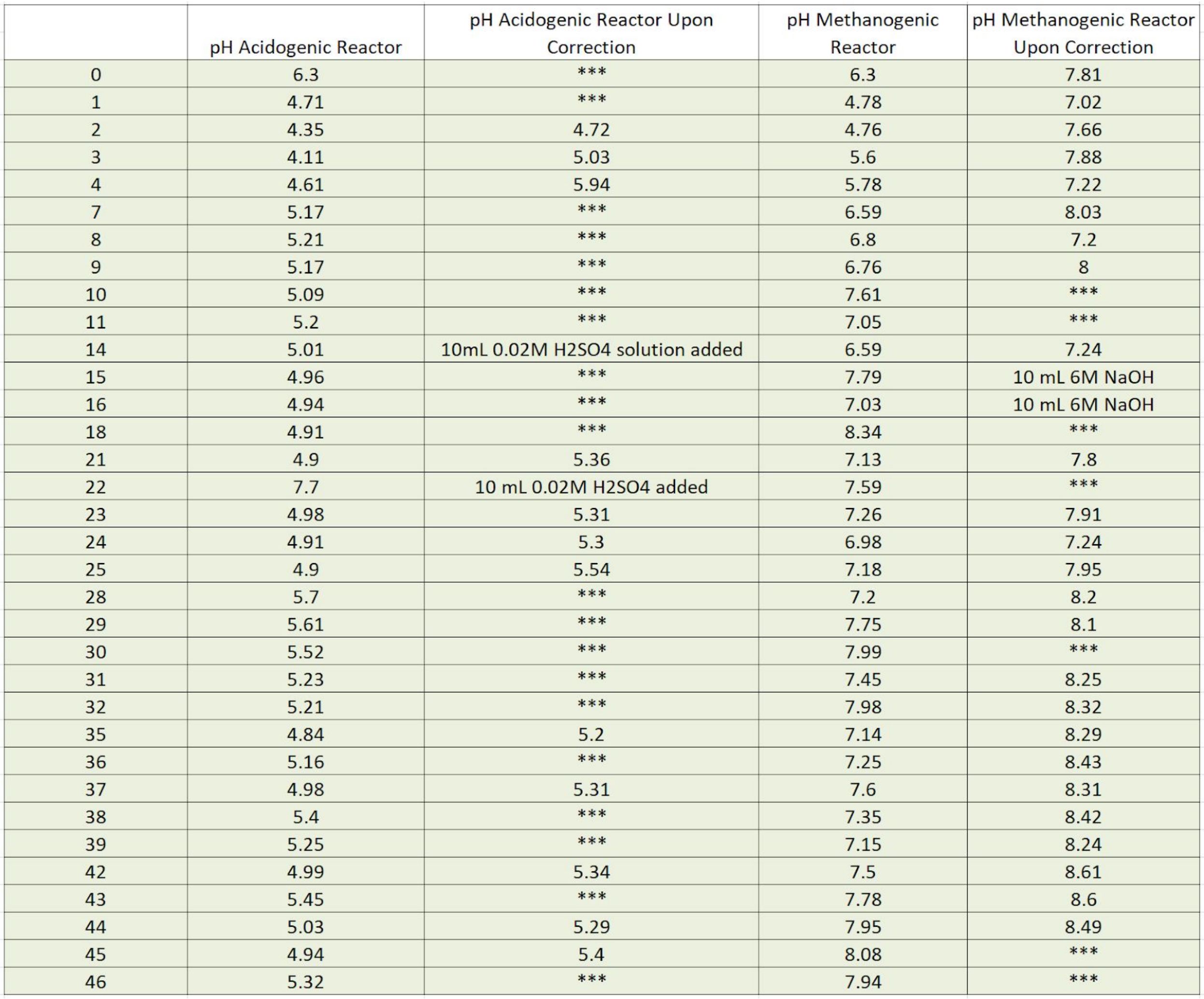
ACIDOGENIC REACTOR

Day	Gas Volume (mL)	Accumulated Gas Volume (mL)
1	180	180
2	103	283
3	321	604
4	100	704
7	300	1004
8	355	1359
9	271	1630
10	189	1819
11	488	2307
14	557	2864
15	252	3116
16	315	3431
17	815	4246
18	130	4376
21	550	4926
22	95	5021
23	350	5371
24	633	6004
25	125	6129
28	58	6187
29	279	6466
30	284	6750
31	203	6953
32	114	7067
35	333	7400
36	199	7599
37	168	7767
38	225	7992
39	133	8125
42	278	8403
43	133	8536
44	240	8776
45	343	9119
46	141	9260

METHANOGENIC REACTOR

Day	Gas Volume (mL)	Accumulated Gas Volume (mL)
1	114	114
2	52	166
3	106	272
4	77	349
7	128	477
8	124	601
9	124	725
10	125	850
11	141	991
14	300	1291
15	63	1354
16	170	1524
17	95	1619
18	100	1719
21	136	1855
22	65	1920
23	80	2000
24	93	2093
25	121	2214
28	125	2339
29	146	2485
30	83	2568
31	104	2672
32	84	2756
35	474	3230
36	93	3323
37	96	3419
38	168	3587
39	82	3669
42	87	3756
43	85	3841
44	152	3993
45	105	4098
46	95	4193

Appendix C: pH Measurements



	pH Acidogenic Reactor	pH Acidogenic Reactor Upon Correction	pH Methanogenic Reactor	pH Methanogenic Reactor Upon Correction
0	6.3	***	6.3	7.81
1	4.71	***	4.78	7.02
2	4.35	4.72	4.76	7.66
3	4.11	5.03	5.6	7.88
4	4.61	5.94	5.78	7.22
7	5.17	***	6.59	8.03
8	5.21	***	6.8	7.2
9	5.17	***	6.76	8
10	5.09	***	7.61	***
11	5.2	***	7.05	***
14	5.01	10mL 0.02M H2SO4 solution added	6.59	7.24
15	4.96	***	7.79	10 mL 6M NaOH
16	4.94	***	7.03	10 mL 6M NaOH
18	4.91	***	8.34	***
21	4.9	5.36	7.13	7.8
22	7.7	10 mL 0.02M H2SO4 added	7.59	***
23	4.98	5.31	7.26	7.91
24	4.91	5.3	6.98	7.24
25	4.9	5.54	7.18	7.95
28	5.7	***	7.2	8.2
29	5.61	***	7.75	8.1
30	5.52	***	7.99	***
31	5.23	***	7.45	8.25
32	5.21	***	7.98	8.32
35	4.84	5.2	7.14	8.29
36	5.16	***	7.25	8.43
37	4.98	5.31	7.6	8.31
38	5.4	***	7.35	8.42
39	5.25	***	7.15	8.24
42	4.99	5.34	7.5	8.61
43	5.45	***	7.78	8.6
44	5.03	5.29	7.95	8.49
45	4.94	5.4	8.08	***
46	5.32	***	7.94	***